Captive Breeding, Seed Production, Grow-out Culture and Biomedicinal Properties of the Commercially Important Sea Urchins (Echinodermata: Echinoidea)

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***Abstract*—**The harvested product of sea urchin fisheries is the gonad of both sexes, commonly known as "Sea urchin Roe”. There are long traditions of consuming sea urchin roe as a high delicacy food in Asian, Mediterranean and Western Hemisphere countries such as Barbados and Chile, and have long been using as luxury foods in Japan. The population of the Asian Pacific Region has been using it for long time as a remedy for improving general living tone and treatment for a number of diseases. Sea urchins have been examined as a source of biologically active compounds with biomedical applications. Sea urchin gonads are also rich in valuable bioactives, such as polyunsaturated fatty acids (PUFAs) and β-carotene. PUFAs, especially eicosapentaenoic acid (EPA, C20:5) (n-3)) and docosahexaenoic acid (DHA C22:6 (n-3)), have significant preventive effects on arrhythmia, cardiovascular diseases and cancer. β-Carotene and some xanthophylls have strong pro-vitamin A activity and can be used to prevent tumor development and light sensitivity. Sea urchin fisheries have expanded so greatly in recent years that the natural population of sea urchins in Japan, France, Chile, the northeastern United States, the Canadian Maritime Provinces, and the west coast of North America from California to British Colombia have been overfished to meet the great demand. This decrease in supply and the continued strong demand have led to a great increase in interest in aquaculture of sea urchins. Most, if not all, sea urchin fisheries have followed the same pattern of rapid expansion to an unsustainable peak, followed by an equally rapid decline. World landings of sea urchin, having peaks at 120,000 mt in 1995, are now in the state of about 82,000 mt. However, over half this catch comes from the recently expanded Chilean fishery for *Loxechinus albus*. In Europe, the sea urchin stocks (*Paracentrotus lividus*) of first France and then Ireland were overfished to supply the French markets. These decreasing patterns clearly reflect the overexploitation of most sea urchin fishery grounds and highlight the need for conservation policies, stock enhancement and fishery management to readjust levels that provide long-term sustainability. Given that demand is unlikely to decline, the economic value of future production will be increased. This also opens new opportunities for alternative methods of production such as on-growing and aquaculture under controlled rearing systems.

***Keywords*—**Sea urchins, Breeding, Seed production, Aquaculture, Bioactive compounds.

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I. Introduction

THE sea urchins are classic objects of research in different fields of biology, ecology, biodiversity, evolution, aquaculture and conservation. At the same time, they are used as raw material to produce foodstuff, in particular, the product of processing gonads known as "Sea urchin Roe", and is considered as a prized delicacy in Asian, Mediterranean and Western Hemisphere countries [1−3]. The gonads of sea urchins either fresh or in the form of processed food have long been using as luxury foods in Japan [4]. The peoples of the Asian Pacific Region has also long been using it as a remedy for improving general living tone, treatment for a number of diseases and strengthening of the sexual potency of men, especially the middle aged [5, 6]. Although, sea urchin gonad has not yet been used as food in Malaysia, it is reported that in Sabah, an indigenous tribe known as ‘Bajau Laut’ eats sea urchin roe with rice after adding spices [3, 7−9]. This delicacy is usually prepared for special events such as Lepa-Lepa Festival, wedding ceremony and other cultural events and is being treated as valuable fishery resources especially by Bajau people [10]. Thus, sea urchins play an important role in providing subsistence income to the local coastal communities. In Japan, sea urchin is known as “uni”, and its roe can retail for as much as AU$450/kg [11]. Sea urchin fisheries have expanded so greatly in recent years that the natural population in Japan, France, Chile, the northeastern United States, the Canadian Maritime Provinces, and the west coast of North America from California to British Colombia have been overfished to meet the great demand [8, 9, 12−15]. World landings of sea urchin, having peaks at 120,000 mt in 1995, are now in the state of about 82,000 mt [16]. These decreasing patterns clearly reflect the overexploitation of most fishery grounds and highlight the need for appropriate conservation policies, stock enhancement, fishery management and aquaculture development to fill the potential gap between demand and supply.

II. Sea Urchins And Bioactive Compounds

Alike many other marine invertebrates, sea urchins have been examined as a source of bioactive compounds with biomedical applications [17]. Nevertheless, the potential of echinoids as a source of biologically active products are largely unexplored and undermined [18]. The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural and chemical features not found in terrestrial natural products. Newly established modern technologies have opened vast areas of research for the extraction of biomedicinal compounds from ocean and seas to treat the fatal diseases. The number of natural products isolated from marine organisms increases rapidly, and now exceeds with hundreds of new compounds being discovered every year [19]. It is expected that some of the secondary metabolites may be pharmacologically active on humans and useful as medicines [20]. Majority of the pharmacologically active secondary metabolites have been isolated from echinoderms [21]. There have been valuable information available for new antibiotic discoveries and give new insights into biologically active compounds in sea urchins; their shells are containing various polyhydroxylated naphtoquinone pigments, spinochromes [22] as well as their analogous compound echinochrome A, which was found to have significant bactericidal effect [23]. The phenolic hydroxyl groups in these molecules also suggested that they could participate in antioxidant activity as was observed in other well-known antioxidant polyphenols such as tea catechins. The similar structured compounds were also found in the shells of sea urchins and thus suggesting that they as well as echinochrome A would act as antioxidant substances similar to other polyphenolic antioxidants in edible plants [24]. Squaric acid ester-based methodology was also used in a new synthesis of echinochrome A pigment, commonly isolated from sea urchin spines [25]. The gonads of sea urchin contain echinochrome A, which have potential antioxidant activity [26]. Sea urchin gonads are also rich in valuable bioactive compounds, such as polyunsaturated fatty acids (PUFAs) and β-carotene [27]. PUFAs, especially eicosapentaenoic acid (EPA, C20:5) (n-3)) and docosahexaenoic acid (DHA C22:6 (n-3)), have significant preventive effects on arrhythmia, cardiovascular diseases and cancer [28]. β-Carotene and some xanthophylls have strong pro-vitamin A activity and can be used to prevent tumor development and light sensitivity [29]. The composition of these valuable components, however, varies greatly among different urchin species and is influenced by their natural diet and physiological processes i.e. reproductive stage [30, 31]. On the other hand, the high levels of AA and EPA recently detected in *Diadema setosum* and *Salmacis sphaeroides* supported the development of aquaculture of sea urchins [32], since PUFAs are important for human nutrition [31]. However, similar to many other marine invertebrates, sea urchins have been and continue to be examined as a potential source of bioactive compounds for nutraceutical and biomedical applications.

III. Sea Urchin Breeding, Seed Production And Aquaculture

It has been documented around 40 edible sea urchin species within more than 900 species that exist worldwide. Among them, 11 species have recently been documented in Malaysia's coral reef communities. These are: *Diadema setosum, D. savignyi, Echinothrix diadema, Salmacis sphaeroides, Parasalenia gratiosa, Toxopneustes pileolus, Astropyga radiata, Echinometra mathaei, Echinothrix calamaris, Salmaciella dussumieri* and *Tripneustes gratilla* [3, 7, 14]. However, very few systematic works have been done on the abundance, distribution and population growth patterns of *D. setosum* and *S. sphaeroides* in Malaysia [7, 15] but no published information on their breeding, nursing, seed production and culture techniques are available. Due to the higher nutritional values of sea urchin gonad, it is very important to develop appropriate techniques for breeding and nursing. In view of this, two projects have been undertaken: (i) to develop a viable methodology for breeding, seed production and culture of *D. setosum* and *S. sphaeroides* in captivity and (ii) to determine the biochemical composition of gonads in a view to develop nutraceutical and pharmaceutical products for biomedical utilizations.

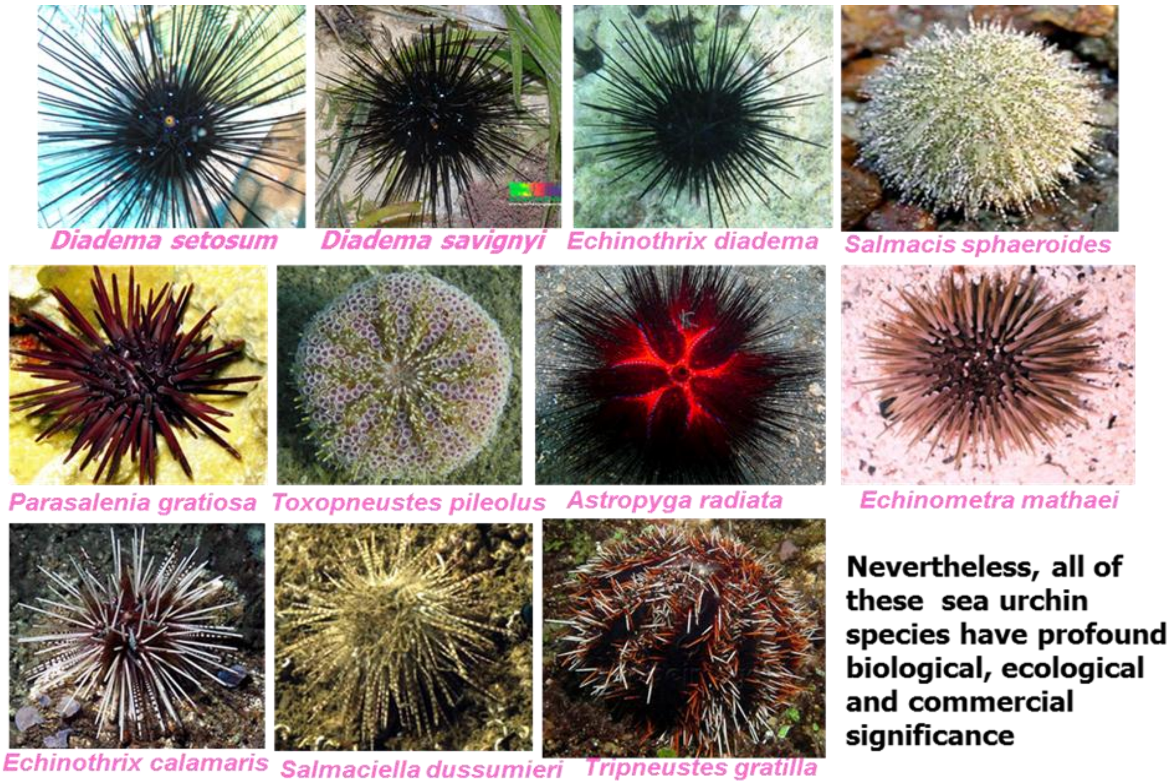

Fig. 1. Eleven species of sea urchins documented in Malaysia's coral reef communities

The sexually matured adults of the sea urchins, *D. setosum* (Fig. 2A) and *S. sphaeroides* (Fig. 2B), weighing from 100 to 150 g, were collected from Pulau Pangkor, Perak and Tanjung Kupang, Johor, respectively, at low tide during their natural breeding season from April to September, 2011. Immediately after collection, the live sea urchins were transported to the Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, where they were maintained in aerated aquaria before used for breeding trials.

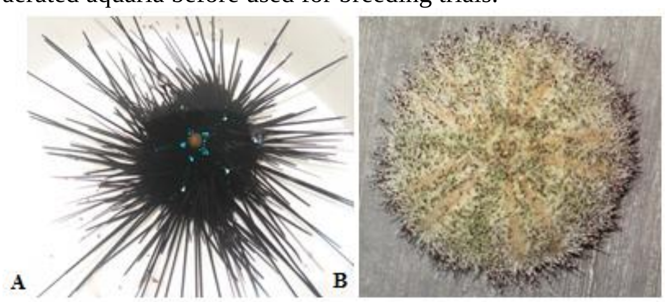

Fig. 2. Matured adults of tropical sea urchins: A) *D. setosum* [7], B) *S. sphaeroides* [14].

Gametes from both female and male urchins were obtained by injecting 0.5 M KCl into the coelomic cavity. Eggs were collected by inverting female urchins over a glass beaker filled with filtered sea water (FSW). Fertilization was done at limited sperm concentration and the resulting embryos and larvae were reared. When the larvae attained feeding stage (four-armed pluteus), they were cultured in glass bottles on a rotating roller with a larval density of 1-2 individual/ml. Larvae were supplemented with a cultured phytoplankton, *Chaetoceros calcitrans* at concentrations of 4,000-8,000 cells per ml of medium daily until attaining metamorphic competence within 1 month post-fertilization (Fig. 3a,b).

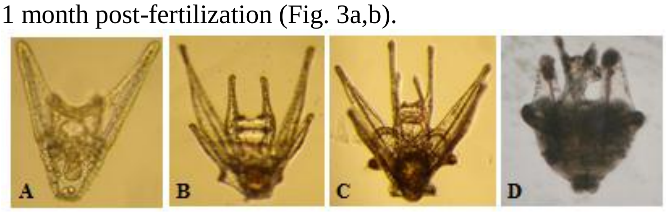

Fig. 3a. Larval developmental stages of *S. sphaeroides*: A) 4-armpluteus, B) 6-arm pluteus, C) 8-arm pluteus, D) Competent larva with complete rudiment growth [14].

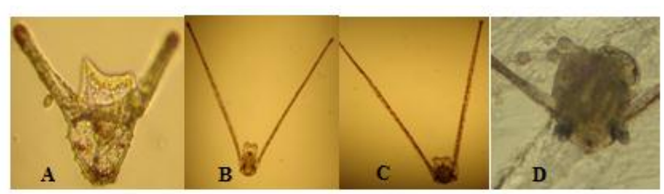

Fig. 3b. Larval developmental stages of *D. setosum*: A) 4-arm pluteus, B) POA (Postoral arm)-elongated stage-1, C) POA-elongated stage-2, D) Competent larva with complete rudiment growth and development.

Induction of metamorphosis was performed on coralline red algal extracts + *Chaetoceros* diatom (50:50) in petri dishes (9.0 x 3.0 cm) containing FSW. Majority of the competent larvae were metamorphosed to young juveniles within 1 day post-settlement and then cultured on coralline algal stones in aerated aquaria for three months by which time they attained appropriate juvenile sizes (Fig. 4a,b) for stocking in grow out culture aquaria.

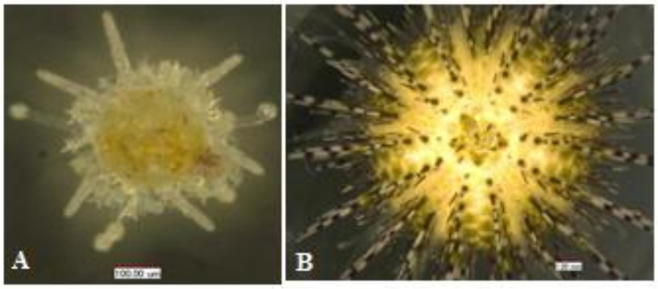

Fig. 4a. Juveniles of *S. sphaeroides*: A) 1-day-old juvenile, B) 3-month-old juvenile.

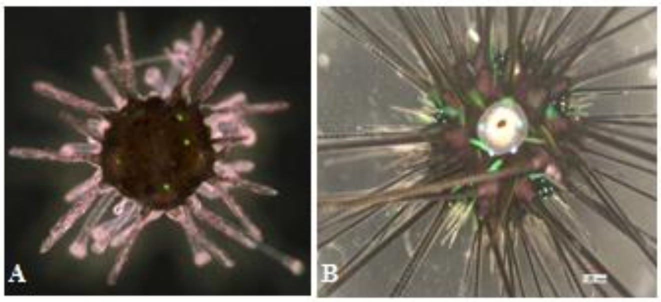

Fig. 3b. Juveniles of *D. setosum*: A) 1-day-old juvenile, B) 3-month-old juvenile.

To develop the appropriate aquaculture techniques, the 3-month old juvenile urchins are reared in aquaria (46 x 30 x 30 cm) at different stocking densities and algal feeding regimes. After two years of rearing in captive condition, all the urchins attained adult sizes with adequate matured gonads for harvesting (Fig. 5).

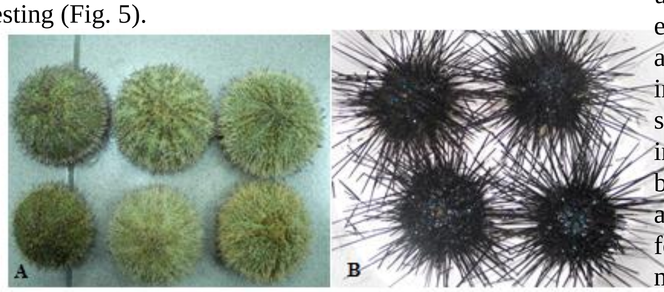

Fig. 5. Sexually matured adult sea urchins after the culture period of two years in captive aquaria-rearing condition: A) *S.sphaeroides*, B) *D. setosum.*

This study demonstrates the first successful culturing of *S. sphaeroides* and *D. setosum* through the detailed larval development, metamorphosis and juvenile rearing in captivity. Therefore, the results obtained from the designated project will immensely be helpful towards the development of breeding, seed production and culture techniques of commercially important sea urchins and other marine invertebrates, which are yet to be fully determined and explored in the Malaysian coral reef communities. In addition, development of appropriate rearing and culture techniques would immensely be helpful to produce adequate quantities of nutraceutical and pharmaceutical products from these high-valued Echinoderms. In contrast to the Japanese systems where hatchery-reared juveniles are mainly released to managed areas of seafloor [17, 33, 34], researchers in other countries have experimented with a wide range of grow-out systems for juvenile and adult urchins, ranging from relocation from poor to good feeding grounds [35] to the ranching of urchins caged on the seafloor [36]. Wild-collected adults of many species have been held in a variety of tank and sea-cage systems for roe conditioning [2, 17, 37−43]. Hatchery-reared juveniles have been grown in suspended culture [17, 44] in closed recirculation systems [45] and in dammed rock pools in southern Ireland. A sea-cage cultivation system of stacking baskets suspended from a ladder-like structure over which a work barge or raft can operate is being developed by Norwegian researchers [46]. The juveniles of most sea urchin species usually reach to marketable size within the culture period of 1–3 years.

Systems that accelerate growth to marketable size while producing a uniform size class would give an economic advantage. One possible route in obtaining sustainable and environmentally friendly systems for sea urchin culture is to further examine their potential in integrated aquaculture systems. They have already been shown to thrive in polyculture with the Atlantic salmon [47] and to have a role in land-based integrated systems [48]. However, many species are true omnivores, so the potential for their integration into systems where natural prey items, for example, mussels, are already produced should be fully determined and explored [17].

IV. Conclusion

From the views, reports and publications of other scientists/researchers works, it is almost apparent that sea urchin fisheries have a poor record of sustainability, as evidenced by the declines recorded in Japan, Maine, California and South Korea among others, as well as by the ad hoc and/or ineffective management in many sea urchin fisheries. Very few stocks have been formally assessed, meaning it is near impossible to qualify declines as the fish-down of accumulated biomass, which does not arrest the productivity of the stock, or as a case of over-fishing in which case its productivity may be forced into permanent decline. Small scale management is mentioned time and again as offering the most promise for ensuring long term sustainability. The strong and consistent spatial structure inherent in sea urchin stocks combined with excessive effort from mobile fleets and inappropriately large scale, and therefore ineffective management all contribute to declining production in many of the world's sea urchin fisheries. This is particularly the case for the world's largest sea urchins fishery in Chile where the risks of collapse cannot be discounted. Given that this fishery alone contributes upwards of 55% of the global harvest, a significant decline in Chile's fishery would likely lead to structural realignment in the market and higher prices for mid-range products until aquaculture production ramped up. There is also general agreement that some form of exclusive access as a prerequisite condition to promote meaningful enhancement and intelligent harvesting to maximize roe value will provide the best hedge against uncertainties in fisheries productivity and market stability.

In order to make sea urchins fisheries profitable, the following actions are suggested:

- Complete the life cycle in culture;
- Improve larval diets and shorten larval life;
- Provide suitable settlement substrates that maximize survival at metamorphosis and of the post-larval stages;
- Refine artificial diet formulations for juveniles and adults to maximize growth rates and survivorship and produce gonads of the desired taste, texture, flavor and color;
- Optimize grow-out facilities for juveniles and adults either at sea (in containers or 'ranched') or land based;
- Attend to packing, food hygiene, transport and marketing requirements;
- Regulations regarding fishing methods, fishing areas and protection of company investments need to be developed;
- Better surveillance of sea urchin density to guarantee a steady flow of raw materials;
- Areas need to be thinned out to get the best possible product for the market, this is also necessary for the kelp forest to grow back;
- More capital needs to be directed towards investing in technology for processing to reduce labour costs and preserve product quality;
- Improved cooperation between fishermen and processors, when marketing and selling the sea urchins.

In the short term it is likely that global production of sea urchin roe from wild fisheries will decline, with the major production being provided by those fisheries that have supported active management strategies to readjust effort and contain catches to levels that provide long-term sustainability. Given that demand is unlikely to decline; future production will be increasingly valuable. This will also open the new opportunities for alternative production methods such as on-growing and aquaculture under captive conditions

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