Echinoderms: Their Culture and Bioactive Compounds

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Abstract. Of the five extant classes of echinoderms, it is the sea urchins (Echinoidea) and the sea cucumbers (Holothuroidea) that are both commercially fished and heavily overexploited. In sea urchins, it is the gonad of both males and females, normally referred to as 'roe', that is a sought-after food. In the sea cucumber, the principal product is the boiled and dried body-wall or '*bêche-de-mer*' for which there is an increasing demand. Many sea urchin and sea cucumber fisheries still have no management system or restrictions in place, and for those that do, the prognosis for catches to continue even at a reduced level is poor. Cultivation of these species increasingly becomes a necessity, both for stock enhancement programs and as a means to meet market demand. Sea urchin culture has been practised on a large scale in Japan for many decades, and effective methods for the culture and reseeding of species in these waters have been long established. Juvenile urchins are produced in their millions in state-sponsored hatcheries, for release to managed areas of seafloor. Outside of Japan, sea urchin cultivation is still a fairly recent practice, less than 10 years old, and largely still at a research level, although a range of species are now being produced in a variety of different culture systems. It is essential that the culture systems are adapted to be species-specific and meet with local environmental constraints. Sea cucumber cultivation originated in Japan in the 1930s and is now well established there and in China. Methods for mass cultivation of the tropical *Holothuria scabra* are now well established and practised in India, Australia, Indonesia, the Maldives and the Solomon Islands, with the focus of the research effort for both temperate and tropical species being centred on the production of juveniles in hatcheries for the restoration and enhancement of wild stocks. Like many other marine organisms, echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications. Sea cucumber has been valued in Chinese medicine for hundreds of years as a cure for a

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wide variety of ailments. Some more recently isolated compounds, mainly from sea cucumbers and starfish, and including those with antitumour, antiviral, anticoagulant and antimicrobial activity, are summarised below. When wild stocks decline, the demand created in the market place raises the price of the product and, consequently, culturing is more likely to become viable economically. As this review shows, there have been dramatic advances in the culture methods of sea urchins and sea cucumbers in the last 10–15 years, to the extent that one can conclude that currently the major obstacles to successful cultivation are indeed economic rather than biological. Hence the future of the echinoculture industry is closely linked to that of the fisheries, whose fate will ultimately determine the market forces that will shape this growing industry.

1
Why Cultivate?

Of the five extant classes of echinoderms, it is the sea urchins (Echinoidea) and the sea cucumbers (Holothuroidea) that are both commercially fished and heavily overexploited. In sea urchins, it is the gonad of both males and females, normally referred to as ‘roe’, that is a sought-after food. The largest market is Japan, but there are also undersupplied markets in Europe. In the sea cucumber, the principal product is the boiled and dried body-wall or ‘*bêche-de-mer*’, for which there is an increasing demand from China, where it is valued as food but also for its curative properties. There is also a trade in sea cucumbers for home aquaria and biomedical products (Bruckner et al. 2003), and at one time there was fishing activity for starfish (Asteroidea), which were processed for fish or poultry meal or for fertiliser (Sloan 1984). The extent and demise of the world’s sea urchin and sea cucumber fisheries have been reviewed by Sloan (1984), Conand and Byrne (1993) and Keesing and Hall (1998), and more comprehensively for sea urchins by Andrew et al. (2002) and for sea cucumbers by Bruckner et al. (2003).

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 peaked at 120,000 t (metric tonnes) in 1995, are now in the region of 90,000 t/year. However, over half this catch comes from the recently expanded Chilean fishery for *Loxechinus albus*; which also appears to have peaked, the catch now being sustained by the discovery of new fishing grounds. The other major sea urchin fisheries, in terms of tonnage landed, are in Japan, Maine (USA), British Columbia (Canada) and California (USA) (Andrew et al. 2002). In Europe, the sea urchin stocks (*Paracentrotus lividus*) of first France and then Ireland were overfished in the 1980s to supply the French markets and those stocks have never recovered (Barnes and Crook 2001; Barnes et al. 2002). There are large populations of edible urchins in Scotland (*Echinus esculentus* and *Psammechinus miliaris*) and Norway (*Strongylocentrotus droebachiensis*), but these stocks are unsuited to commercial fishing as their roe content is either too low or too variable (Hagen 2000; Kelly 2000; Kelly et al. 2001; Sivertsen 2004).

The global landing of sea cucumbers was estimated as 13,000 t dried weight (130,000 t live weight) in 1995. The largest fisheries today are in Indonesia and the Philippines; however, in the last decade, the number of countries and species involved in the trade have increased in both tropical and temperate regions and the fishery has spread to non-traditional areas such as Mexico, the Galapagos and North America. Bruckner et al. (2003) list 29 species from 50 countries that are fished for import into Hong Kong, many of which will be re-exported to China and other trade centres in Singapore and Chinese Taipei.

Regulations are now imposed on some sea urchin and sea cucumber fisheries, although few are based on formal stock assessments. For example, in the state of Maine, on the east coast of the USA, the sea urchin fishery is 'zoned', in part based on the reproductive/spawning cycle along the coast. Regulation limits fishing effort; there is a limited entry scheme to the fishery, restrictions on gear size, fishing times and minimum and maximum landing sizes. However, researchers believe even this diminished catch is likely to prove unsustainable (L. Harris, University of New Hampshire, USA, pers. comm.).

In contrast, the Japanese sea urchin fishery has endured for more than 50 years, yielding more than 13,000 t in 1998. Six species account for the bulk of the commercial landings, but it is important to note that there is government subsidy for urchin stock enhancement and this is used as a management tool to conserve and rebuild stocks (Andrew et al. 2002).

Many sea cucumber fisheries show evidence of overexploitation or have already collapsed and some still have no known management or restrictions, including the world's largest fishery in Indonesia (Bruckner et al. 2003). Possible management measures suggested for sea cucumber fisheries include the imposition of minimum landing sizes, closed seasons, no-take marine protected areas, bag limits, prohibition of night fishing for nocturnal species and restrictions on the use of SCUBA for harvesting. However, the artisanal nature of the fishery makes implementation of such measures difficult. Bruckner et al. (2003) propose that listing sea cucumbers in the Convention on International Trade in Endangered Species (CITES) Appendix II might be an appropriate way to ensure that harvest to international markets is conducted in a sustainable manner, without detriment to the target species or their ecosystem. Compounding the impact of overexploitation of wild echinoderm populations is the increasing conviction among researchers that recruitment of juvenile sea urchins is both sporadic and unpredictable (see Lawrence 2001 for reviews; also Kelly 2000; Harris et al. 2001), and the fact that some species of sea urchin are extremely long-lived (Ebert 1998). Sea cucumbers are a valuable source of income for many coastal communities, particularly among the developing nations of the Indo-Pacific, but as with sea urchins, their high value and sedentary, shallow-water habit have left them vulnerable to overexploitation through unregulated and illegal fishing activity. Once their density is reduced below a critical mass, populations may take as long as 50 years to recover (Dalzell et al. 1996; Battaglene 1999; Bruckner et al. 2003).

As the prognosis for catches to continue at the current level is poor, cultivation of these species increasingly becomes a necessity, both for stock enhancement programs and as a means to meet market demand. In addition, as wild stocks decline, cultivation is more likely to become viable economically. The need for effective culture methods is now reflected in increased research effort. Culture of sea urchins for reseeding is practised on a large scale in Japan and to a lesser extent in South Korea and the Philippines (Andrew et al. 2002). Sea cucumbers are cultured for reseeding on a large scale in China. Outside these countries most echinoculture is at a research or semi-commercial scale. There is no commercial-scale cultivation of starfish, brittle stars (ophiuroids) or feather stars (crinoids).

2 Sea Urchin Aquaculture

2.1 Life History and State of the Art

Edible sea urchins are among the orders of regular Echinoidea (Lawrence 2001), dioecious (separate sexes) and broadcast spawners; mature individuals shed gametes to seawater where fertilisation occurs. The eggs develop to form pluteus larvae, which, after a period of planktonic development, feeding on microalgae, settle to a substrate and undergo metamorphosis to form tiny juvenile sea urchins. The estimated time for these individuals to reach market size (40–50 mm horizontal test diameter) is commonly in the order of 1–3 years, again varying according to species.

Sea urchin culture has been practised on a large scale in Japan for many decades, and effective methods for the culture and reseeding of species in these waters have been long established. Juvenile urchins are produced in their millions in state-sponsored hatcheries, for release to managed areas of seafloor. The nationally co-ordinated reseeding program initiated in the 1960s has now developed to the extent that over 66 million juveniles were released onto reefs in 2000; of these, over 80 % were *Strongylocentrotus intermedius* (Agatsuma et al. 2004). The effectiveness of the reseeding program has not been easy to assess, in part because of the difficulty in discriminating between reseeded and wild individuals. In Hokkaido prefecture, reseeding of *S. intermedius* began in 1985 with the release of 1 million juveniles, increasing to over 60 million in 1996. However, for the first 8 years of this program, the total harvest of urchins from this area continued to decline and it is only since 1992 that the catch has begun to stabilise. The contribution of released sea urchins to the overall catch has been estimated to be between 62 and 80 % (Agatsuma et al. 2004). There are also much smaller-scale reseeding programs operating in South Korea and on Luzon Island in the Philippines (Andrew et al. 2002).

Outside of Japan, sea urchin cultivation is still a fairly recent practice, less than 10 years old. There have been researchers and companies developing methods for sea urchin (*Paracentrotus lividus*) cultivation in southern Ireland for over 20 years (Leighton 1995), and until relatively recently a concentrated effort into developing 'closed-cycle' land-based systems in France, also with *P. lividus* (Grosjean et al. 1998). Echinoculture (*Psammechinus miliaris*, *E. esculentus*, *P. lividus*) has been conducted in Scotland since 1995 and there are also established research teams on the east coast of North America – Florida, Alabama, Maine, New Hampshire, New Brunswick and Newfoundland – working on *S. droebachiensis* and *Lytechinus variegatus*; on the west coast of North America, including California and British Columbia (*S. droebachiensis*, *S. franciscanus*, *S. purpuratus*); in Chile (*Loxechinus albus*); Norway (*S. droebachiensis*); Israel (*P. lividus*); and in New Zealand (*Evechinus chloroticus*). This list is not intended to be exclusive or exhaustive but to illustrate the extent of the research base. The biology and ecology of these and other edible echinoids have recently been reviewed (Lawrence 2001).

While including discussion of the basic methodologies employed, both in and outside of Japan, this chapter will concentrate on examining some of the more recent advances and innovations in sea urchin culture, outside of Japan, with reference to the particular challenges remaining for the culturist at each stage of the sea urchin life cycle.

2.2 Sea Urchin Larviculture

Echinoids have been successfully raised in the laboratory for over 100 years (MacBride 1903). The reproductive periodicity for many echinoid species is well described; temperate water species in culture tend to have one spawning period per year (Himmelman 1977; Byrne 1993; Kelly 2000; Fig. 1) and brood stock are collected locally. Gravid individuals are induced to spawn either by temperature shock or, commonly, by injection of 0.5 M KCl to the coelom via the peristomal membrane. The concentration of sperm allowed to mix with the eggs must be controlled to optimise fertilisation and development success rates. The fertilised eggs hatch in approximately 10–15 h, depending on the species, to release a ciliated blastula which develops to the four-armed, then six-armed, then eight-armed pluteus larvae (Fig. 2). To raise large numbers of larvae in a commercial context the culture techniques must be refined in terms of food quality and quantity, larval density and water quality; and then shown to be effective once scaled up to large batches of larvae (>100,000). Static (no through-flow) aerated systems with a variable number of complete or

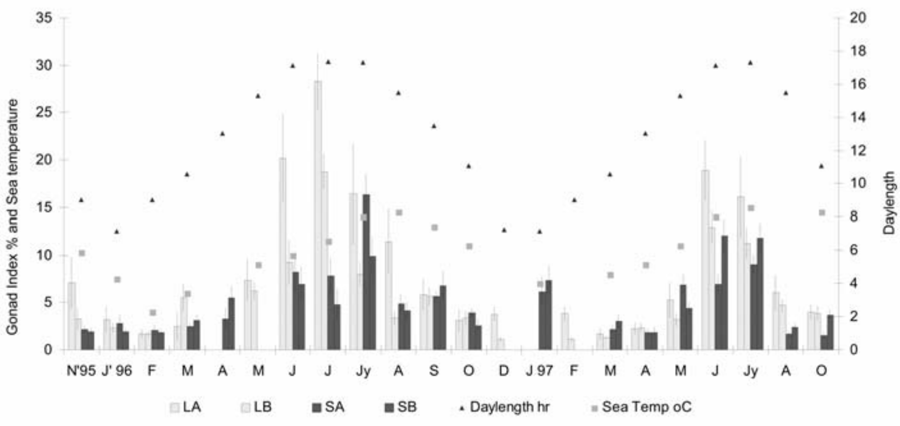

Fig. 1. Annual reproductive cycles (Nov 1995 to Oct 1997) of the sea urchin *Psammechinus miliaris* in a Scottish sea loch as described by the gonad index (GI). GI was calculated as wet weight of gonad divided by drained wet weight of sea urchin expressed as a percentage (n=10) for two littoral (*LA* and *LB*) and two subtidal (*SA* and *SB*) populations. Seawater temperature (°C) and day length (h) ▲ are illustrated. *Error bars* represent 95 % confidence limits. (Kelly 2000)

partial water changes throughout the larval life have been widely used (Fenaux et al. 1988; Leighton 1995; Grosjean et al. 1998; Kelly et al. 2000). In large-scale culture in Japan, partial exchange systems (Sakai et al. 2004) and continuous flow systems are used, the water flow being increased as the larvae develop (Hagen 1996). Upwelling silos, of the type used for fragile halibut yolk-sac larvae, have been tested on a small scale (M. Russell, Villanova University, USA, pers. comm.) and may also prove suitable for the large-scale culture of sea urchin larvae.

The planktonic diatom *Chaetoceros gracilis* is widely used as larval food in sea urchin hatcheries in Japan (Sakai et al. 2004). Many studies have compared different species or combinations of species of microalgae as larval foods for other species of sea urchin. Some species of microalgae used regularly and with success include *Isocrysis galbana* (Gonzalez et al. 1987), *Cricosphaera* (*Hymenomonas*) *elongata* (Fenaux et al. 1988) and *C. carterae* (Leighton 1995), the diatom *Phaeodactylum tricornutum* (Grosjean et al. 1998) and *Dunaliella tertiolecta* (Kelly et al. 2000; Jimmy et al. 2003). One noteworthy observation from these studies is there is no one optimal larval diet; different sea urchin species are reported to respond best to different algae. Of course, the biochemical and therefore nutritional value of the same species of microalgae, grown in different laboratories, may not be identical. However, it seems likely that there are true species-specific differences in echinoid larval dietary requirements.

Echinoid larvae demonstrate considerable plasticity in their morphology in response to varying food ration and quality. Growing larvae must increase

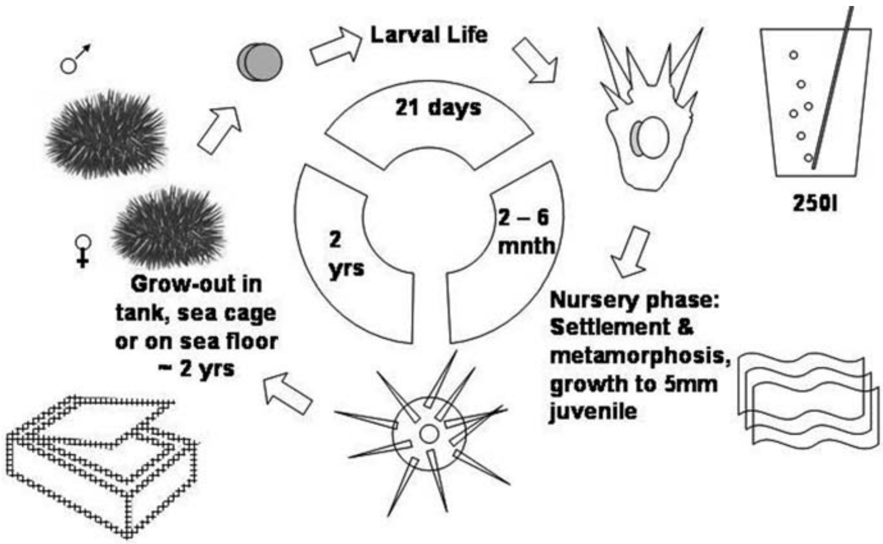

Fig. 2. Life cycle of the regular echinoid *Psammechinus miliaris* in culture. Gravid adults shed their gametes to seawater where fertilisation and first cleavage of the developing embryo occur within hours. Over the next 21 days, the planktonic larvae, maintained in aerated 250-l containers of seawater and fed microalgae, develop to a point where they are competent to settle. Newly metamorphosed juveniles are maintained on PVC wave plates coated with diatoms. At approximately 5-mm test diameter they are weaned to other foods (soft macroalgae or artificial diets) and transferred to a grow-out system where they mature to market size (40–50 mm test diameter)

the ciliated band length in order to increase feeding capability (McEdward 1984; Strathmann et al. 1992). Ciliated band length is increased by increasing arm length and by developing additional pairs of larval arms. The relative proportions of the larval body, e.g. post-oral arm length to larval body length, can therefore be a useful indicator of the nutritional status of larvae in culture (Fig. 3). Underfeeding will increase arm length relative to body length and overfed larvae may show a reduction in the length of the larval and in particular post-oral arms (Kelly et al. 2000; Jimmy et al. 2003).

One labour-intensive aspect of larval culture is the need for the simultaneous production of microalgae as live feed. However, sea urchin larvae may prove suited to culture using artificial diets, as research on *Lytechinus variegatus* (J.M. Lawrence, University of South Florida, USA, pers. comm.) has shown. It is the lipid or fatty acid component that is lost or destroyed in some forms of preserved algae. For example, their loss renders spray-dried microalgae a relatively poor food source for bivalve larvae which require poly- and highly unsaturated fatty acids (PUFAs and HUFAs) (Caers et al. 1998). Some species of sea urchin larvae have been shown to grow well when fed the green microalga *Dunaliella tertiolecta* (Kelly et al. 2000, Jimmy et al. 2003), which is

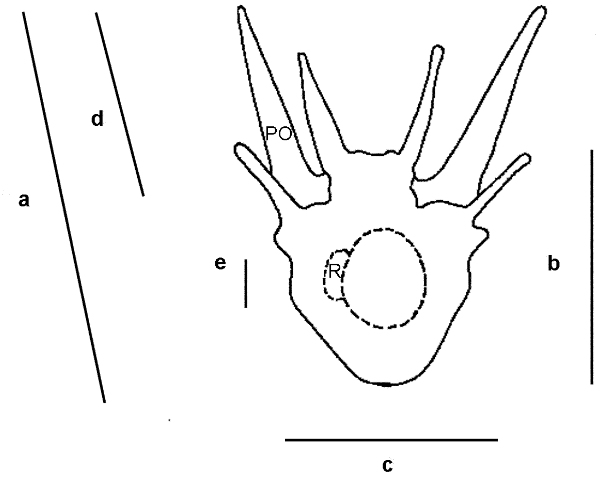

Fig. 3. Relative proportions of the echinoid larva that can be used as a measure of its nutritional status. *a* Larval length; *b* larval body length; *c* larval width; *d* post-oral arm length; *e* rudiment length; *R* echinorudiment; *PO* post-oral arm

known to be deficient in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Therefore, sea urchins may prove suited to culture using spray-dried or other preserved algal preparations.

2.3 Metamorphosis and the Post-Larval Stage

Sea urchin juveniles have been produced on a commercial or semi-commercial scale by hatcheries in Japan, South Korea, Ireland, Norway, Scotland and in British Columbia, Canada. When deemed competent to settle (judged by the size and state of development of the echinorudiment; Fig. 3), sea urchin larvae are presented with a substrate likely to induce metamorphosis, but which will subsequently serve as a food source for the early juvenile. Most culturists use a natural biofilm or a specially seeded diatom substrate created from species isolated locally and grown on a PVC wave plate. Optimising diets for the early juveniles and/or the replacement of diatom biofilms with artificial diets is probably one of the most challenging areas left to research. The variation in size and subsequent variation in growth rates of post-larvae remain a bottleneck in the supply of hatchery-reared juveniles. Hatchery-reared juveniles are robust enough to survive transfer to sea cages or other grow-out systems from a small size (5-mm test diameter) (Kelly 2002; Sakai et al. 2004). At this point they are weaned onto other diets, soft macroalgae or artificial diets, depending on the grow-out system.

2.4 Sea Urchin Grow-Out Systems

In contrast to the Japanese systems where hatchery-reared juveniles are mainly released to managed areas of seafloor (Hagen 1996; Sakai et al. 2004), researchers in other countries newer to echinoculture have experimented with a wide range of grow-out systems for juvenile and adult urchins, ranging from relocation from poor to good feeding grounds (Moylan 1997) to the ranching of urchins caged on the seafloor (Cuthbert et al. 1995). Wild-collected adults of many species have been held in a variety of tank and sea-cage systems for roe conditioning (Lawrence et al. 1997; Cook et al. 1998; McBride et al. 1998; Fernandez and Boudouresque 2000; Hammer et al. 2000; Kelly et al. 2001; Kennedy 2002; Pearce et al. 2002a). Hatchery-reared juveniles have been grown in suspended culture (Kelly 2002, and also at Instituto de Fomento, Pesquero, Hueihue, Chile) in closed recirculation systems (Grosjean et al. 1998) and in dammed rock pools in southern Ireland (J. Chamberlain, Dunmanus Seafoods Ltd., pers. comm.). 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 (Aas 2004). The time taken for juveniles of most species to reach market size is in the order of 1–3 years.

Systems that accelerate growth to market size while producing a uniform size class would give an economic advantage. One possible route to obtaining sustainable and environmentally friendly systems for urchin culture is to further examine their potential in integrated systems. They have already been shown to thrive in polyculture with the Atlantic salmon (Kelly et al. 1998) and to have a role in land-based integrated systems (Shpigel et al. 2004). 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 explored.

2.5 Juvenile and Adult Somatic Versus Gonadal Growth

Sea urchins can produce gonads with viable gametes years before they reach the typical ‘adult’ size for their species (Jensen 1969; Kelly 2001). Such small/young urchins are frequently termed ‘juvenile’ despite the fact they may have gonads, and young *Psammechinus miliaris* can successfully reproduce in their first year (Kelly 2001). However, it is likely once gametes start to form that urchins begin to partition ingested energy differently (Hagen 1998; Guillou and Lumingas 1999; Otero-Villanueva et al. 2004), and ultimately diets should be designed that promote somatic growth in urchins that are below market size, rather than encourage the sequestering of nutrients as gonad biomass.

2.6 Artificial Diets

The use of artificial diets has been widely adopted by researchers outside of Japan as they generally produce better growth rates than seaweeds. Seaweeds, potentially a cheap source of feed, have the disadvantage of being of variable quality and in variable supply over a season. In some regions, the large-scale harvesting of seaweeds would be regarded as an environmentally unsound practice.

Artificial diets are required to raise sea urchins in monoculture from juveniles to market size, for use as a finishing diet to perfect roe quality in urchins from polyculture systems and for use in enhancing the roe of fished urchins with unmarketable roe content. There is a large body of literature on the formulation of artificial diets for juvenile and adult sea urchins, many of which have been tested in comparison to seaweed as a reference diet. Most artificial diets contain a selection of soybean meal and cereals, either with or without animal-origin proteins and lipids (Cook et al. 1998; Fernandez and Boudouresque 2000; Spirlet et al. 2001), and range from simple moist or agar-bound diets (Klinger et al. 1994; Goebel and Barker 1998) to pellets extruded in commercial processing equipment (Lawrence et al. 1997; Pantazis et al. 2000; Olave et al. 2001). The impact of differing protein levels (de Jong-Westman et al. 1995; McBride et al. 1998; Hammer et al. 2000), the relative value of different protein sources (Pearce et al. 2002a), the necessity of minerals (Kennedy 2002) and effect of binder type (Pearce et al. 2002b) have all been examined. There now appears to be a consensus emerging from the literature that there is little advantage to feeding protein levels in excess of 30 %, and that lipid levels between 4 and 8 % are satisfactory. Although relatively little is known of the lipid biosynthetic pathways in sea urchins, they appear to have some capability for the elongation and desaturation of fatty acids (Bell et al. 2001; Kennedy 2002), and the inclusion of more expensive animal-origin oils is not essential for growth in some species (Pantazis et al. 2000; Kennedy 2002), although Floreto et al. (1996) suggested they may benefit juvenile growth in *Tripneustes gratilla*. It is also very likely there are species-specific differences in dietary needs in the juvenile and adult stages.

An inherent outcome of feeding an artificial formulation is a change in the biochemical composition of the urchin and its gonads (Fernandez 1997; Liyana-Pathirana et al. 2002), which will affect both flavour and colour. Free amino acids are the major factors influencing taste (Murata et al. 2002), and fatty acids and carotenoids are important in the development of 'off flavours', post-mortem (Liyana-Pathirana and Shahidi 2003).

Further research is required to better elucidate how each dietary component influences gonad biochemistry and the relation to gonad flavour. Some diets have been tested on more than one species, for example the 'Wenger' diet (Watts et al. 1998; Olave et al. 2001); however, further trials of one pre-defined diet on a range of sea urchin species would amplify species-specific differences in nutritional needs and assist researchers in optimising artificial diets for each species in culture.

2.7 Carotenoids in Sea Urchin Diets

Roe colour is a critical factor in the commercial product; poor or variable gonad colour at point of sale has a detrimental effect on the value of all species. Therefore cost-effective diets that positively enhance roe colour in adult urchins are key to the success of the industry. Sea urchins do not synthesise carotenoid pigments de novo, so the coloration of their gonad is the result of selective accumulation and modification of pigments from their diet. However, relatively little information is available on the way the primary dietary sources of carotenoids, be they of vegetable or animal origin, influence roe colour in the echinoids of commercial importance. For a review of the occurrence and distribution of carotenoids in echinoids, see Matsuno and Tsushima (2004).

In addition to several studies on the efficiency of pigment transfer from diets to gonads (Havardsson and Imsland 1999; McLaughlin and Kelly 2001; Robinson et al. 2002), the effect of carotenoids from natural and artificial diets on gonad development has been researched (Plank et al. 2002). As well as influencing colour, carotenoids are thought to have a role in biological defence (Kawakami et al. 1998) and reproduction (George et al. 2001). Studies using the same diet formulations and with the same pigment sources (β-carotene from a spray-dried microalgal preparation) (Robinson et al. 2002; Kelly 2004) have a different effect in different urchin species, an indication of species-specific pathways of carotenoid metabolism and expression.

Artificial diets do alter gonad carotenoid composition, but as carotenoid composition changes with sex, season, nutritive state and reproductive stage (Griffiths and Perrott 1976; Borisovets et al. 2002; Young et al. 2004), further research is required to unravel the complexities of pigment metabolism and expression in echinoids.

2.8 Sea Urchin Harvest Protocol, Spoilage and Shelf Life

Japan remains the world's largest consumer of sea urchin roe, and roe exported to Japan is usually delivered processed as chilled, frozen or canned produce. However, there is another undersupplied market in Europe; here, and in France particularly, the market demands a whole urchin. Therefore, harvest protocols should be developed that guarantee the shelf life and quality of sea urchins that are marketed intact. In Europe, sea urchins must conform to the EC Directive on Shellfish Hygiene (statutory instrument 994) and

Food Safety (fishery products and live shellfish). Although there is no requirement for classification of growing water, as with bivalves, other shellfish produce for human consumption must meet the End Product Standard for shellfish toxins and bacterial contamination by *Escherichia coli*. There have been comparatively few published studies (Cook 1999) on the impact of handling and packing protocols on the viability of whole, harvested sea urchins. Spoilage will begin as soon as the physical condition of the sea urchin begins to deteriorate; bacteria are the most important cause of seafood spoilage and spoilage rates are temperature dependent (Dalgaard et al. 2002). For many seafood species, increasing the temperature from 0 to 4 °C doubles the rate of spoilage and cuts the shelf life in half. Sanitation in the handling process is also important. Information on the spoilage rate of sea urchin gonads in situ would enable growers to guarantee the shelf life of urchins, when appropriately packed.

2.9 Disease in Cultured Sea Urchins

There are reports of catastrophic sea urchin die-offs attributable to pathogenic water-borne microorganisms (Lessios et al. 1984; Scheibling and Hennigar 1997), and of heavy infestations by a parasitic nematode in Norwegian populations of *S. droebachiensis* (Sivertsen 1996). The appearance of contagious disease typically accompanies the intensification of culture effort. In Japan, where sea urchins have been in culture the longest, there are reports of bacterial diseases affecting juveniles maintained in tanks (Tajima and Lawrence 2001), the outbreaks related to high summer and low spring seawater temperatures. The symptoms include green or black lesions on the body surfaces, spine loss, discoloration of the peristomal membrane and tube feet that are limp or unable to attach to surfaces. Several bacterial strains have been isolated as the causative agents and methods for their control reported. However, as yet, there is no substantial reporting of contagious sea urchin diseases in cultures in other countries.

3 Sea Cucumber Aquaculture

3.1 Life History and State of the Art

The sea cucumber species targeted for culture belong to two families, the deposit-feeding Aspidochirotida, which includes the Holothuriidae and the Stichopodidae, and the suspension-feeding Dendrochirotida, which includes the genus *Cucumaria*. The sea cucumber species in cultivation are dioecious, broadcast spawners, the fertilised eggs developing into planktonic larvae before settling and undergoing metamorphosis to the juvenile sea cucumber. The average life span of a sea cucumber is thought to be 5–10 years and most species first reproduce at 2–6 years. A number of species are reported to reproduce asexually by fission, and this has been examined as a technique to propagate commercially important species (Reichenbach et al. 1996). They also have the capability to eviscerate part or all of their internal organs as a defence against predation, the shed organs being rapidly regenerated.

Sea cucumber cultivation originated in Japan in the 1930s and juveniles of the temperate species *Stichopus japonicus* were first produced in 1950 (Battaglene 1999). In the last 15 years, commercial production in Japan has accelerated, where annually an estimated 2.5 million juveniles are released. In China, cultured rather than fished *S. japonicus* now account for around 50 % of the country's estimated annual production of 2375 t of dry sea cucumber. Methods for mass cultivation of the tropical *Holothuria scabra* are now well established and practised in India, Australia, Indonesia, the Maldives and the Solomon Islands (Battaglene 1999). Other tropical species in culture include *Actinopyga mauritania* (Ramofafia et al. 1996) and *H. fuscogilva* (Ramofafia et al. 2000), with the focus of the research effort centred on the production of juveniles in hatcheries for the restoration and enhancement of wild stocks.

3.2 Sea Cucumber Larviculture

Brood stock, collected from the wild, is most commonly induced to spawn through thermal stimulation, by raising the seawater temperature in holding tanks by 3–5 °C for 1 h. *S. japonicus* broodstock is collected in spring, when mature (Hagen 1996). In general, *H. scabra* has a bi-annual peak in gonadosomatic index, indicating two spawning periods a year, but closer to the equator a proportion of the population spawns year-round (Battaglene 1999). Fertilisation occurs spontaneously once the gametes are allowed to mix in seawater; the fertilised eggs are held in suspension by aeration and egg development is rapid. In *H. scabra* the larval life cycle is around 14 days at 28 °C, including the feeding or auricularia stage, the doliolaria or non-feeding stage and settling pentacula stage (Fig. 4). As with larval sea urchins, holothurian larvae are fed a mixture of microalgal species, with the number of algal cells provided gradually being increased over the larval life. *H. scabra* larvae grow well on a diet of the red microalgae *Rhodomonas salina* and the diatom *Chaetoceros calcitrans* (Battaglene 1999). Further refinement of larval culture techniques is required to improve larval survivorship in this and other species of sea cucumbers.

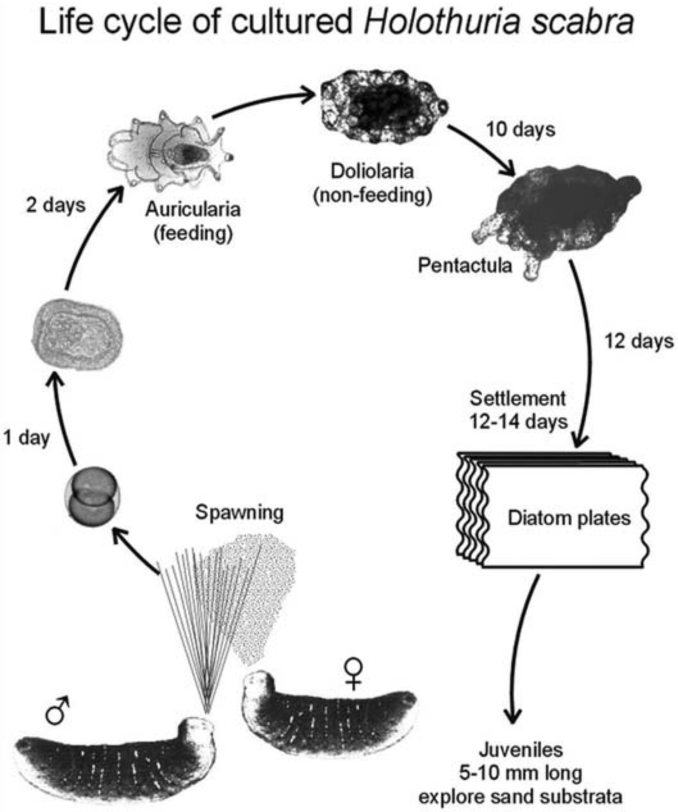

Fig. 4. The 14-day larval cycle of cultured sandfish (*Holothuria scabra*) at a water temperature of 28 °C. (Reproduced with permission from Battaglene 1999)

3.3 Metamorphosis and the Post-Larval Stage

Metamorphosis and settlement are challenging stages in the culture of sea cucumber juveniles. Competent pentacula larvae are provided with a substrate of bacteria and diatoms, which provide the appropriate settlement cues, and to which they adhere with their buccal podia. Typically, *S. japonicus* is settled on PVC plates coated with small periphytic diatoms such as *Navicula*, *Amphora*, *Achnanthes* and *Nitzchia* sp. The plates are coated in outdoor tanks in direct sunlight, although the light intensity, nutrient enrichment and copepod levels must be controlled to produce suitable plates (Ito and Kitamura 1997). Leaves of the sea grass (*Thallassia hemprichii*) are the preferred settlement substrate of *H. scabra* and soluble extracts of the leaves have been shown to induce settlement onto clean plastic surfaces (Mercier et al. 2000).

Post-settlement juvenile sea cucumbers are grown either on diatom-coated plates, held in fine mesh bags in tanks (method used for *S. japonicus* in Japan and China) or on the bottom of tanks, where juveniles of 10–20 mm are transferred to a fine sand substrate and fed a diet supplemented by algal extracts or powdered algae (methods for *H. scabra* developed in India; Battaglene 1999). Throughout the juvenile stage it is necessary to periodically detach the juveniles from the substrate for grading, transfer between tanks or to supply fresh substrates. KCl (1 %) in seawater is an effective agent for detaching *H. scabra* from settlement surfaces (Battaglene and Seymour 1998).

3.4 Sea Cucumber Growth to Maturity

After a 6-month, on-growing nursery phase, and at a length of 2–8 cm, juvenile *S. japonicus* are released to managed areas of the seafloor. They are recovered after 1 year when they measure approximately 20 cm (Hagen 1996). There is a lack of information on growth rates and survivorship in tropical species, and, as with all *Holothuria*, measurements of growth are complicated by their ability to change shape, eviscerate and retain water and sediment in the gut and coelomic cavity. However, Battaglene et al. (1999) suggest there should be no impediment to the large-scale production of juvenile *H. scabra* for stock enhancement programs provided they can be released at a size of 6 cm and with a weight of 20 g.

3.5 Disease in Cultured Holothurians

There is little published information on parasites and diseases of cultured holothurians. Copepods and ciliates are the main predators of the auricularia, copepods also compete with juveniles for food and in some hatcheries have been controlled by the use of pesticides (Battaglene 1999). Routine filtering of seawater and the regular transfer of juveniles to clean tanks can prevent copepod infestation (Battaglene 1999). Fungal infections of the skin are also a problem in the cage culture of wild-collected *H. scabra* in Indonesia.

4 Bioactive Compounds from Echinoderms

Like many other marine organisms, echinoderms have been, and continue to be, examined as a source of biologically active compounds with biomedical applications. Sea cucumber has been valued in Chinese medicine for hundreds of years as a cure for a wide variety of ailments. More recently isolated compounds, mainly from sea cucumbers and starfish, are summarised below.

4.1 Triterpene Glycosides

Triterpenes are mainly synthesised by higher plants, but in animals, cholesterol is an example of a triterpene-like structure. Triterpenes and a group of plant steroids are broadly classified together as saponins (glycosidic surfactants) which affect the solubility of membrane proteins. A well-described toxic effect of plant saponins, when given in high doses, is the haemolysis of red blood cells by the disruption of their membranes.

Novel triterpene glycosides, both sulphated and non-sulphated, have been isolated from sea cucumbers from polar, temperate and tropical regions, some of which have been reported to have significant cytotoxicity against human tumour cell lines (Zou et al. 2003), virucidal activity (Maier et al. 2001), anti-tumour and antiviral activity (Rodriguez et al. 1991), antifungal activity (Murray et al. 2001; Chludil et al. 2002) and to cause haemolysis by membrane disruption (Kalinin et al. 1996).

4.2 Glycosaminoglycans: Chondroitin Sulphate

Glycosaminoglycans (GAGs) (mucopolysaccharides) are polymers of acidic disaccharides containing derivatives of the amino sugars glucosamine or galactosamine. Sulphated polysaccharides abound in vertebrate tissues, and some invertebrate species are a rich source of sulphated GAGs with novel structures. The anticoagulant and antithrombotic characteristics are among the most widely studied properties of the sulphated polysaccharides, for example the anticoagulant GAG heparin is an important therapeutic agent in the prevention and treatment of thrombosis. A replacement agent for heparin is sought as there are problems with both allergy to heparin and heparin resistance. Recently isolated sulphated polysaccharides from the body wall of sea cucumbers, fucosylated chondroitin sulphates (FucCS), have structures analogous to heparin and have been investigated for possible biological activity in mammalian systems. Tapon-Bretaudiere et al. (2002) found that FucCS from a sea cucumber promoted the proliferation of blood vessels and had a concomitant capacity to prevent venous and arterial thrombosis. Mourao et al. (1996), Mourao and Pereira (1999) and Li et al. (2000) have described novel FucCS from sea cucumbers that possess anticoagulant activity in vivo and as such are promising drugs for antithrombotic therapy.

Sea cucumbers have long been used in traditional Chinese medicine for prevention of disease and as a longevity tonic. Products containing sea cucumber chondroitin sulphate are now available through natural product outlets for the treatment of arthritic pain and to promote healthy joints and mobility (Natural Products website, 2004, http://www.psoriasis.com/seacucumber.html).

4.3 Neuritogenic Gangliosides

Sphingolipids are structural lipids where the parent structure is sphingosine (a long-chain amino alcohol) rather than glycerol. Glycosphingolipids (GSLs) contain at least one monosaccharide residue and they are found in the plasma membranes of all animal and some plant cells. Where sialic acid (*N*-acetyl neuraminic acid) is present, these compounds are termed gangliosides. Gangliosides are found in highest concentration in the nervous system where they can constitute 5 % of the lipid. Many new and biologically active gangliosides have been described from starfish. To cite but a few, by way of examples, Higuchi et al. (1991, 1993, 1995) describe compounds with neuritogenic and antitumour activity from *Asterina pectinifera*, *Asterias amurensis* and *Astropecten latespinosus*. New neuritogenic gangliosides have also been described from sea cucumbers, for example *Stichopus japonicus* and *Holothuria* species (Yamada et al. 2001; Kaneko et al. 2003).

4.4 Antimicrobial Activity

Results from some recent studies suggest that echinoderms are a potential source of novel antibiotics. Haug et al. (2002) found antibacterial activity in different body parts of the sea urchin *Strongylocentrotus droebachiensis*, the starfish *Asterias rubens* and the sea cucumber *Cucumaria frondosa*. Antibacterial and antifungal activity has been found in alcoholic extracts of a range of holothurian species from the Tamil Nadu coast, India. The bacteria *Aeromonas hydrophila*, *Escherichia coli*, *Enterococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio harveyi* and the fish-borne mould *Aspergillus* sp. were inhibited at varying levels by extracts of the sea cucumbers *Actinopyga miliaris*, *Holothuria atra* and *H. scabra* (Abraham et al. 2002). Antibacterial activity has also been found in extracts of the body wall, coelomocytes and eggs in a variety of species (Stabili and Pagliara 1994; Stabili et al. 1996; Haug et al. 2002).

4.5
Other Types of Bioactive Compounds: Branched-Chain Fatty Acids, Lectins, Opsonins, Analgesics and Anti-ulcer Compounds

Branched-chain fatty acids or fatty alcohols have been reported to possess antitumour activity in various tumour models. Yang et al. (2003) reported that a branched-chain fatty acid, 12-methyltetradecanoic acid (12-MTA), isolated from a sea cucumber, inhibited proliferation of prostate cancer cell lines in culture via apoptosis. The authors suggest that this agent may be a novel adjunctive therapy for selected malignancies including prostate cancer.

Lectins are proteins that possess binding sites for specific mono- and oligosaccharides. A lectin has been isolated from sea cucumber which exhibited cytotoxicity against mouse cancer cells and human lung cancer cells (Gana and Merca 2002). Opsonins are proteins known to bind to the surface of many kinds of pathogens and tag them as targets for phagocytosis. A new opsonin-like molecule in the coelomic fluid of the sea cucumber *H. leucospilota* has been reported (Xing and Chia 2000). A crude water extract of a Malaysian sea cucumber has been shown to possess analgesic activity with a relatively long duration of action (Yaacob et al. 1994). Anti-ulcerative effects on rat stomach cells have been demonstrated in an aqueous/ethanolic extract of *Stichopus japonicus* body-wall muscle (Migas and Klemenchenko 1990).

4.6
Regeneration of Nerve Tissue and Arm Regrowth in Crinoids

Sea cucumbers, starfish and crinoids (feather stars or sea lilies) are well known for their striking regenerative potential. Crinoids can rapidly and completely regenerate arms lost following self-induced or traumatic amputation. Thus they provide a valuable experimental model for investigation of the regenerative process from the macroscopic to the molecular level (Candia Carnevali and Bonasoro 2001; see Candia Carnevali, this Vol.) and for the identification of the genes involved in the process of neural regeneration (Thorndyke et al. 2001). Echinoderm regeneration also provides a convenient model for examining the effects of persistent micropollutants on the developmental physiology (cell proliferation, morphogenesis, differentiation, tissue renewal) of marine animals. The regeneration response of the crinoid *Antedon mediterranea* is especially sensitive to endocrine disrupters such as polychlorinated biphenyls (PCBs), and exposure to these chemicals induces significant variations in the timing and mode of arm regeneration (Candia Carnevali et al. 2001).

5
Sustainable Development

5.1
The Research Requirement

Although there has been a recent increase in research effort into echinoculture, the technology developed for many species outside of Japan has thus far largely been at a research scale and it is likely to require some adaptation to allow commercial operations. Being able to guarantee a supply of seed (juveniles) for on-growing underpins any successful aquaculture operation, and seed supply may prove to be a bottleneck initially as the growing industry scales up. Not all the technologies developed so far (and in particular diet formulations) may be totally transferable between species, and further refinements will be needed.

The aspects still requiring further research for commercial cultivation of sea urchins can be summarised as the need to:

- complete the life cycle in culture;
- improve larval diets and shorten larval life;
- provide suitable settlement substrates that maximise survival at metamorphosis and of the post-larval stages;
- refine artificial diet formulations for juveniles and adults to maximise growth rates and survivorship and produce gonads of the desired taste, texture, flavour and colour;
- optimise 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.

The first three points in the list are also relevant for developing sea cucumber cultivation where there is a similar bottleneck in seed supplies. Countries already marketing fished produce will have experience in transport and marketing; others will have to establish trade outlets.

There is potentially the need to culture a range of echinoderm species for the growing market in compounds for biomedical research, and culture could play a conservation role in meeting the needs of the home aquarium trade.

5.2
Environmental Considerations

While major reseeding/sea ranching programs, such as that for sea urchins in Japan and for sea cucumbers in China, may be an appropriate way to enhance stocks in areas formerly depleted by fishing, releasing large numbers of captive-bred animals into the wild will undoubtedly impact the genetic composition of those populations. The FAO recommends a minimum breeding population size of 50 for short-term conservation and 500 for longer-term conservation. Relatively small numbers of brood stock are used in hatcheries (Agatsuma 2004; Sakai et al. 2004), and the release of their juveniles will decrease the genetic diversity in local populations. Similarly, where cultured urchins are caged on the seafloor or in suspended culture, their gametes will still be shed to the surrounding seawater. Therefore, consideration should be given to both (1) the desire to genetically manipulate brood stock for better growth characteristics of their progeny and (2) the preservation of genetic diversity in sea urchin populations (Robinson 2004a).

Echinoculture is now poised to expand at a time when globally the aquaculture industry is receiving bad publicity, accused of a range of negative environmental impacts. Therefore, to succeed, echinoculture must develop effectively in a framework of increased legislation affecting businesses operating in the marine environment. This may provide the incentive for polyculture of sea urchins or sea cucumbers with other species that feed at different trophic levels. In such systems, the echinoderms, feeding on uneaten feeds, detritus or seaweeds grown on waste nutrients from the polyculture systems, may serve to reduce the environmental impact of the aquaculture activity (Hagen 1996; Kelly et al. 1998; Shpigel et al. 2004).

5.3 Economic Considerations

It is a general trend that aquaculture operations for marine species do not start until the (wild) fished stock has been diminished to a point where earnings and lifestyle of the people involved are affected (Robinson 2004b). When wild stocks decline, the demand created in the market place raises the price of the product and consequently culturing is more likely to become viable economically. As this review of culture methods has shown, there have been dramatic advances in the culture methods of sea urchins and sea cucumbers in the last 10–15 years, to the extent that one can conclude that currently the major obstacles to successful cultivation are indeed economic rather than biological. For example, it is the cost of producing seed, infrastructure for grow-out systems and artificial diets for growing juveniles to market size rather than the technical difficulty of these operations that will constrain the growth of the industry. At present, it is the reseeding operations that are cost-effective, the 'cost' of the grow-out period being borne by the environment. One of the few examples, outside of Japan, of farmed urchins reaching the market place comes from Southern Ireland, where hatchery-reared juveniles are transplanted to rock pools where they can be fed drift algae, until they reach market size (J. Chamberlain, Dunmanus Seafoods Ltd., pers. comm.).

Hence the future of the echinoculture industry is closely linked to that of the fisheries, whose fate will ultimately determine the market forces that will shape this growing industry.

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