CHAPTER 2

Care and Maintenance of Adult Echinoderms

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Table 1
Summary of Species Information Relating to Collection of Echinoids (Sea Urchins) and Their Breeding Season

<table>
<thead>
<tr>
<th>Species</th>
<th>Size</th>
<th>Depth</th>
<th>Substrate</th>
<th>Distribution</th>
<th>Reproductive season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthocidaris crassispina</td>
<td>&lt;7 cm</td>
<td>0-70 m</td>
<td>rock, shell</td>
<td>Japan, Southern China</td>
<td>June-August</td>
</tr>
<tr>
<td>Arbacia punctulata</td>
<td>&lt;5.6 cm</td>
<td>0-230 m</td>
<td>rock, shell</td>
<td>Cape Cod–Florida, Texas, Yucatan, Cuba, Jamaica, West Indies</td>
<td>May-September</td>
</tr>
<tr>
<td>Echinus exculeatus</td>
<td>15-16 cm</td>
<td>0-40 m</td>
<td>rock</td>
<td>British Isles</td>
<td>February-June</td>
</tr>
<tr>
<td>Hemicentrotus pulcherminus</td>
<td>&lt;5 cm</td>
<td>0-40 m</td>
<td>rock, coarse gravel</td>
<td>Japan, Northern China, Korea, North Carolina</td>
<td>January-March</td>
</tr>
<tr>
<td>Lytechinus variegatus</td>
<td>&lt;7.6 cm</td>
<td>0-55 m</td>
<td>sand, gravel</td>
<td>Florida, Bahamas, West Indies</td>
<td>December-July</td>
</tr>
<tr>
<td>Loxechinus albus</td>
<td>&lt;7.5 cm</td>
<td>0-15 m</td>
<td>rock, gravel</td>
<td>Chile, Peru</td>
<td>July-September</td>
</tr>
<tr>
<td>Paracentrotus lividus</td>
<td>&lt;7 cm</td>
<td>&lt;3 m</td>
<td>rock, seagrass</td>
<td>Mediterranean, Western Atlantic, Britain &amp; Ireland</td>
<td>April-May</td>
</tr>
<tr>
<td>Piannenechinus milliarius</td>
<td>&lt;5.5 cm</td>
<td>0-10 m</td>
<td>coarse gravel</td>
<td>Britain &amp; Ireland</td>
<td>February-November</td>
</tr>
<tr>
<td>Pseudocentrotus depressus</td>
<td>&lt;3 cm</td>
<td>0-50 m</td>
<td>rock</td>
<td>Japan</td>
<td>October-December</td>
</tr>
<tr>
<td>Strongylocentrotus droebachiensis</td>
<td>&lt;8.3 cm</td>
<td>0-1-160 m</td>
<td>rock</td>
<td>Arctic-New Jersey, Alaska–Puget Sound, Alaska–Baja California</td>
<td>January–April</td>
</tr>
<tr>
<td>Strongylocentrotus franciscanus</td>
<td>&lt;12.7 cm</td>
<td>0-91 m</td>
<td>rock</td>
<td>Alaska–Baja California</td>
<td>February-August</td>
</tr>
<tr>
<td>Strongylocentrotus intermedius</td>
<td>&lt;8 cm</td>
<td>0-40 m</td>
<td>rock</td>
<td>Pacific coasts of Asia and Siberia</td>
<td>August-October</td>
</tr>
<tr>
<td>Strongylocentrotus nudus</td>
<td>&lt;8 cm</td>
<td>0-180 m</td>
<td>rock</td>
<td>Pacific coasts of Asia and Siberia</td>
<td>September-November</td>
</tr>
<tr>
<td>Strongylocentrotus purpuratus</td>
<td>&lt;10.2 cm</td>
<td>0-160 m</td>
<td>rock</td>
<td>Alaska–Baja California</td>
<td>November-June</td>
</tr>
<tr>
<td>Tripneustes gratilla</td>
<td>&lt;12 cm</td>
<td>0-30 m</td>
<td>hard substrate</td>
<td>Circumtropical extending to tropical areas in Indian and West Pacific Ocean</td>
<td>July-September (in Japan)</td>
</tr>
</tbody>
</table>

*The reproductive seasons have been compiled for sea urchins from different populations. In different geographical areas, breeding seasons can therefore vary slightly but will be within the reported range. Further information on British species can be found at http://www.marlin.ac.uk/.*

are stored. Such prolific and orderly gametogenesis depends upon the subdivision of the germinal epithelium into small groups of interrelated somatic and germinial cells (Walker, 1979, 1982; Walker et al., 2001). Subdivisions increase the surface area in the interior of an otherwise sac-like gonadal lumen and provide a mechanism for supplying nutrients to smaller groups of germinial cells among the
Uniquely among the echinoderms, sea urchin gonads grow in size not only because gametogenesis increases the size and/or numbers of germinal cells present but also because somatic cells within the germinal epithelium, the nutritive phagocytes (NP), store extensive nutrient reserves before gametogenesis begins (see the green sea urchin Web page, http://zoology.unh.edu/faculty/walker/urchin/gametogenesis.html). In sea urchins of both sexes, a glycoprotein termed “major yolk protein” (MYP) is the principal nutrient stored in NP (Brooks and Wessel, 2002; Unuma et al., 2001). This protein was originally identified as the most abundant component of yolk granules in sea urchin eggs (Yokota and Sappington, 2002), but is predominantly found prior to gametogenesis in the NPs of both ovaries and testes (Unuma et al., 2003). MYP is mobilized from NP during gametogenesis, transported in an unknown way to gametogenic cells, and used in the synthesis of new protein, nucleic acids, and other constituents of ova and spermatozoa (Unuma et al., 2003).

Nutritive phagocytes are surrounded by oogonia, oocytes, and subsequent gametogenic stages. During oogenesis, follicle cells are not present and ovarian follicles do not exist in sea urchins as they do in sea stars (Walker, 1982; Walker et al., 2001). Meiotic divisions of sea urchin post-vitellogenic primary oocytes occur as each is released into the ovarian lumen for storage. As a result, meiosis in sea urchin primary oocytes does not occur at one discrete time (as it does during spawning of the primary oocytes of sea stars), but rather continues during several months as successive groups of primary oocytes become meiotically competent (Shirai and Walker, 1988; Smiley, 1990; Walker et al., 2001). Following oogenesis, fully mature sea urchin ova are stored within the ovarian lumen for periods varying from days to weeks prior to their release at spawning (Pearse and Cameron, 1991; Walker, 1982; Walker and Lesser, 1998; Walker et al., 2001). Stages of spermatogenesis are similarly associated with NP as they progress from spermatogonial stem cell clusters that are located near the testicular wall to the lumen where spermatozoa are stored prior to spawning (Pearse and Cameron, 1991; Walker, 1982; Walker and Lesser, 1998; Walker et al., 2001). (Fig. 1).

B. Sea Star Models from North America, Europe, and Japan

Adult sea star model organisms from around the world (Table II) have been instrumental in understanding the initiation of meiosis in primary oocytes and in determining the biochemistry and physiology of molecules that are involved. Such studies depend upon the nature of oogenesis in sea stars that differs substantially from that of sea urchins. During oogenesis in sea stars, individual primary oocytes are enveloped by a single layer of follicle cells, resulting in ovarian follicles. During vitellogenesis, each ovarian follicle supplies nutrients derived from extragonadal sources to primary oocytes (Schroeder et al., 1979). A single meiotically arrested primary oocyte is contained within each ovarian follicle. Fully developed ovarian follicles are stored within the ovarian lumen until ovulation, when primary oocytes synchronously complete meiosis (Kishimoto, 1998, 1999). Three interrelated chemical messengers have been implicated in this annual event. These are
Fig. 2 Gametogenesis in sea stars: (2a) Ovary of a sea star early during vitellogenesis, showing ovarian follicles containing primary oocytes in various stages of growth (notice black follicle cell nuclei on the surface of the primary oocytes); (2b) Ovary of a sea star later in vitellogenesis with maturing ovarian follicles released into the lumen and still surrounded by follicle cells; (2c) Testis of a sea star prior to the initiation of meiosis in primary spermatocytes which are arranged along the axes of axial somatic cells forming separate spermatogenenic columns (SC); (2d) Testis of a sea star near the completion of annunal spermatogenesis, showing reduced spermatogenenic columns (SC) and new spermatozoa stored in the testicular lumen (NS). C = coelom in 2a-d. Scale bar = 100 μm.

reflects the growth of NP as well as the progress of gametogenesis, the size of gonads in both sexes of sea stars does indicate the progress of gametogenesis. In sea stars, nutrients are mobilized from remote sources like the pyloric caeca and are transported to the gonads during gametogenesis.

C. Echinoderm Gametogenesis in Plastic Sections for Light and Electron Microscopy

1. Quick Fixation Method

Small pieces of gonadal tissue are placed in 2.5% glutaraldehyde in 0.45 μm filtered H₂O at room temperature for 1 hour to overnight.

Fixative is removed and gonads are rinsed (×2) in 2.5% NaHCO₃ (pH 7.2) for 10 min and finally brought to distilled H₂O prior to embedding. To avoid damage, do not move gonads from one fluid to another during this process; instead, remove fluids from the tissues by decanting or aspiration.
available from a variety of manufacturers) supplied with battery-operated mino-
mizers (Hypark Specialty Corporation Inc., Minnetonka, Minnesota) that cool
and aerate seawater. During transport, temperate species like *Strongylocentrotus
droebachiensis* may be placed on a bed of macroalgae that is cooled with bags of
ice or gel coolants (e.g., North America—*Laminaria saccharina*; Europe—*Laminaria
saccharina*, *Fucus vesiculosus*, *Ulva* spp.; Japan—*Sargassum* spp.). When macroalgae
are not available, moistened towels or newspapers can be substituted (Yokota,
2002). Some sea stars, like *Asterias vulgaris*, have relatively flaccid bodies that leak
coelomic fluid and must be transported in seawater. Others like *Asterina pectini-
fera* can be treated like sea urchins. If echinoderms are ready to spawn, it is
advisable to keep each one in separate Zip-Loc bags, since a single individual
can trigger mass spawning. Following transport and prior to feeding and experi-
m entation, echinoderms should be acclimated to the tank conditions at their
destination for 48 to 72 hours.

Commercial harvesting of sea urchins is usually tightly regulated by local
governments. It is essential to contact local Departments of Marine Resources
to ascertain local regulations before attempting to collect these species or you
could be prosecuted!

### B. Commercial Suppliers

Sea urchins are harvested worldwide as a source of the sushi termed “uni”
(Andrew *et al.*, 2002; Keesting and Hall, 1998; Williams, 2001) and can usually
be purchased from local fishermen along the coast. Problems associated
with utilizing such urchins depend upon the methods used in their collection. Such
individuals may be severely compromised if collected by dredging. Some com-
mercial suppliers (e.g., Connecticut Valley Biological and Carolina Biological)
will collect animals by hand, a method that is less efficient, so prices may be
higher. It is also important to point out that by using field-collected echinoderms,
it may be difficult to obtain individuals of similar quality, primarily because
of seasonal and environmental variability at different collection sites. Before
using them in experiments, it may be necessary to maintain field-collected
animals for a period of time under similar conditions, including feeding them a
similar diet.

In Japan, there are many semi-governmental hatcheries that produce seedlings
of sea urchins (Hagen, 1996). When surplus stocks of sea urchins are available,
they can be purchased at reasonable prices. The benefit of buying the young sea
urchins from these facilities is that they are of the same age and size. The disadvan-
tage of buying sea urchins from the hatcheries is that they must be cultured
before they reach sexual maturity. Buying adult sea urchins from aquaculturists
would be most convenient. However, in Japan and the United States, sea urchin
aquaculture ventures are in their infancy and elsewhere in the world they do not
exist.
Fig. 3  Large-scale, land-based facilities: (a) Toboggan system for maintaining large numbers of sea urchins from fertilization to sexual maturity (from Grosjean et al., 1998); (b) Trough system for maintaining sea urchins throughout their life cycle (from Devin, 2001).
A. Physical and Chemical Factors

1. Temperature

In combination with other physical, chemical, and biological factors, temperature can be important in the husbandry of adult sea urchins. The optimum temperature for most echinoderms depends upon the time of year and should approximate the ambient temperature at which they were collected as closely as possible. Temperate echinoderms (e.g., *Strongylocentrotus droebachiensis*) show a negative correlation between feeding and temperature (Garrido and Barber, 2001). Subtropical species (e.g., *Lytechinus variegatus*) can acclimate to elevated temperatures (Klinger et al., 1986), while other species (e.g., *Eucidaris tribuloides, Strongylocentrotus franciscanus*) (Lares and McClintock, 1991; McBride et al., 1997) seem to be unaffected by mild changes in temperature and their feeding rates and absorption efficiencies remain unchanged. Most echinoderms, however, show a positive relationship between feeding absorption rates, and temperature. Thus, gross feeding and assimilation rates in such echinoderms in their natural environment are highest in spring and summer and decline in the winter (Fernandez and Boudouresque, 2000).

2. Photoperiod

Although exceptions occur (Bay-Schmith and Pearse, 1987; Cochran and Engelmann, 1975; Ito et al., 1989; Sakairi et al., 1989; Spirlet et al., 1998; Yamamoto et al., 1988), changing photoperiod can be correlated with the simultaneous initiation of gametogenesis and the mobilization of nutrients from NP in both sexes of a number of species of sea urchins and with mobilization of nutrients from pyloric caeca and the subsequent initiation of gametogenesis in sea stars (Pearse et al., 1986a, b; Walker and Lesser, 1998; Walker et al., 2001). In the Gulf of Maine, the onset of shortening day length in the fall results in the initiation of gametogenesis in the northern sea star, *Asterias vulgaris*, and the green sea urchin, *S. droebachiensis* (Pearse and Walker, 1986; Walker and Lesser, 1998). This occurs at photosynthetically active irradiances (PAR: 400–700 nm) found at approximately 10 m of depth (Walker and Lesser, 1998). Fall photoperiod is so influential in the green sea urchin that one can manipulate the ambient light regime and promote out-of-season gametogenesis in urchins maintained in the laboratory (Walker and Lesser, 1998). Green sea urchins were brought into the laboratory (in March, following natural spawning), fed the Lawrence pelleted diet *ad libitum* (Lawrence et al., 2001), and subjected to a photoperiod advanced by four months. During this study, temperatures and salinities for experimental urchins mirrored those recorded at the collection site. Experimental urchins had a significantly higher gonad index (GI) in March, April, and May (18 ± 6%) compared with field urchins (11 ± 3%). Subsequently, experimental urchins had a mean monthly GI of 25 to 30%, while the mean GI for field urchins was 11 to 13%. Germ-line stem cell mitosis and gametogenesis began in June in experimental male and female urchins, 4 to 5 months earlier than in field urchins.
4. Pollutants

Another important factor to consider in the maintenance of sea urchins is the accumulation of undesirable compounds in the culture system. In closed seawater systems, where water is recirculated, excretion products such as ammonia and phosphates may become a problem. Despite the use of a biofiltration device, not all of these compounds may be eliminated. Testing for harmful compounds should occur regularly. Phosphates are a chronic problem in recirculating seawater systems, since they may leak from dietary pellets and can also be produced by the animals themselves. Accumulation of phosphates (Böttger et al., 2001) or crude oil (Temara et al., 1999) can decrease feeding, which would result in smaller nutritive phagocytes and subsequently the production of fewer or lower quality gametes (Fig. 4). Aquaculture in near-shore facilities presents an even larger problem resulting from the effects of anthropogenic pollutants. Phosphates (Böttger and McClintock, 2002), cadmium (Au et al., 2001a,b; Gnezdilova et al., 1985, 1987; Vashchenko et al., 1993), and oil (Krause, 1994) are known inhibitors of gametogenesis. It may therefore be necessary to limit near-shore aquaculture to areas that are strictly monitored for environmental contaminants or to design land-based running seawater systems that reduce the amounts of pollutants that animals may encounter.

B. Biological Factors

1. Diet

The quantity and quality of food provided to echinoderms can substantially alter their morphology, physiology, and reproduction (Ebert, 1980; Fernandez and Boudouresque, 1997; George, 1996; Gonor, 1973; Levitan, 1991; Walker and

![Fig. 4](image_url)  

The effects of chronic phosphate exposure on the ovaries of *Lytechinus variegatus* (Lamarck): (4a) Well-developed nutritive phagocytes (NP) and young vitellogenic oocytes can be seen in the ovary of animals maintained in artificial seawater; (4b) An ovary of the animals maintained in an organic phosphate has poorly developed nutritive phagocytes (NP) and is devoid of oocytes. C = coelom in 4a–b. Scale bar = 200 μm.
2. Care of Echinoderms

absorption efficiencies. Animal-based diets will result in excellent somatic and
gonadal growth for most echinoderms.

2. Diseases

A variety of pathogens and parasites from protozoans to chordates (Jangoux,
1987a,b, 1990) can infect echinoderms. Perhaps most interesting to molecular and
 cellular biologists is the lack of any evidence for diseases involving cellular
 proliferation (e.g., cancer) in any echinoderm (Wellings, 1969). Ultimately, inves-
tigation of this remarkable feature of the biology of echinoderms may yield
information on the progress and prevention of cancer in other organisms.

Sea urchins from a variety of geographical areas suffer from the spectacular sea-
urchin balding disease (Jangoux, 1990; Scheibling and Stephenson, 1984). The
disease progresses from discoloration of the epidermis surrounding the spines, to
loss of spines and other appendages followed by loss of epidermis and superficial
dermal tissues, and finally to destruction of the skeleton and appearance of lesions
on the aboral surface (Maes and Jangoux, 1984; Maes et al., 1986; Scheibling and
Stephenson, 1984). This communicable disease is caused by the marine bacteria,
Vibrio anguillarum and Aeromonas salmonicida (Gilles and Pearse, 1986; Maes and
Jangoux, 1985) and can result in mass mortalities affecting 10 to 90% of sea urchin
populations in nature and in culture (Bourdouresque et al., 1980, 1981; Pearse
et al., 1977). Once infected, sea urchins do not seem to recover, and there is no
known cure for this communicable disease.

It is not likely that small-scale aquaculture will have a problem with diseases,
unless infected individuals are brought in from the field. Infections may be
avoided by treating individuals for 1 to 2 hours with Gentamycin (10 mg l\(^{-1}\)),
Neomycin (30 mg l\(^{-1}\)), Novobiocin (30 mg l\(^{-1}\)), or Sulfisoxalole (0.25 mg l\(^{-1}\))
(Böttger and McClintock, in prep.). There is a major problem with bacterial
infections in mass aquaculture, where millions of juvenile organisms (seed stock)
have been lost from disease in culture (Tajima and Lawrence, 2001), with no
treatment known at present.

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