Methods for generating triploid green sea urchin embryos: An initial step in producing triploid adults for land-based and near-shore aquaculture

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1. Introduction

In the last fifteen years, a fishery for the green sea urchin, Strongylocentrotus droebachiensis, has become the seventh largest marine fishery overall in the Northeastern United States and the third largest in Maine behind lobsters and aquacultured Atlantic salmon (Andrew et al., 2002). Early unregulated harvesting and continued over-fishing have drastically depleted once abundant field populations throughout the Gulf of Maine. Developing sustainable alternatives to direct harvest of the green sea urchin are vital to the future of this commercial enterprise. It is of significant interest to commercial aquaculture to produce adult triploid sea urchins for aquaculture in land-based facilities or near-shore lease sites. In land-based facilities, the taste of urchin gonads would not be negatively influenced by the initiation of gametogenesis (Unuma and Walker, 2009, 2010). In lease sites, urchins would feed on naturally available food sources and growth of somatic tissues (nutritive phagocytes, NPs) in the germinal epithelia of gonads in such animals would not rely on the use of expensive formulated feeds and would naturally develop taste preferred by consumers.

Triploid bivalve mollusks and fish may have increased somatic growth and provide year-round marketable products because they are sterile (Allen and Downing, 1986; Barber and Mann, 1991; Cox et al., 1996; Eversole et al., 1996; Hand et al., 1998; Ruiz-Verdugo et al., 2000; Pferrer et al., 2009). Sterility presumably depends on the failure of three sets of chromatides to successfully form synaptonemal complexes prior to meiosis I (Liu et al., 2004). Another benefit of sterility in triploids is the reduced risk of genetic cross-contamination when triploids are released into natural populations of diploid individuals (Purdom and Lincoln, 1973; Purdom, 1973; Gjedrem, 1976; Allen and Downing, 1986). Triploidy can be induced in salmon (Salmo salar) and rainbow trout (Salmo gairdneri) by heat shock (Thorgaard et al., 1979) or by treatment of newly fertilized "eggs"
ova were allowed to settle by gravity, collected by pipetting and repeated 9 times. After ten gentle passages through the nylon mesh, mature adults (injected through the peristomial membrane into the coelom of the

2.2. Spawning and gamete collection

Spawning in the laboratory on the campus of the University of New Hampshire occurred within 2 days of collection. Spawning was induced using 2 ml of 0.5 M KCl per animal injected through the peristomial membrane of the mature adults (Stephens, 1972). Our methods have yielded the only viable triploid embryos ever generated from any species of sea urchin and are a successful first step in an effort to generate adult triploid green sea urchins. We discuss scaling up our laboratory methods for embryos to the hatchery environment and also address issues unique to the reproductive biology of sea urchins that will be vital for commercial land-based and near-shore aquaculture ventures to consider if triploid adult green sea urchins can ultimately be produced.

2. Materials and methods

2.1. Animal collection and maintenance

One hundred adult northeastern green sea urchins (Strongylocentrotus droebachiensis) between 50 and 75 mm in diameter were collected by SCUBA in March, 2010 from a lease site in the Piscataqua River (Portsmouth, New Hampshire). They were transported in ambient seawater to the Coastal Marine Laboratory of the University of New Hampshire, maintained in a two tiered A-frame tank system at the ambient water temperature (5–10 °C) and salinity (33 ppt) of collection within the facilities flowing seawater system and fed ad libitum with a commercially available formulated feed (Wenger International, Sabetha, KS) prior to the induction of spawning. Spawning in the laboratory on the campus of the University of New Hampshire occurred within 2 days of collection.

2.2. Spawning and gamete collection

Spawning was induced using 2 ml of 0.5 M KCl per animal injected through the peristomial membrane into the coelom of the mature adults (Carpizo-Ituarte et al., 2002; Foltz et al., 2004), and gametes were collected (dry for sperm and in calcium-free seawater for eggs) following established protocols described by Strathmann (1987).

2.3. Removal of the jelly coat and vitelline membrane

Eggs were denuded (by removal of both the jelly coat and vitelline membrane) using three different procedures, mechanical, proteolytic and acidic (Table 1). (1) For mechanical removal of extracelluar structures, ova were resuspended in filtered seawater (FSW) at 10% vol/vol. The suspension was gently passed into a glass beaker through a nylon mesh screen (210 μm) pre-wetted with FSW (Foltz et al., 2004). The mesh was rinsed with FSW and this process was repeated 9 times. After ten gentle passages through the nylon mesh, ova were allowed to settle by gravity, collected by pipetting and

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**Table 1**

Methods for denuding proteolytic, mechanical and acidic), fusion and fertilization for producing triploid Strongylocentrotus droebachiensis embryos, including all washes conducted and times of treatments. One liter Ca-free SW was produced by addition of 27.8 g NaCl, 0.754 g KCl, 7.3 g MgCl\(_2\) \(\cdot\) 6H\(_2\)O, 4.31 g MgSO\(_4\) \(\cdot\) 7H\(_2\)O and 1.24 g H\(_3\)BO\(_3\) to 1000 ml ddH\(_2\)O.

<table>
<thead>
<tr>
<th>Step</th>
<th>Denuding</th>
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<tr>
<td>1. Wash</td>
<td>Proteolytic</td>
<td>Two washes with 100 ml Ca-free SW containing 60.6 g CaCO(_3) for 10 min</td>
<td>Fertilization: 1 ml diluted sperm into 100 ml Ca-free SW</td>
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<td>Fertilization cut-off after 10 min</td>
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<td>4. Wash</td>
<td>Mechanical</td>
<td>10 ml eggs in 90 ml Ca-free SW, 10 0.2 g cysteine and 120 mg poly(argin) passed 10 times through 210 μm nylon mesh</td>
<td>Fertilization: 1 ml diluted sperm into 100 ml Ca-free SW</td>
<td>Fertilization cut-off after 10 min</td>
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(= secondary oocytes) with cytochalasin B (Barber and Mann, 1991; Nell et al., 1996) or with 6-dimethylaminopurine (6-DMP) (Desrosiers et al., 1993; Gerard et al., 1994; Nell et al., 1996; Zhang et al., 1998; Norris and Preston, 2003; Liu et al., 2004). Following fertilization, methods described for these organisms block second meiotic division and result in retention of the second polar body yielding triploid embryos that develop into sterile triploid adults.

Since methods that block release of the second polar body are not possible for sea urchins because both meiotic divisions occur within the ovary and prior to ovulation and fertilization, triploids have never been produced for any species of sea urchin (Walker et al., 2005, 2006). Retrieval of primary oocytes from dissected green sea urchin ovaries is not practical for commercial purposes because most oocytes obtained in this way are immature and meiotically incompetent (Wessel et al., 2004; Walker et al., 2005) and the numbers of embryos that might result from suppressing polar body release would be extremely low.

In this study we describe methodology for producing triploid green sea urchin embryos (n = 63) and maintaining them to the prism pluteus stage with spicules (Stephens, 1972). Our methods have yielded the only viable triploid embryos ever generated from any species of sea urchin and are a successful first step in an effort to generate adult triploid green sea urchins. We discuss scaling up our laboratory methods for embryos to the hatchery environment and also address issues unique to the reproductive biology of sea urchins that will be vital for commercial land-based and near-shore aquaculture ventures to consider if triploid adult green sea urchins can ultimately be produced.
resuspended in calcium-free seawater. (2) For proteolytic removal, ova were treated with calcium-free seawater containing 0.2% cysteine and 1.2 mg ml\(^{-1}\) pronase for 30 min (Bennett and Mazia, 1981a,b) followed immediately by exposure to 1 M urea containing 0.1 mM CaC\(_2\), after which the denuded ova were resuspended in calcium-free seawater. (3) Acidic removal was accomplished by exposing ova for 20 min to acidified FSW at pH 5. Following exposure, ova were washed three times and resuspended in calcium-free seawater (Bennett and Mazia, 1981a,b). In all of these treatments, the resulting jelly water (possibly containing elements of the jelly coat and essential to inducing the acrosomal reaction in spermatozoa) was collected, maintained on ice and used later during fertilization (Foltz et al., 2004).

To confirm that the jelly coat had been removed, subsamples of denuded ova from all three treatments described above were stained with Azure B (which stains the jelly coat) and were viewed with a Zeiss Axioplan II MOT equipped with an AxioCam MR camera and AxioVision 4.3 software (Carl Zeiss, Inc., Thornwood, NJ). Following fertilization, success in removal of vitelline membrane was determined by the absence of a fertilization membrane that is derived from an existing vitelline membrane by the addition of structural proteins from the cortical granules of the fertilized ovum.

2.4. Fusion of denuded ova

Two green sea urchin ova (1n) denuded as above were fused to generate the "diploid" entities used for fertilizations. Calcium-free seawater diluted to 75% of its original volume with distilled H\(_2\)O and CaC\(_2\) was added to reach a working concentration of 25 mM. 6.75 \(\mu\)l of poly(Arg) (120,000 MW, Sigma in 1 ml DMSO) was added to 10 ml of the diluted seawater and denuded ova were suspended in this medium (Table 1).

Contents tested for fusion efficiency were: (1) 1.5 ml micro-centrifuge tubes, (2) 96 well microtiter plates, (3) 6 ml round-bottom tubes and (4) 15 ml Falcon tubes. Following treatment ova were washed with 1.5 mg/ml arginase in calcium-free seawater and returned to sterilized FSW. After 55 min, diameters of ova containing two pronuclei were measured with an ocular micrometer. This procedure was repeated eight times.

2.5. Fertilization of fused ova

All ova (fused and un-fused) were fertilized in new tripour beakers (4.5 cm internal diameter) by adding 15 drops of undiluted sperm to 10 ml of sterile FSW and adding 1 ml of diluted sperm and 10 ml of jelly-water (collected as described above during mechanical removal of the jelly coat and vitelline membrane) to fused ova and 40 ml FSW (Table 1). Ova were distributed in a single layer at the bottom of the beaker after addition of spermatozoa; mixing was accomplished using a plastic pipet. Each time fused ova were fertilized, batches of untreated and un-fused ova were simultaneously fertilized to serve as controls. When >95% of the control ova had produced a fertilization membrane, resulting zygotes in all treatments were washed three times and returned to labeled containers with FSW.

2.6. Zygotes and development of embryos

Resulting zygotes were allowed to develop at 8 °C for 24 h until the control embryos had reached the blastula stage when chromosome numbers were first counted. A sub-sample of 30 blastulae was withdrawn from all treatments and embryos were scored into four groups depending on their developmental stage (blastula, undeveloped, delayed or abnormal development). The same embryos were also sub-divided based on the presence or absence of the fertilization membrane and their diameters were measured (\(\mu\)m) to account for unsuccessful removal of the vitelline membrane. Subsequently, control diploid and triploid embryos at the gastrula stage (when chromosome numbers were again counted) were transferred to a local hatchery and placed in 300 l vats filled with seawater at ambient local temperatures.

2.7. Chromosome counts

Embryos resulting from control fertilizations and after fusion and subsequent fertilization were collected before and after gasulation and treated according to Eno et al. (2009). To prepare chromosomes at gastrula and pluteus developmental stages, both fused and unfused embryos were treated for 2 h with colchicine (Sigma) at a final concentration of 1 mg/ml to suspend cell division at metaphase, collected by centrifugation (1000 rpm for 5 min), suspended in 1 M urea and dissociated into their component cells by pipetting. The dissociated cells were collected by centrifugation, treated with 8% sodium citrate for 10 min and fixed in methanol: acetic acid (3:1). The fixed cells were stained with DAPI to stain their chromosomes, squashed manually on microscope slides and viewed with a Zeiss Axioplan II MOT, an AxioCam MR camera and AxioVision 4.3 software (Carl Zeiss, Inc., Thornwood, NJ).

2.8. Statistical analysis

Data were expressed as mean±SEM. Differences among values for: (1) removal of the jelly coat and vitelline membrane, (2) fusion by the different methods used and (3) fertilization and development of resulting embryos were evaluated using descriptive statistics and a One-Way ANOVA on Ranks followed by a Tukey pairwise comparison (SigmaStat). All statistical analyses were preceded by assessments of the assumption of normality (Kolmogorov–Smirnov Test) and homoscedacity (Spearman Rank Correlation).

![Fig. 1](image-url) Removal of the jelly coat and vitelline membrane: A) percent success ± SEM for removal of the jelly coat and vitelline membrane using the following three treatments: mechanical (passing through a 210 \(\mu\)m mesh screen), proteolytic (pronase and cysteine) and acidic (treatment in seawater at pH 5); B) untreated ova with fertilization membranes; scale bar = 100 \(\mu\)m and C) unfertilized ova with cell membranes touching following treatments to remove the jelly coat and vitelline membrane. Scale bar = 100 \(\mu\)m.
3. Results

3.1. Removal of the jelly coat and vitelline membrane

Mechanical removal of the vitelline membrane did not yield significant success (p = 0.255), but did produce large numbers of ova with incomplete or no removal (42.01%±28.87) or damage (13.32%±9.99). A significant number (p = 0.001) of denuded ova (= successfully removal of both the jelly coat and the vitelline membrane) resulted when ova were subjected to proteolytic (97.92%±1.94 all results±SEM) or acidic treatments (73.54%±6.15) (Fig. 1A–C). Since we required denuded ova to initiate fusion these results indicate that mechanical denuding was neither as successful nor as consistent as that resulting from proteolytic and acidic treatments. Though denuding was most successful following proteolytic treatment, resulting ova were not fertilizable. Acidic removal was used in this study to prepare for fusion of ova and subsequent fertilization.

3.2. Fusion

As determined by the presence of two pro-nuclei and by size measurements, the highest numbers of fusing and fused ova occurred in 1.5 ml microcentrifuge tubes (41.44%±7.45 of ova achieved fusion and 15.81%±4.6 were completing fusion, all results±SEM) (Fig. 2A–C). When compared with results from the other containers used, the numbers of viable fused ova were significantly higher in microcentrifuge tubes (p = 0.023). For example, fusion of ova in Falcon tubes (15 ml) resulted in 25.01%±6.2, fused ova, however, since these tubes were generally filled with more ova, a large number were crushed (up to 30.57%±7.65). Also, both 96-well plates and 6 ml round bottom tubes, yielded low numbers of completed fusions (6.59%±5.4 and 14.24%±7.81 respectively), with no significant difference between containers (p = 0.67).

3.3. Fertilization

Ova that had been successfully denuded either by proteolytic or acidic treatment did not develop a fertilization membrane (100%±0 and 78.26%±1.9 respectively, all results±SEM). For example, only 21.74%±5.4 of ova successfully developed a fertilization membrane following acidic treatments (Fig. 3) while the majority of fertilized eggs did not develop a fertilization membrane. Following mechanical treatment, only 24.14%±4.3 of ova did not produce a fertilization membrane, while 75.86%±10.4 of ova had a fertilization membrane, a result that was not significantly different (p = 0.058) from untreated ova where fertilization membranes were evident in 93.1%±7.3 of ova.

3.4. Laboratory rearing, development and size of embryos

In 10 separate trials subsequent to fertilization, proteolytically denuded ova (100%±0, all results±SE) did not undergo cleavage to 2-cell stage (Fig. 4A). Mechanically denuded ova resulted in a large number of zygotes that did not cleave (34.84%±9.56), although a few blastulae did result (10.32%±1.45). Acidically denuded ova produced a significantly higher numbers of normal blastulae lacking both the jelly coat and vitelline membrane (p < 0.001) than any other treatment (60.64%±1.39).

Removal of the jelly coat and vitelline membrane by mechanical and acidic treatments yielded significantly larger (p < 0.001) blastulae (317 μm±12.13 and 321.2 μm±10.54 respectively) than untreated ova or ova that were not denuded and then treated mechanically or with acidified seawater (199.2 μm±1.01, 201.3 μm±2.3 and 200.9 μm±2.4 respectively) (Fig. 4B).

3.5. Transferring laboratory methodology to a hatchery

On two occasions, subsamples of control diploid and of triploid gastrulae were transferred from the laboratory to two 3001 round Plexiglas vats at a local sea urchin hatchery and maintained at ambient temperature and salinity in seawater with aeration via an air stone. In these vats, triploid gastrulae developed to the prism pluteus stage more slowly than diploid gastrulae with a delay of approximately 30 h. In these trials, both control diploid and triploid embryos...
membrane had significantly larger blastomeres and blastulae. All results ± SEM.

3.6. Chromosome counts

Chromosome counts were made according to methods developed specifically for strongylocentrotid sea urchins and described in Eno et al. (2009). Normal green sea urchins have 42 chromosomes in their diploid genomes (n = 21) and include two large, eight medium and ten small pairs plus one putative sex pair (Fig. 5A). Resulting triploid blastulae and gastrulae contained 63 chromosomes (a diploid set and an extra maternal set of chromosomes) with representatives from all of the documented size classes (Fig. 5B) in subsamples of 50 gastrulae, 77.42 ± 1.57% were triploid while 21.58 ± 1.16% were diploid.

4. Discussion

We discuss: Scaling up our successful laboratory methodology for the production of triploid green sea urchin embryos in a hatchery environment

Generation of triploids in animals that are fertilized as primary oocytes (molluscs) or as secondary oocytes arrested at metaphase of second meiotic division (fish) is a relative straightforward process involving the treatment of oocytes with chemical or physical shock to prevent release of the second polar body. Generating triploids in sea urchins cannot be accomplished using this methodology since haploid ova are released from the gonopores and fertilized in the water column. Previous studies have indicated that fusion of the haploid ova of sea urchins can be accomplished by a variety of means (Wilson, 1953; Bennett and Mazia, 1981a,b; Richter et al., 1981; Sekirina et al., 1983; Vassettzky and Sekirina, 1985; Vassettzky et al., 1986). Results of these early studies did not yield triploids, nor have they been adapted for use in aquaculture for any sea urchin and they are probably not useful since we have shown that proteolytic or chemical removal of extracellular structures results in non-fertilizable ova.

Scaling up our methods for the generation of triploid green sea urchin embryos from the research laboratory to a commercial hatchery environment is underway. Each step in this process is discussed below to assist those interested in commercial application of our methods.

4.1. Scaling up our current successful laboratory methodology for the production of triploid green sea urchin embryos in a hatchery environment

This suite of procedures prepares 1n ova (that exist in the ovary before ovulation, Walker et al., 2005) for fusion and subsequent fertilization. The procedure for spawning sea urchins with 0.5 M KCl is outlined in Foltz et al. (2004). In nature, sea urchin ova are fertilized ecto-somatically in the water surrounding feeding adults. As a result, both sexes produce prodigious numbers of gametes (millions) that are more than sufficient to yield hundreds of thousands of embryos using our methods. Removal of the jelly coat and the vitelline membrane is necessary before fusion of ova can take place. The jelly coat is removed mechanically during filtration. We describe three methods for removal of the vitelline membrane. Acidic removal was the most successful and was used in this study to prepare ova for fusion and subsequent fertilization. It should also be possible to remove the vitelline membrane using the Cleland reagent (Epel et al., 1970) although we did not attempt this method in our study.

4.1.1. Spawning, gamete collection and removal of the vitelline membrane

This suite of procedures prepares 1n ova (that exist in the ovary before ovulation, Walker et al., 2005) for fusion and subsequent fertilization. The procedure for spawning sea urchins with 0.5 M KCl is outlined in Foltz et al. (2004). In nature, sea urchin ova are fertilized ecto-somatically in the water surrounding feeding adults. As a result, both sexes produce prodigious numbers of gametes (millions) that are more than sufficient to yield hundreds of thousands of embryos using our methods. Removal of the jelly coat and the vitelline membrane is necessary before fusion of ova can take place. The jelly coat is removed mechanically during filtration. We describe three methods for removal of the vitelline membrane. Acidic removal was the most successful and was used in this study to prepare ova for fusion and subsequent fertilization. It should also be possible to remove the vitelline membrane using the Cleland reagent (Epel et al., 1970) although we did not attempt this method in our study.

4.1.2. Fusion, fertilization and embryo culture

We determined that fusion of denuded ova occurred optimally in 1.5 ml microcentrifuge tubes (41.44% ± 7.45 of ova achieved fusion and 15.81% ± 4.6 were completing fusion). Each of these tubes contains approximately 1000 ova. To scale up this laboratory procedure, 500 tubes would yield 250,000 triploid embryos. In our hands, embryos reached the gastrula stage after 3 days in laboratory culture (8 °C) and early prism pluteus after 6 days in the hatchery post fertilization (Fert 3/7/2010; gast 3/11/2010; prism pluteus 3/13/2010). Diploid green sea urchin plutei begin feeding after the mouth has formed and appropriate phytoplankton (Isochrysis galbana, Dunaliella tertiolecta and Rhodomonas lens) must be cultured in adequate quantities to supply feeding embryos as soon as the mouth is formed. It is important to point out that the triploid embryos we generated have gastrulated successfully. In sea urchins, most new expression of zygotic genes begins at gastrulation. Development to the gastrula stage depends upon the expression of genes or use of proteins placed in the oocytes during oogenesis and before meiosis (Davidson, 1987). Expression of novel, lethal combinations of genes united during fertilization are potentially fatal at gastrulation. The fact that triploid embryos did gastrulate successfully confirms that “maternal stored RNA” remains and is accessible during early development and the observation that triploid embryos transferred to a commercial hatchery developed successfully to prism stage
suggests that the equivalence of zygotic RNA transcribed from the triploid cell nucleus was available and translated successfully during development.

4.1.3. Chromosome counts

This process easily differentiates triploids from embryos with an alternative ploidy and provides a means for monitoring the success of the fusion process prior to transfer of embryos to a hatchery environment. The protocol is described in detail by Eno et al. (2009) and employs a simple compound microscope at 60–100× magnification.

4.1.4. Transfer to hatchery, feeding embryos and maintenance to metamorphosis

This portion of the scaling up procedure depends on the availability of a hatchery. The hatchery used in this study is maintained by Dr. Larry Harris and located in Portsmouth, New Hampshire. In our case, parallel trials for control diploid gastrulae and for triploid gastrulae were carried out in 300-liter vats containing filtered and UV-sterilized seawater. Control diploid and triploid embryos were both cultured at 10 °C with aeration provided by an air stone to create current within the vat. Stacking density was 4 embryos/ml of medium with an ultimate final density expected to be 300,000 to 600,000 settled juveniles per vat.

Larvae were fed a combination of Isochrysis galbana, Dunaliella tertiolecta and Rhodomonas lens daily and overfeeding was avoided to yield achieve minimum mortality (Kelly, 2002). Still in this initial trial, mortality resulted for both diploid and triploid embryos following the prism pluteus stage. We suspected that the mortality that we observed for both diploid and triploid embryos resulted from over-aeration in these small containers that prevented normal filtering feeding by the plutei, but we do not have evidence to back up this idea. Normally in this hatchery, successful development of diploid embryos to metamorphosis and settlement occurs at 21 days in containers three to five times larger than the ones we used in these trials. Resulting diploid juveniles are then maintained for 5 to 7 days before extraction and transfer to juvenile culture systems at the hatchery. In future efforts to generate triploids, the use of larger tanks, with less vigorous aerations should yield triploid embryos that will also develop beyond the prism pluteus stage of development.

4.1.5. Maintenance of triploid juveniles in land-based aquaculture ventures and out-planting and maintenance of juveniles in near-shore lease sites

Because their sterility can reduce the energy required for reproduction, triploid adult sea urchins should prove useful for both land-based and near shore aquaculture ventures. It should be recognized that there is anecdotal evidence that some individuals view the use of triploid organisms negatively.

Diploid juvenile green sea urchins are typically maintained in recirculating or flow-through culture systems at hatcheries until they are approximately 10–20 mm in diameter (unpublished data from Dr. Larry Harris, UNH). Juvenile urchins can then be maintained in land-based aquaculture systems or be transferred to near-shore lease sites until they reach harvest size at 52 mm diameter. Previous and on-going studies show that transfer to lease sites during winter months when predators are dormant results in higher survival and under appropriate culture conditions, juveniles reach transfer size within 6 months. Sea urchins remain within lease sites if adequate food is available (Dumont et al., 2006; Lauzon-Guay et al., 2006).

Since reversion of presumably triploid organisms to reproductively active individuals has occurred in some cases, especially for male triploid fish and shellfish (Nell et al., 1996; Piferrer et al., 2009), follow up studies to monitor this potential outcome for triploid sea urchins are essential. While the behavioral issues recognized in fish that result from matings between triploid males and diploid females would not be an important issue for sea urchins, broadcasting gametes from triploid sea urchins might be even more important (Kirk et al., 2004). These precautionary studies should be carried out for both land- and near shore-based aquaculture ventures using triploid sea urchins, since such reversion may have genetic consequences for wild sea urchin populations (Kirk et al., 2004).

4.2. Issues unique to the reproductive biology of sea urchins that will be vital for commercial near-shore aquaculture ventures to consider if triploid adult green sea urchins can ultimately be produced

Gametogenesis and intra-gonadal nutrient storage and utilization are linked processes in sea urchin reproduction (Walker et al., 2005, 2006). Initial increase in size of sea urchin gonads of both sexes results as somatic cells within the germinal epithelium called nutritive phagocytes (NPs), store extensive nutrient reserves prior to gametogenesis (principally the major yolk protein, MYP, Unuma et al., 2003). Urchin gonads of both sexes that contain predominantly NPs are preferred as a commercial product (Walker et al., 2005; Unuma and Walker, 2009, 2010). In the green sea urchin, shorter day-lengths characteristic of fall photoperiod are closely correlated with initiation of gametogenesis and commercial quality of these gonads declines as gametes of both sexes are produced (Walker and Lesser, 1998; Unuma and Walker, 2009, 2010). Several studies of a variety of sea urchins have addressed the possibility that photoperiod can be manipulated at various times of the year to suspend gametogenesis in order to emphasize the desirable size and sensory qualities present in green
sea urchin gonads harvested from the wild in the fall when NPs are maximally developed and result in the best tasting commercial product (Walker and Lesser, 1998; Walker et al., 2005, 2006; Dumont et al., 2006; Böttger et al., 2006; Unuma and Walker, 2009, 2010). While it is possible to delay gametogenesis and to generate large commercially valuable gonads for the green sea urchin that contain predominantly NPs for use in land-based aquaculture (Walker et al., 2005; Böttger et al., 2006), methods for achieving this same result for the near-shore aquaculture of the green sea urchins have not yet been developed. Generation of sterile adult triploids is advantageous for culture of green sea urchins in their natural environment where photoperiod is ambient and where diploid green sea urchins initiate gametogenesis in the fall.

By generating adult triploid green sea urchins, we would begin to experimentally unlink gametogenesis and intraglandular nutrient storage in NPs and over-emphasize the nutrient storage phase of the green sea urchin annual gametogenic cycle. Gonads in triploid sea urchin of both sexes should be sterile and would not produce gametes in response to the autumn photoperiod cue (Walker and Lesser, 1998). We do not know if autumn photoperiod will or will not lead to mobilization of nutrients from NPs in adult triploid sea urchins maintained in the wild, resulting in reduction in the quality of the commercial product. Experiments designed to test this idea can only be accomplished when adult triploid green sea urchins are available. However, it is reassuring that in nature, adult green and other sea urchins continue to incorporate copious nutrients in NPs following the autumn photoperiod cue and the initiation of gametogenesis (Walker et al., 2005; Unuma et al., 2010). An additional benefit of near-shore aquaculture of adult triploid green sea urchins is that they will consume food in their natural environment and as a result, their gonads should develop taste that is expected by the industry.

Acknowledgments

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References


C.W. Walker is a Professor of Molecular, Cellular and Biomedical Sciences at the University of New Hampshire, Durham, NH. He received a BS from Miami University, Oxford, Ohio and an MS and PhD from Cornell University, Ithaca, New York. His interests include molecular aspects of gametogenesis and also aquaculture of the green sea urchin and molecular biology of the p53 pathway in hemocyte cancer of the soft shell clam, Mya arenaria. His laboratory was the first to clone c-myc and p53 genes from any invertebrate and opsin from the green sea urchin.

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