

## FINAL REPORT

**PROJECT CODE: 03-15**

**SUBCONTRACT/ACCOUNT NO: 556806**

**PROJECT TITLE: Developing and Testing Novel Methodology for Land- and Near Shore-Based Aquaculture of the Green Sea Urchin.**

**FUNDING LEVEL:** Year 1: January 1, 2004-December 31, 2004 - **\$68,321**  
Year 2: January 1, 2005-December 31, 2005 - **\$46,891**  
Year 3: January 1, 2006-December 31, 2006 - **\$54,438**

### **PARTICIPANTS (FUNDED TO DATE):**

**Dr. Walker** – PI, project coordinator, coordinator of laboratory components, evaluating gonadal histology; **Mr. Devin** – CoPI/ Consultant/Service Provider- land and near shore husbandry and technology transfer; **Dr. Böttger** – Post-Doctoral Student - Production of triploid sea urchins; **Mr. Tamaki** - President and Founder, I.S.F. Trading Co. Inc. - Taste testing of green sea urchins.

### **PROJECT OBJECTIVES:**

**Objective I.** To use invariant photoperiod to produce green sea urchins for the winter and summer Japanese markets with gonads that do not initiated gametogenesis, contain only nutritive phagocytes and are of high commercial quality (Years 1-2).

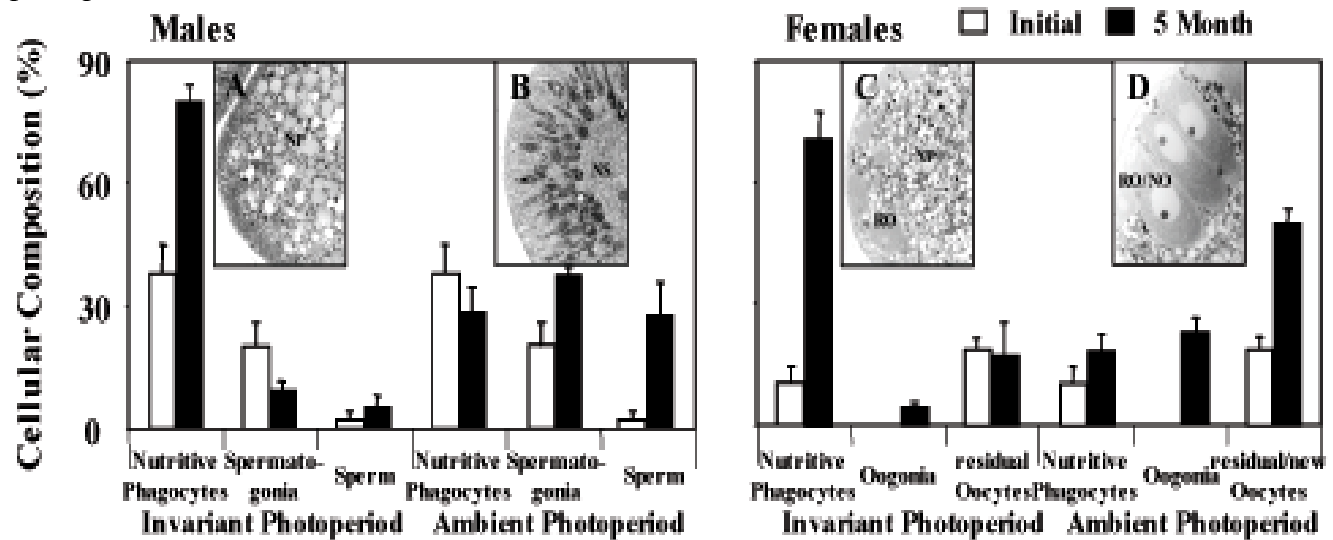
**Objective II.** To develop sterile triploid green sea urchins for use in either commercial land- or near shore-based aquaculture that contain only nutritive phagocytes and are of high commercial quality (Years 1-3).

### **PROGRESS AND PRINCIPLE ACCOMPLISHMENTS:**

**OBJECTIVE I:** We have conducted a study related to Objective I and aimed at producing green sea urchins for the winter Japanese market. We used one trough system (holding approximately 500 animals) and five cages (each containing 100 sea urchins) maintaining urchins respectively in invariant (July, using specially designed lighting systems) photoperiod in the lab and at ambient photoperiods at the lease site of the Darling Marine Center, Maine. During the six months (July-December, 2004) of this experiment, urchins were fed an extruded diet prepared for this purpose by Wenger Mfg. Inc. At the end of the experiment, gonad indices had increased significantly in both treatments, from 4.79% initially to 23.47% for ambient and 20.5% for invariant photoperiod treatments. Following these results we had taste testings of ten animals from each treatment conducted by Mr. Atsuchi Tamaki of I.S.F. Trading Co. Inc., who evaluated urchins maintained at ambient photoperiod as having usable roe of 17% (compared to total body weight), consistent color/ texture and sweet taste. Animals maintained at invariant photoperiod, however, were evaluated as having usable roe of 10%, inconsistent color/texture and a bitter taste. The taste results have to lead to the following considerations regarding sea urchin aquaculture. First of all, the index that we commonly use for scientific experiments with sea urchin gonads (wet weight gonad/wet weight whole animal \*100) does not necessarily reflect the final value of roe sold on the market. During the scientific evaluations all small amounts of gonad will be extracted, however, not so in the sea urchins harvesting process, which is reflected in the amount of usable roe, as determined through Mr. Tamaki. Second, the extruded Wenger diet will yield gonads of large size compared to preliminary experiments, where animals fed a diet of *Laminaria saccharina* for five months and yielded gonad indices of 16.67% under ambient and 15.02% under invariant light regime. However, these gonads were not considered to be of good taste and color and will thus not yield a high market

price (discrepancies between animals maintained under ambient and invariant photoperiod are a result of sea urchins feeding on encrusting organisms as well as the extruded diet in the ambient photoperiod treatment).

Results for stereology of gonads (**Fig. 1**) showed a significant increase in the volume fraction of nutritive phagocytes under invariant photoperiod from initial values (37.92% in males and 10.36% in females) to those in urchins at the end of the study (80.34% in males and 73.8% in females). There was no change in the volume fractions of nutritive phagocytes in urchins maintained under ambient photoperiod. Sizes of nutritive phagocytes increased in males and females from both invariant and ambient photoperiod treatments. When expressed as the nutrient portion of the nutritive phagocytes (excluding developing gametes in both sexes), we again found an increase in males and females under invariant and ambient photoperiod. However, the nutrient portion of NPs in individuals under invariant photoperiod were significantly higher than those of individuals under ambient photoperiod. Volume fractions of gonial cells increased from 20.12%± 5.51 in males and 0% in females initially to 37.81% ± 1.78 in males and 22.56%± 1.04 in females in individuals maintained for five months under ambient photoperiod.

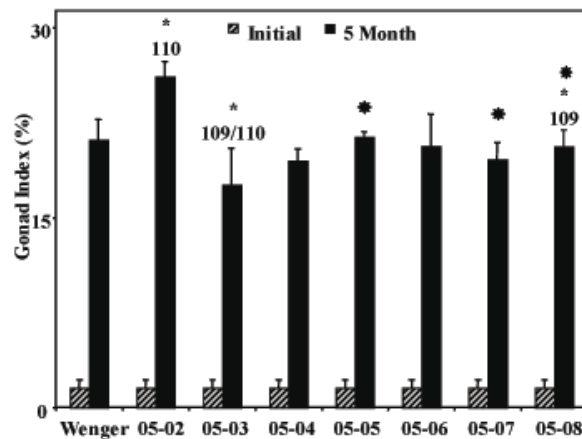


**Figure 1** - Volume fractions (%) of the NPs, gonial cells, oocytes and sperm of green sea urchins evaluated for males and females at time zero and after culture for five months under initial and ambient photoperiod. Animals were sacrificed in July (initial) and after 5 months (December). Pictures (A) and (B) for males and (C) and (D) for females represent invariant and ambient photoperiod respectively. NP = nutritive phagocyte, NS = new sperm, RO = residual oocyte, RO/NO = residual and new oocytes.

Under invariant photoperiod, there was a decrease in the volume fraction of gonial cells to 9.74% ± 2.11 in males and no change in females. Volume fractions of luminal spermatozoa and of primary oocytes within NP incubation chambers also increased in individuals maintained under ambient photoperiod from 2.15% ± 1.34 to 28.04% ± 7.85 in males and from 18.62% ± 2.61 to 49.67% ± 3.64 in females. No significant change in volume fractions of spermatozoa or residual oocytes in individuals maintained under invariant photoperiod was recorded. Size frequencies (%) of oocyte diameters increased significantly in urchins maintained under both invariant and ambient photoperiod. Under invariant photoperiod the mean oocyte diameter of residual oocytes from last years gametogenesis increased to 93.51 μm ± 3.66 (from 56.21 μm ± 2.23 initially). Under ambient photoperiod, mean oocyte diameters increased to 125.96 μm ± 7.34. These results indicate that we can manipulate the cellular composition of sea urchin gonads using invariant photoperiod to a composition that is favored by the Japanese consumers. However, the commercial diets currently available produce roe of an unpalatable taste,

which lead us to test new experimental diets (produced through cold extrusion) formulated and manufactured at Texas A&M by Addison Lawrence and Steve Watts.

For this experiment we tested seven new diets and compared them to the currently available commercial diet (used previously) in the aquaculture module, feeding 50 urchins on each diet for 5 months. After five months there we noted an increase in gonad indices with all diets, including the commercially available diet (**Fig. 2**). Three of the new diets were scored as marketable by our taste tester, their color evaluated as very good, and while taste scores are still not as high as from naturally harvested urchins, we are making progress in identifying a diet that will produce roe of high market value.



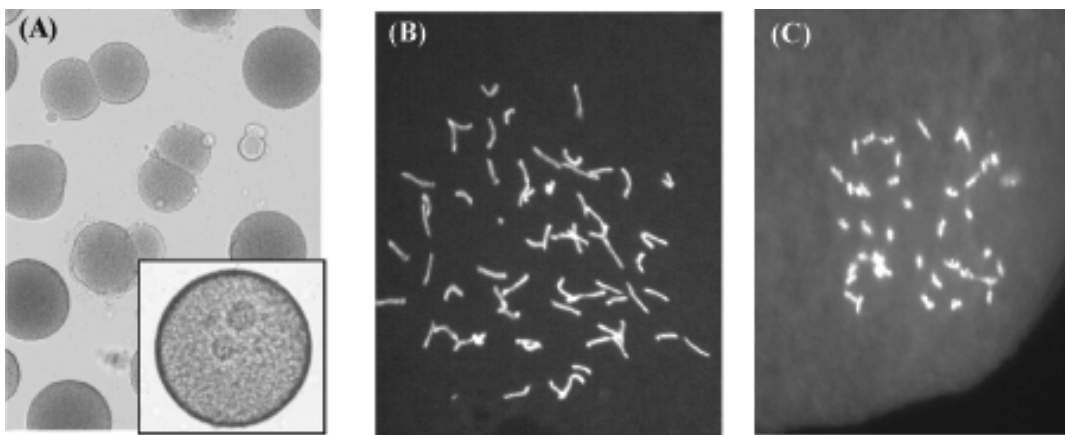
**Figure 2** - Gonad indices (% wet weight) for green sea urchins fed eight different feeds (commercially available Wenger and experimental feeds formulated and prepared by UAB and Texas A&M). \* = consistent color, # = orange/yellow color on the Maine DMR chart (laboratory dissection), \* = deemed marketable by the taste tester (Atchan Tamaki, ISF Trading).

**Objective II:** We have successfully removed the jelly coat and vitelline membranes and achieved maximum fusion rates for 2 fully mature ova. Removal of the vitelline membrane was accomplished by spawning female sea urchins (injection of 2 ml 0.5 M KCl) and incubating the eggs for 30 minutes in calcium-free seawater with 0.2% cysteine and 1.2 mg/ml pronase. Denuded ova were then treated with either polyethylene glycol and poly(Arg). Polyethylene glycol did not yield fusion. Ova treated by dilution to 75% of their original volume with dH<sub>2</sub>O plus CaCl<sub>2</sub> to a final concentration of 25mM and followed by a 10-fold with a solution of 0.0075 mg/ml poly(Arg) in calcium-free seawater consistently yielded >40% fusion of eggs in 1.5 ml microcentrifuge tubes following 50 minute exposure. However, when fertilization of these fused ova was attempted no development was accomplished, leading us to revise our treatments of the ova.

We have therefore modified the procedure for the removal of the jelly coat and vitelline membrane using a mechanical and an acidic method. The mechanical method entails an egg suspension of 1:10 eggs in calcium free seawater (which will weaken the jelly coat) that is filtered through a 210µm nylon mesh 9-10 times. The mesh size had to be slightly larger than the eggs (164.21µm ± 6.12) to ensure that the eggs were not damaged in the process. For the acidic methodology eggs were incubated for 50 minutes in filtered seawater at pH 5. Of the three methods we have now employed for the removal of the jelly coat (including the chemical removal with pronase that seemed to affect subsequent fertilization), the chemical treatment is still the most successful (98.1% ± 1.9 SE), however, since it seems deleterious to fertilization we have chosen the acidic removal, which had a success rate of 79.7% ± 6.2 removal over the less reliable mechanical removal (47.8% ± 17.8).

We then followed fertilization and development through to gastrula stage, when we measured the size of normal (fertilized without removal of vitelline membrane and fusion) and potential triploid embryos (that had undergone acidic removal of the jelly coat and successful fusion). Results showed that normal embryos were significantly smaller  $197.5 \mu\text{m} \pm 5.71$ , than embryos that had undergone fusion ( $234.5 \mu\text{m} \pm 2.34$ ).

Triploid embryos were consistently obtained from these fusions based on their size (normal embryos were significantly smaller  $197.5 \mu\text{m} \pm 5.71$  than embryos that underwent fusion  $234.5 \mu\text{m} \pm 2.34$ ) and chromosomal number (**Fig. 3**). These embryos were raised to normal blastula stage only (**Böttger et al., in preparation**).



**Figure 3** - Production of triploid eggs and blastulae. (A) Eggs undergoing fusion, inset fused eggs with two pronuclei prior to fertilization; (B) 63 chromosomes in a triploid blastula (fused egg fertilized by normal sperm) at 24 hrs after fertilization; (C) 42 chromosomes in a normal blastula 24 hrs after fertilization.

**WORK PLANNED:** At this point we are planning to disseminate our work, through publications, presentations and updating the website for Dr. Charles Walker.

**IMPACTS:** The development of techniques for successful large-scale production of urchins with suppressed gametogenesis (following either **Objective I or II**) will be a considerable boon to the sea urchin aquaculture in the following ways. Large-scale land-based culture of sea urchins depends upon understanding and manipulating gametogenesis to yield gonads of maximum size, texture and taste. The use of invariant photoperiod to produce such gonads is simple and can be employed in small or large-scale aquaculture ventures at a variety of sites on the East coast. Production of triploid sea urchins would permit similar results in near-shore lease sites where photoperiod cannot be controlled.

**SUPPORT:**

Year	NRAC-USDA Funding	University	Industry	Other	IOther	Total	Total Support
2004	\$68,321	30,542	0	0	0		98,863
2005	46,891	10,970	0	0	0		57,861
2006	43,618	10,820	0	0	0		54,438
Total	158,830	51,459	0	0	0		210,289

**PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED:**

**Publications**

Böttger, S. Anne, Celeste C. Eno and Charles W. Walker. 2006. Novel methodology for generating triploid green sea urchins – Applications for open-ocean aquaculture. *Aquaculture* 261:1422-1431.

Suspension of gametogenesis in green sea urchins experiencing invariant photoperiod – Applications for Aquaculture. S. Anne Böttger, Michael G. Devin and Charles W. Walker. Submitted to Aquaculture.

Böttger S.A., C.W. Walker and T. Unuma. 2004. Care and maintenance of adult echinoderms. In: *Methods in Cell Biology*, Volume 74, Development of sea urchins, ascidians and other invertebrate deuterostomes: Experimental approaches. Academic Press. pg.17-38.

## **Presentations**

Two novel culture approaches for sea urchin aquaculture. To be presented at the Annual NACE, Mystic, CO, December 2006.

Novel methodology for generating triploid green sea urchins – Applications for open-ocean aquaculture. To be presented at the 12<sup>th</sup> International Echinoderm Conference, Durham, NH, August 2006

Application of photoperiod manipulation and new extruded diets in aquaculture of the green sea urchin (*Strongylocentrotus droebachiensis*). NSA Annual Meeting, Monterey, CA, February 2006

Current echinoderm research: Novel approaches using photoperiod manipulation and triploidy for culture of the green sea urchin. GOMMEA Annual Meeting, Portland, ME, November 2005.

Application of novel technologies in sea urchin aquaculture: Photoperiod manipulation and triploid production. Research Seminar, June 15, 2005. The University of Maine at Orono, ME.

Culture of the green sea urchin: Novel approaches using photoperiod manipulation and triploidy. Research Seminar, March 2, 2005. Roger Williams University, RI.

Assessment of two novel culture methods for land- and near shore based aquaculture of the green sea urchin (*Strongylocentrotus droebachiensis*). Annual NACE, Concord, NH, December 2004.

Normal and altered gametogenesis in the green sea urchin *Strongylocentrotus droebachiensis*: Implications for aquaculture. Sea urchin 2003, International conference on fisheries and aquaculture, March 24-29, 2003, Puerto Varas, Chile.