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Culture Protocols and Production of Triploid Purple-Hinge Rock Scallops

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Abstract: The goal of this ongoing research and outreach is to expand the West Coast shellfish industry through creation of triploid seed and demonstration of efficient culture methods for the native purple-hinge rock scallop (*Crassadoma gigantea*). Shellfish aquaculture is a low trophic level means of seafood production that provides many benefits to coastal communities and the environment, while at the same time increasing the supply of locally produced safe and nutritious seafood. There is a strong desire to develop native species for aquaculture development to diversify the shellfish industry and help to avoid concerns often voiced today about the use of non-native species. While new native species of shellfish for aquaculture are highly sought after, there are also genetic concerns associated with rearing native species for aquaculture using hatchery-reared seed that may have undergone significant domestication selection or been produced from distant broodstock populations. This may occur as a normal consequence of rearing in the hatchery environment or through highly directed selection, crossbreeding, or other means to genetically change the production characteristics of the organism. These risks are significant and must be addressed to realize the potential for growth of the U.S. west coast shellfish industry. Issues associated with potential genetic risk to wild rock scallop populations could be resolved through the creation of tetraploid scallop stocks, which could be mated to diploids, producing 100% triploid offspring, or by using chemical means. Scallops were successfully spawned and cultured at the Taylor Shellfish hatchery in Washington State and at the Bodega Marine Laboratory of UC Davis in Bodega Bay, California. Growth and survival of larvae was highly variable among batches and populations, despite broodstock maintained in common conditions and similar larval dietary rations. Causes for this variability and generally low larval survival are being investigated. Initial efforts to culture scallop larvae relied mainly on C-Isochrysis sp. These efforts produced weak larvae with low survival and nutrition was identified early as a limiting factor. Subsequent efforts relied on a mixed diet of C-Isochrysis sp., Nannochloropsis sp., Pavlova sp., Chaetoceros sp., and Thallasiosira sp. The optimal timing for production of 3N scallops by inhibition of second polar body extrusion using 6-DMAP has been determined to be a 20 min treatment 55 - 60 min post fertilization at 17 °C. The optimal dosage of 6-DMAP for production of 3N scallops by inhibition of second polar body extrusion has been determined to be 425 uM.

Key words: Purple-hinged rock scallop, *Crassadoma gigantea*, triploid, 6-DMAP

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A bacteriological study was carried out in a scallop hatchery in western Norway. The hatchery had suffered severe mortalities during the larval stages, and prophylactic use of antibacterial agents was necessary to produce larvae. A number of bacterial strains were isolated from the hatchery. A challenge test was performed with the isolates. Six of the strains caused mortalities not statistically different from *Vibrio pectenicida*, a known scallop pathogen. From 16s rDNA analysis on these strains, the phylogenetic tree indicated two groups of apparent pathogens; one strain that resembles the Alteromonas/Pseudoalteromonas group and a cluster of strains that resemble the species *Vibrio splendidus*. Since the antibacterial agent used in the hatchery was chloramphenicol, which is now banned in Norway and Europe, application of alternative antibacterial agents were investigated. In this study the minimum inhibitory concentration (MIC) values of chloramphenicol, florfenicol, fluemequine, the combination trimethoprim/sulfadiazine, oxytetracycline, oxolinic acid and Pyceze on bacterial strains isolated from scallop larvae were investigated. Based on these MIC values, procedures for the treatment of scallop larvae with antibacterial agents were evaluated. Since the therapeutic procedures will be used in a marine environment, the antagonizing effect of seawater on some of the antibacterial agents was measured. For fluemequine and trimethoprim/sulfadiazine the average MIC values increased significantly when using seawater with salinity of 25 ppt compared to 2% NaCl.

(4) Caines J. and Crocker K. 1999: Hatchery production of sea scallop spat (*Placopecten magellanicus*) in Newfoundland, Canada.

A commercial hatchery for production of sea scallop spat was commissioned in 1995 at Belleoram, Newfoundland, by the provincial Department of Fisheries and Aquaculture. Four annual production seasonal cycles have been completed to date, where spat have been transferred to grow-out at shellfish farms. The annual yield from the hatchery has been 2 – 25% of the planned target of 20 million spat per year. This presentation will report our experiences in 1998 regarding algal culture, broodstock conditioning, larval growth, spat settlement and growth, and bacteriological monitoring of water quality. Algae (flagellates and diatoms) were grown in a continuous culture system purchased from Seasalter Shellfisheries in England. The intent was to provide scallop larvae and spat with a diet of mixed species of varied lipid and carbohydrate content. The following flagellates and diatoms were cultured in 500-L polyethylene bags under continuous light at 22 degree C with the addition of CO sub(2): *Isochrysis galbana, T.Iso, Pavlova lutheri, Tetraselmis suecica, Chaetoceros calcitrans, C. ceratosporum, C. muelleri, Thalassiosira pseudonana, T. weissflogii, and Chroomonas salina*. The growth rate was approximately 0.30 and 0.35 divisions/day for the flagellates and diatoms respectively.

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The successful induction of triploid embryos or larvae has been performed in *Patinopecten yessoensis* during the past two decades. However, no research has been reported about the performance of triploid *P. yessoensis* cultured in the field. This study induced triploidy in *P. yessoensis* by hypotonic shock and compared the growth and reproductive performance of triploids and diploids reared under commercial conditions for up to 24 months. The main results of this study are as follows: Triploid scallops were smaller in size and weight compared with diploids and had a retarded absolute growth rate (AGR). After 24 months of cultivation, the mean shell height, shell length, shell width, and body weight of triploids were 9%, 10%, 9%,

and 25% less than diploids, respectively ($P < 0.01$). Although normal in sex ratio, the reproductive potential of triploids was significantly reduced. Only 87% of the triploids exhibited sex-discernible gonads during the breeding season. None of the male triploids spawned, and the percentage of female spawners among the triploid population was only 27% of that for the diploid population. The relative fecundity of triploid females was only 4% of diploid females. Triploid eggs produced mostly aneuploid larvae and had an extremely small chance of generating viable offspring when fertilized by sperm from diploid males. Most aneuploid larvae died before the D-shaped stage, and no survival exceeded seven days. The potential to yield viable offspring from the triploid population was estimated to be only 4% of that of the diploid population. Despite the growth disadvantage of triploids, this study may support, in part, the energy reallocation hypothesis because triploid AGR was similar to diploid AGR (2% variance) during the sexual maturation season. Our results also indicate that there would be no growth advantage, but instead a disadvantage, for triploid *P. yessoensis* growing at the experiment site. In addition, this research provides the first evidence that viable triploid molluscs can be induced by hypotonic shock, of which the practical and evolutionary implications are also discussed.

(6) Cogswell A. T., Roach S. E., and MacDonald B. W., 2006: Triploid bay scallops (*Argopecten irradians*): induction methodology, early gonadic development and growth. Canadian Technical Report of Fisheries and Aquatic Sciences, 2635pp.

Triploid (3N) Pacific oysters (*Crassostrea gigas*) account for more than 50% of total oyster production in the US. The success of this species has created an interest in producing triploids of other commercial shellfish species, including scallops, clams and mussels. Here, we report on 3N induction trials with the bay scallop, *Argopecten irradians*. The most commonly employed 3N induction technique involves exposing early embryos to chemicals (e.g., Cytochalasin B (CB)). CB inhibits the release of the second polar body immediately following fertilization, causing retention of both pairs of female chromosomes in addition to the male chromosome set. It does this by disrupting actin polymerization. Four concentrations of CB were evaluated for ability to produce 3N larvae. The efficacy of current and proposed 3N induction techniques (i.e., 4N x 2N crosses) and the commercial potential of 3N bay scallops are discussed.

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Mass induction of triploidy in the catarina scallop (*Argopecten ventricosus*) results in low success in the percentage of triploids produced. To understand whether this is a treatment effect affecting all eggs equally, families were individually induced to triploidy with cytochalasin-B (CB), comparing their survival from egg to D-larvae and spat and the percent of triploidy within families. Differences in percent triploidy success were evident between families, obtaining some with no triploids and some with high triploidy. Among the possible causes for these differences are quality of eggs, different developmental rates, and differences in susceptibility to the treatment (CB or DMSO) itself. Regardless of those differences, overall triploidy production was increased by inducing individually eggs of each scallop rather than in mixed egg batches. In the first experiment, it was improved by 17%, and in the second experiment by 42%, as indicated by the weighted mean of triploids among the families and when compared with previous results with this same species, where triploidy success was 58%. In a second experiment with three different families, the growth and fecundity of triploid and diploid catarina scallop were evaluated. The growth superiority of triploids was confirmed. The results indicated that triploid catarina scallop had a significantly reduced fecundity when compared with diploid scallop. The reduced fecundity appears to be mostly of a random nature, possibly associated with a reduced capability to produce balanced gametes. Whereas the successful production of tetraploid catarina scallop from fertile triploid scallop is in principle possible, the low number of eggs shed by triploid catarina scallop could diminish that success rate, even more if single triploid females are required to optimize tetraploid induction.