Performance of Mass Produced Diploid vs Triploid Tropical Oyster, *Crassostrea iredalei* Faustino

MASAZURAH, A.R.[1/], MOHD. SALEH, M.T.[2/] and DEVAKIE, M.N.[1/]

[1/]*Fisheries Research Institute, Batu Maung, 11960 Penang, Malaysia.* masazurah@fri.gov.my
[2/]*National Prawn Fry Production and Research Centre (NAPFRE), 08500 Pulau Sayak, Kedah, Malaysia*

Abstract: A study was conducted on the growth and survival rates of diploid and triploid tropical oyster (*Crassostrea iredalei)* larvae cultured in the hatchery at NAPFRE and grown out thereafter, at the Teluk Bayu River in Batu Lintang, Kedah. Triploidy was induced on strip-spawned eggs using Cytochalasin B (CB) at optimal concentration of 0.75 mg/l. Chromosome analysis showed that 81% triploid embryo (chromosome count) were produced during the induction. A comparison of the mean length and width (± SE) was found to be significantly different at $p< 0.05$ for the triploid and diploid oyster larvae. The triploid larvae attained a mean length of 336 ± 3.41 µm as compared to the diploid which measured 263 ± 3.41 µm, while the mean width was 293 ± 3.03 µm and 229 ± 3.03 µm for the triploid and the diploid larvae, respectively. A fifteen month study was conducted to compare the growth and survival rates of the diploid and triploid oyster spat cultured at the Teluk Bayu River in Batu Lintang, Kedah. Although the triploid oysters attained a larger mean length of 79.3 ± 0.97 mm as compared to the diploid oysters which measured 70.8 ± 1.02 mm, they were not significantly different ($p>0.05$). A comparison of the mean total weight at the end of the experiment was found to be significantly different ($p <0.05$) for the triploid and diploid oysters, which was 94.0 ± 1.29 g and 69.1 ± 1.4 g, respectively. It was observed that the triploid oysters were on the average, 36% heavier than their diploid siblings after 15 months of culture. However, when marketable sized oysters were analysed for their ploidy level (gill tissue cell nuclei diameter) only 53% were found to be triploids.

Keywords: *Crassostrea iredalei*, triploid, growth performance, survival, larvae, spat

Abstrak: Satu kajian telah dijalankan ke atas tumbesaran dan kadar kemandirian larva tiram diploid dan triploid tropika (*Crassostrea iredalei*) yang dikultur di hatcheri di NAPFRE dan seterusnya dibesarkan di Sungai Teluk Bayu, Batu Lintang, Kedah. Triploidi diaruhkan ke atas telur yang dilurut keluar menggunakan Cytochalasin B (CB) pada kepekatan optima 0.75 mg/l. Analisis kromosom menunjukkan 81% embrio triploid terhasil daripada aruhan ini. Perbandingan purata panjang dan lebar (± SE) menunjukkan perbezaan yang signifikan ($p<0.05$) untuk larva tiram triploid dan diploid. Larva triploid menjangkau purata panjang 336 ± 3.41 µm dan lebar 293 ± 3.03 µm berbanding dengan larva diploid, 263 ± 3.41 µm panjang dan 229 ± 3.03 µm lebar. Kajian selama 15 bulan dijalankan untuk membandingkan kadar tumbesaran dan kemandirian tiram-tiram ini di lapangan.Walaupun tiram triploid mencapai purata panjang 79.3 ± 0.97 mm yang lebih dari tiram diploid 70.8 ± 1.02 mm, perbezaan ini adalah tidak signifikan ($p>0.05$). Perbandingan purata berat keseluruhan di penghujung kajian mendapati ada perbezaan yang signifikan ($p<0.05$) antara tiram triploid (94.0 ± 1.29 g) dan diploid (69.1 ± 1.4 g). Tiram triploid didapati secara puratanya 36% lebih berat dari tiram diploid selepas diternak selama 15 bulan. Walaubagaimanapun, apabila dijalankan penentuan tahap ploidi pada tiram-tiram yang mencapai saiz pasaran, cuma 53% sahaja yang dipastikan sebagai triploid.

Introduction

Production of triploid is gaining popularity worldwide for the purpose of shortening their growth period. Unlike diploids, triploids lack reproductive capacity (Beaumont and Fairbrother, 1991) and the poor gonadal development have been associated with either partial sterility (Utting *et al.*, 1996; Ruiz-Verdugo *et al.*, 2000 and 2001) or total sterility (Allen and Downing, 1986) Sterility in turn, has been known to trigger better growth rates, meat quality, large adductor muscle and apparently better taste (Tabarini, 1984; Allen and Downing, 1986; Beaumont and Fairbrother, 1991; Ruiz-Verdugo *et al.*, 2000). From the conservation point of view, triploids are known to eliminate potential risks of genetic pollution posed by animals escaping from farms (Liu *et al.*, 2004).

Over the last two decades, induction of triploidy in bivalves, especially oysters had been the main focus (Liu *et al.*, 2004). Stanley *et al.* (1981) were the first to induce triploidy in oysters, targeting the American oyster *Crassostrea virginica*. Since then, extensive studies have been conducted worldwide on temperate oyster species. Commercial benefits of triploidy have been evaluated in the Pacific oyster, *Crassostrea gigas*; the Eastern oyster *C. virginica*; the Sydney rock oyster *Saccostrea glomerata* and the

European flat oyster *Ostrea edulis* but the technique has only been commercialised for the Pacific oyster (Allen and Bushek, 1991; Nell, 2002). On the other hand, very little work has been done on the tropical oyster species except for a few preliminary induction trials in Thailand, on *Saccostrea cucullata* (Jarayabhand *et al.*, 1994), S. *commercialis* (Navanarasest *et al.*, 1993) and the mangrove oyster, *C. lugubris* (Roongratri and Youngvanichset, 1988).

The tropical slipper oyster, *C. iredalei* (Faustino) in Malaysia, is known to spawn throughout the year with peak seasons in April-June and October to December, periods which coincide with the tropical monsoons (Devakie *et al.*, 1993). During these seasons, changes in water temperature and salinity trigger the oysters to spawn actively thus rendering the meat condition lean and not presentable for the market. Triploidy was thus considered the option to resolve this problem. Several trials using strip spawned gametes to determine the optimal chemical (Cytochalasin-B) concentration to induce triploidy in this oyster have been carried out and it was found that concentrations of 0.75 and 1.0 mg/L yielded the highest level of triploidy (Hand *et al.*, 2003; Mohd. Saleh *et al.*, 2004). The present study was undertaken to evaluate the performance of triploid *C. iredalei* in terms of growth and survival in the hatchery and field as compared to diploids and as well as to develop techniques on the mass production of triploid oysters for pre-commercial purposes.

Materials and Methods

Gamete production

Oyster broodstocks were procured from the Setiu Lagoon, Terengganu, which were grown on rafts at the Teluk Bayu River in Bt. Lintang, Kedah. A total of 10 pieces of broodstock were sampled randomly from the raft and opened with the oyster knife to determine the condition of the gonad. Oysters with running ripe gonads were brought back to the hatchery for spawning. About 25 millions eggs were produced by strip spawning 16 pieces of oysters (7 females and 9 males). The eggs were pooled together, counted and distributed to treatment beakers of 1 liter capacity. The eggs were divided among the replicate beakers (triplicate: 3 for triploid and 3 for diploid) at 4 million/L. Fertilisation was done by adding pooled sperm (50 ml/beaker) into all the beakers containing eggs and stirred.

Triploid induction

Triploid induction was carried out using standard method by Allen *et al.* (1989). When 45-50% of the eggs had extruded the 1st polar body (usually 15-25 min after fertilisation), the beakers were treated with cytochalasin B (CB) at 0.75 ppm. When the eggs attained trochophore stage, they were sampled for ploidy analysis.

Ploidy determination

Standard methods were employed for ploidy analysis (Allen *et al.*, 1989). The ploidy level of trocophore larvae from each treatment were determined by direct chromosome counts. At the end of the experiment, a total of 30 spat (size 0.5-2.0 cm) were sampled randomly from both the triploid and diploid batches to determine the percentage of triploid spat produced.

Larval and spat culture

The remaining eggs in beakers were counted and stocked in the larval rearing tanks. Six rearing tanks of 2 tons capacity of water were used to stock the treated eggs at the density of 2-3 eggs/ml. The treated eggs were cultured until they attained the setting stage and the growth and survival rates of the diploid and their triploid siblings were monitored.

Water change

Total water change was observed on every alternate day until the larvae attained the eyed larvae stage. At this point water change was done on a daily basis. This is to retain the setting larvae on a screen (the opening size of the screen used were 200 μm) for transfer to the settling tanks.

Feeding

Feeding of algae commenced on the second day of culture onwards. *Isochrysis galbana* was used for the first 7-10 days and mixtures *of I. galbana* and *Chaetoceros calcitrans* thereafter, till the eyed stage. Algae density given ranged from 10-30 x10^3 cells/ml for the first week and then was increased from 30-70 x 10^3 cells/ml from the second onwards. Once the larvae set, mixtures of *I. galbana, C. calcitrans* and *Skeletonema costatum* were fed at a density of more than 100 x10^3 cell/ml as the filtering rate of the spats were becoming more efficient.

Water quality monitoring

Physical parameters such as temperature and salinity were monitored daily using thermometer and refractometer, respectively.

Sample for growth and survival

For growth, a total of 50 larvae/tank were sampled from each tank for length and width measurements on alternate days. And as for survival rate, during each water change, counts were made from aliquots of 1ml of larvae.

Larval settlement

Once the larvae attained the pedivelliger stage (eye spot observed), the larvae tend to retain on screen with a mesh size of 200 µm. These larvae were then transferred to the setting tanks that were lined with HDPE plastic sheet. Plastic strips (cultch) were also hung from the water column to create more surface area for the spat. All larvae were considered set after no larvae were observed on screens during water change. These spat were fed with mixed algae and nursed till they attained 5 mm and were counted to determine the setting rate of the spats.

Field grow-out

Some 37,500 triploid oysters (mean size of 5 mm) were transplanted to Teluk Bayu River in Batu Lintang, Kedah for grow-out. They were stocked at 5000 pieces/basket (mesh size of 3 mm). Their growth rates (shell length and weight) and survival was monitored for 15 month. For the purpose of sampling, 200 pieces were stocked in a basket (two baskets for triploid and two baskets for diploid).

Statistical analysis

The data were analysed using the one way analysis of variance (ANOVA) and the comparison of their means using the Tukey's honesty significant differences (HSD) procedure (Analytical Software, 2003). Data in the text, tables and figures are expressed as means SE.

Results

The present study showed that there was a significant difference ($p<0.05$) in the length and width of the triploid and diploid oyster eyed larvae. The mean length (± SE) attained by the triploid and diploid oyster larvae were 336 ± 3.41 µm and 263 ± 3.41 µm, respectively while the mean width (± SE) attained by the triploid and diploid oyster larvae were 293 ± 3.03 µm and 229 ± 3.03 µm, respectively (Fig. 1).

A comparison of the survival rates indicated that there was no significant difference ($p>0.05$) between the triploid and the diploid oyster larvae. The temperature levels monitored indicated slight fluctuations throughout the culture period ranging from 27.3-31.0°C. The salinity on the other hand, remained at a normal range of 28-31 ppt.

Survival rates of the eyed larvae for triploid and diploid were 5% (0.43 million) and 32% (2.81 million), respectively while the setting rate of triploid and diploid spats (3 mm) were 18% (0.08 million) and 5% (0.151 million), respectively. Numbers of oyster spat at a mean size of 5 mm were 37,500 pieces for triploid and 21,900 pieces for diploid (Plate 1). The numbers of remaining oysters after 15 months of culture were about 3,000 pieces (triploid) and 2,000 pieces (diploid).

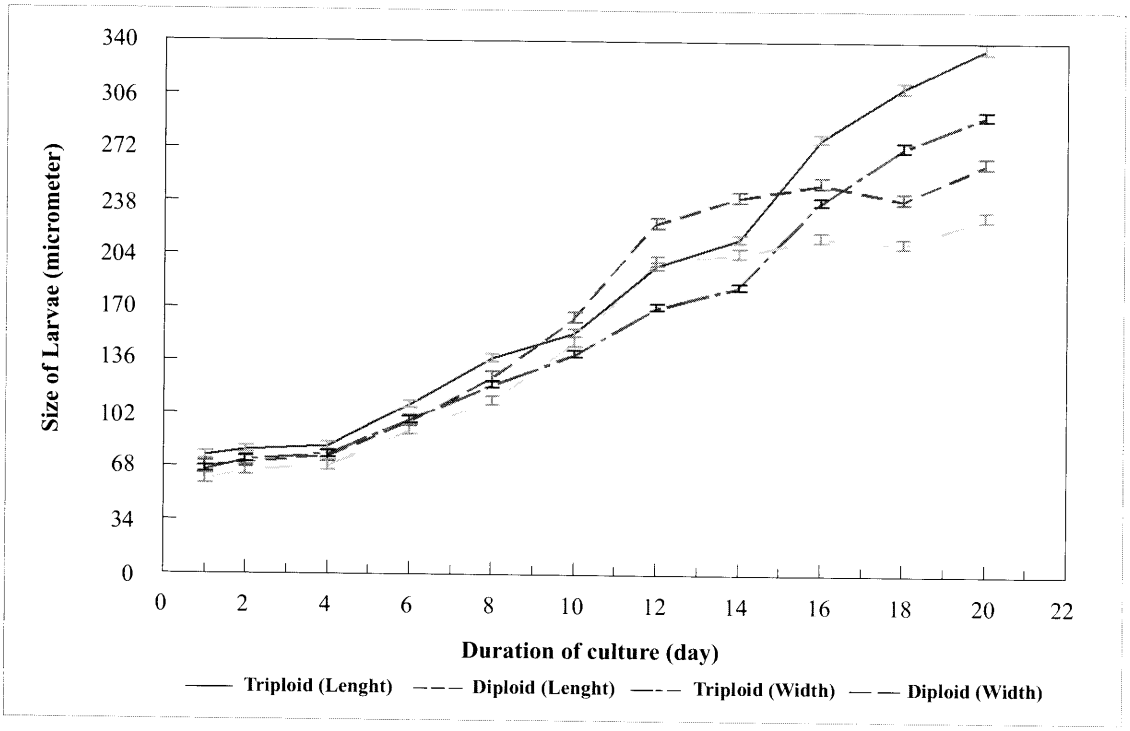

Figure 1 : Growth (length and width) of triploid vs diploid oyster larvae

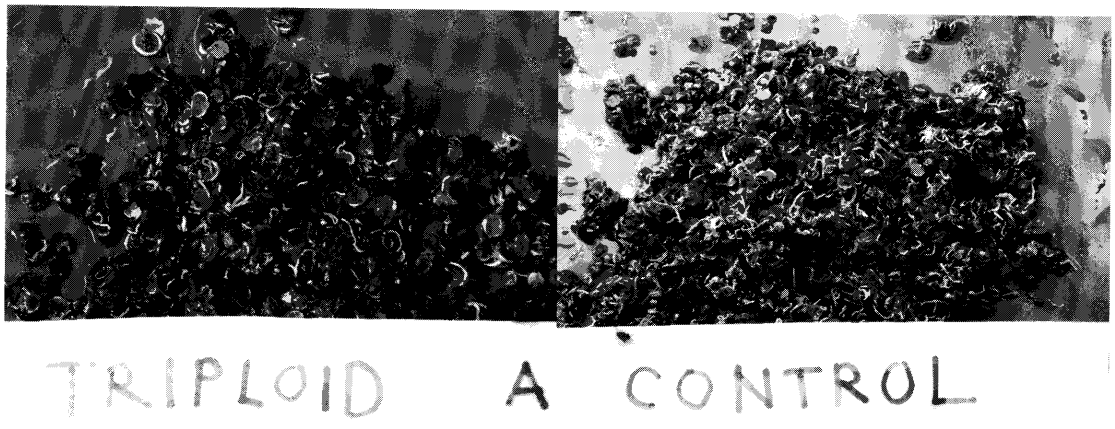

Plate 1: Triploid and diploid (control) spats produced in the hatchery

The study showed that there was no significant difference ($p>0.05$) in the length of the triploid and diploid oysters attained after 15 months of culture. The mean length (± SE) attained by the triploid and diploid oysters were 79.3 ± 0.97 mm and 70.8 ± 1.02 mm, respectively (Fig. 2). However, a discernable trend was noted for the weight gained. There was a significant difference ($p<0.05$) between the weights of the triploid oysters (94.0 ± 1.29 g) as compared to the weight of the diploid oysters which was 69.1 ± 1.4 g (Fig. 3). General observations indicated that the shell length of the triploid oysters appeared bigger and the meat appeared thicker and more succulent (Plate 2). It was noted that triploid oysters were on the average, 36% heavier than their diploid siblings after 15 months of culture. The percentage triploidy ascertained at this stage was 52.0%.

A comparison of the survival rates indicated that there was no significant difference ($p>0.05$) between the triploid (35%) and the diploid oysters (40%). The temperature levels monitored indicated slight fluctuations throughout the culture period ranging from 28.0-30.5°C. The salinity on the other hand, fluctuated widely from 15-30 ppt. Low salinity levels were observed during heavy rains but this species being euryhaline in nature is known to tolerate wide fluctuations in salinity.

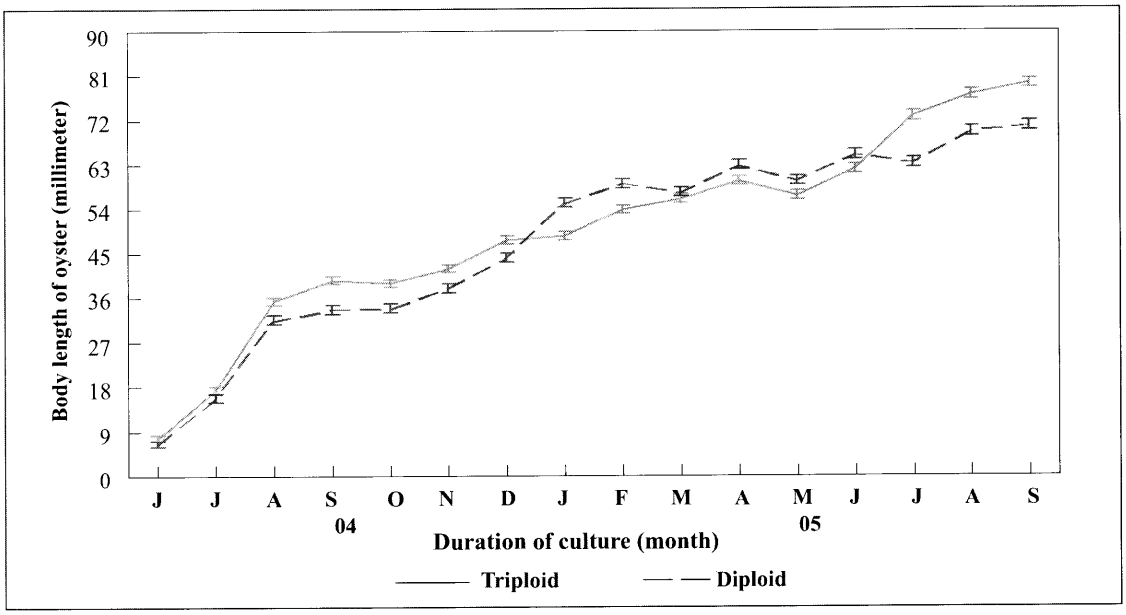

Figure 2: Growth (shell length) of triploid vs diploid oyster spat at Bt. Lintang, Kedah

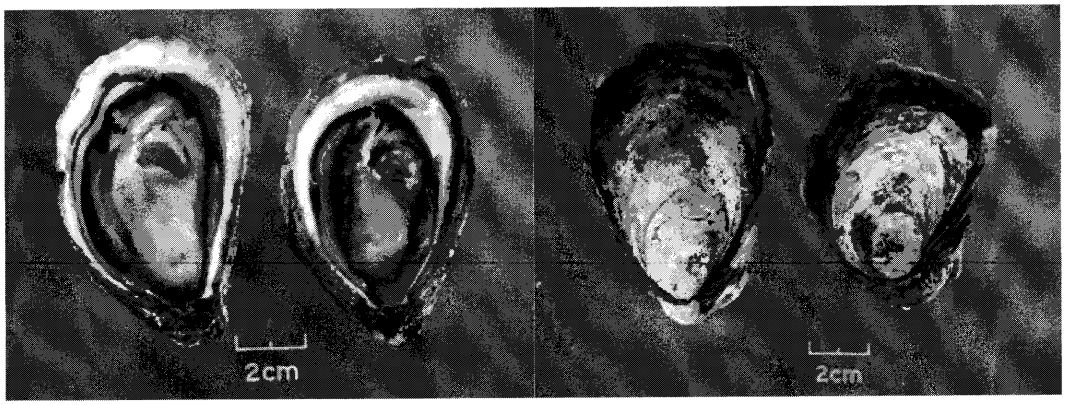

Plate 2: (LEFT) Meat appearance (Left triploid; right diploid)
(RIGHT) External shell appearance (Left triploid; right diploid)

Discussion

In China, triploid Pacific oysters have been in commercial production at least since 1997, while utilising tetraploids for the production of triploids on a pilot scale had commenced in 2000 (Nell *et al.*, 2002). In the west coast of the United States, about one third of the hatchery produced seeds are triploids (Chew, 1994). Though the commercial production of triploid oysters is gaining popularity worldwide, the application of this technique is still under study in the South East Asian region. The present study showed that there is a strong potential for the mass producing triploid oysters in Malaysia. At an experimental level, some 37,500 triploid spats could be produced and when considering mass production, this number can be increased to suit the hatchery capacity. The percentage ploidy level detected after 15 months of culture was quite low (53%) as compared to 80-90% in other countries. This could be due to treatment methods where the number of larvae in the treatment beaker is the determinant factor. Usually they sink to the bottom and this result in some of the larvae being not exposed to the chemical. Although the survival rate of triploid larvae in the hatchery was low (5% as compared to 32% for diploid), this is acceptable because the larvae are exposed to chemical and it can be improved through better handling methods. Likewise in the wild, survival was also low (40%) due to poor management methods. The spat needs to be flushed clean from silt and other organisms routinely and the baskets needs to be changed. Another factor contributing to the low survival rate was heavy rain which tremendously reduced the salinity level to 15 ppt. From the study it was also obvious that the triploids were 36% heavier, meaning although they were exposed to the same environmental conditions, the triploids still exhibit better growth traits.

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References

Allen, Jr., S.K. and Downing, S.L. 1989. Performance of triploid Pacific Oyster, *Crassostrea gigas* (Thunberg): I. Survival, growth, glycogen content and sexual maturation in yearlings. *Journal of Experimental Marine Biology and Ecology*, **102**: 197-208.

Allen, Jr., S.K. and Bushek, D. 1991. Large-scale production of triploid oysters, *Crassostrea virginica* (Gmelin), using stripped gametes. *Aquaculture*, **103**: 241-251.

Analytical Software, 2003. Statistix User Manual, Analytical Software. Statistix 8. Tallahassee, FL. 396p.

Beaumont, A.R. and Fairbrother, J.E. 1991. Ploidy manipulation in molluscan shellfish: A Review. *Journal of Shellfish Research*, **10**: 1-18.

Chew, K.K. 1994. Tetraploid Pacific Oyster offer promise to future production of triploids. *Aquatic Magazines*, **20**: 69-74.

Devakie, M.N., Hall, R. and Angell, CL. 1993. Small-scale Oyster Culture on the West Coast of Peninsular Malaysia. BOBP/REP/63., GCP/RAS/118/MUL. Bay of Bengal Programme, Madras. 30p.

Hand, R.E., Md. Saleh, M.T., Devakie, M.N. Masazurah, A.R. and Roslina, A.N. 2003. Preliminary determination of optimal cytochalasin B concentration for triploid induction in the tropical Oyster (*Crassostrea iredalei*). Poster presentation at the National Fishcrics Symposium, Kota Bharu, Kelantan, 18-20th Feb., 2003.

Jarayabhand, P., Jindalikit, J. and Menasveta, P. 1994. Chromosome manipulation in small oyster *Sasscostrea cucullata* by thermal shock. *Thai Journal of Aquatic Science*, **1(1):** 26-33.

Liu, W., Heasman, M. and Simpson, R. 2004. Induction and evaluation of triploidy in the Australian blacklip abalone, *Haliotis rubra*: a preliminary study. *Aquaculture Research*, **35(15)**: 1487-1494.

Mohd. Saleh, M.D., Masazurah, A.R., Devakie, M.N., Roslina, A.N. and Azhar, H. 2004. Optimal concentration of Cytochalasin B for the induction of triploidy in the tropical oyster *Crassostrea iredalei* embryo and their short-term survival rate. Paper presented at the Fisheries Symposium, 28-29hb Nov. 2004, Sg. Petani, Kedah.

Navanarasest, M., Menasveta, P., Jarayabhand, P. and Rungsupa, S. 1993. Induced settlement, metamorphosis, and triploidy in oyster. *In:* The Proceedings the Third Technical Conference on Living Aquatic Resources. Chulalongkorn University Press, Bangkok, 277-291 pp.

Nell, J.A. 2002. Farming the Triploid Oysters. *Aquaculture*, **210**: 69-88.

Roongratri, N. and Youngvanichset, K. 1988. Triploid induction in the Mangrove oyster (*Crassostrea lugubris*) with cytochalasin B. *Thai Fish. Gaz.*, **44(3):** 223-227.

Ruiz-Verdugo, C.A., Ramirez, J.L., Allen., Jr., S.K. and Ibarra, A.M. 2000. Triploid catarina scallop (*Argopecten ventricosus* Sowerby II, 1842): growth, gametogenesis and suppression of functional hermaphroditism. *Aquaculture,* **186**: 13-32.

Ruiz-Verdugo, C.A., Allen., Jr., S.K. and Ibarra, A.M. 2001. Family differences in success of triploid induction and effects of triploidy on fecundity of catarina scallop (*Argopecten ventricosus*). *Aquaculture*, **201**: 19-33.

Stanley, J.G., Allen Jr., S.K. and Hidu, H. 1981. Polyploidy induced in the American oyster, *Crassostrea virginica* with cytochalasin B. *Aquaculture*, **12**: 1-10.

Tabarini, C.L. 1984. Induced triploidy in the bay scallop *Argopecten irradians* and its effects on growth and gametogenesis. *Aquaculture*, **42**: 151-160.

Utting, S.D., Millican, P.F. and Laing, I. 1996. The breeding potential and biochemical composition of triploid Manila clams, *Tapes philippinarum* Adams and Reeve. *Aquaculture Research*, **27**: 573-580.