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Note

Induction and evaluation of triploidy in edible oyster, *Crassostrea madrasensis* (Preston) - an approach to enhance bivalve aquaculture

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ABSTRACT

Induced triploidy can be used for enhancing production of commercially valuable bivalve species. Triploid individuals, having an extra set of chromosomes (3n) typically exhibit reduced gametogenic activity leading to better growth since the energy used for reproduction is reallocated for somatic growth. In India, research on genetic manipulation of the edible oyster, *Crassostrea madrasensis* by triploidy inducement and assessment of triploid performance as a strategy to increase production has been underway since 1998. This article outlines the potential of using triploidy in bivalve culture for better returns.

Molluscs contribute two third of the total aquaculture production and Asia accounts for two third of the molluscs produced globally (Nair, 1999). Bivalves are the most important of all molluscs, since oysters, mussels and clams are consumed live or processed and also exported (Samuel, 1988). World bivalve production has increased substantially in the last fifty years, from about 1 million t in 1950 to about 13.2 million t in 2003.

Culture of filter feeding bivalves, which are low in food chain, is a low energy input aquaculture activity, which can be successfully adopted by traditional fishermen. Oyster culture is practised in many countries. Mussel farming technology has been extensively adopted and is successful along the south-west coast of India. An important requirement for successful commercial farming of bivalves is faster growth with good quality meat.

Use of triploidy has been suggested as means for enhancing growth and meat quality in bivalves. Triploid molluscs were first produced at the University of Maine in early 1980’s and afterwards triploidy has been induced in a variety of marine bivalves (Stanley *et al.*, 1984; Mason *et al.*, 1988; Hand *et al.*, 2004; Thomas *et al.*, 2006). Triploidy can be induced in bivalves by suppressing meiosis I or II (Beaumont and Fairbrother, 1991). Methods successfully employed to achieve this include the use of physical, thermal and chemical inhibition of the polar bodies. Suppressing meiosis II was reported to yield higher percentages of triploids and better larval survival (Hand *et al.*, 1998).

Advantages of triploidy listed by Nell *et al.* (1994) are increased growth rate, higher dry meat weight, higher condition index values and disease resistance. Triploid bivalves may have advantages not only in superior growth, but also in better product quality. Oysters are usually harvested when the condition of the meat is at the optimum level. The condition factor indicates the fullness of meat in the shell cavity and this is an important factor to be considered for harvesting. The harvesting period is limited to pre-spawning seasons, because meat quality decreases due to spawning. As triploid oysters are sterile, harvesting could be done throughout the year. Thus the application of triploidy in bivalves hold more promise than for any other group of aquaculture species.

Edible oyster (*Crassostrea madrasensis*) farming has picked up fast in Kerala, following the production of oyster seed through a hatchery system by CMFRI in 1982 (Nayar *et al.,* 1984; Muthiah *et al.,* 2000) and transfer of the oyster farming techniques to coastal fishers through demonstration programmes. First time in India, triploidy in *C. madrasensis* was induced using different physical and chemical agents like heat and cold shock, 6- Dimethyl aminopurine (6-DMAP) and Cytochalasin- B (CB) (Mallia, 2004). Further, this was also the first attempt in India on the use of the safe chemical viz. 6-DMAP for inducing triploidy in any species. Successful induction of triploidy in *C. madrasensis vis* a *vis* the diploids have been demonstrated at CMFRI (Mallia, 2004; Mallia *et al.,* 2006, Thomas *et al.,* 2004; 2006).

Both I and II meiotic triploidy were induced in larvae by treating with 6-dimethyl-aminopurine (100 µM for 8'). Larvae were reared in hatchery as per Thomas *et al.* (2004). After settling, spat were kept in separate rearing cages of 40 X 40 X 10 cm webbed with synthetic twine and enriched with velon screen. The cages were suspended from a rack erected in an intertidal area in the Tuticorin Bay. Every 3 months, 20 oysters were measured for length, breadth and weight following standard procedures. Growth performance of both I meiotic and II meiotic triploid oysters were evaluated in this study by comparing with that of diploids (Mallia *et al.,* 2006). Progressive increase in the length, breadth and weight of the triploid and diploid oysters are given in Table 1. Both type of triploids showed better performance compared to the diploids (Fig. 1). The mean growth rate in respect of length of I meiotic triploid oysters was 3.87 mm/month whereas diploid and II meiotic oysters registered 2.99 and 2.45 mm respectively. The mean values of the observation were statistically analyzed for significant differences ($P<0.05$) by ANOVA using SYSTAT 7.0. The differences in diploids and both types of (I and II) triploids were found to be significant. Higher dry meat weight of 1.45g was observed in I meiotic triploids compared to 0.86 and 0.19g in II meiotic triploids and diploids respectively (Fig. 2), which works out to be 126% more than the diploids. These observations indicate that triploidy leads to enhanced growth performance. Triploidy induction studies by Nell *et al.* (1994) in Sydney rock oysters (*Saccostrea commercialis*) have also revealed significant differences in morphometric characteristics of triploids and diploids. According to Muthiah *et al.* (2000), the estimated production of diploid oyster from

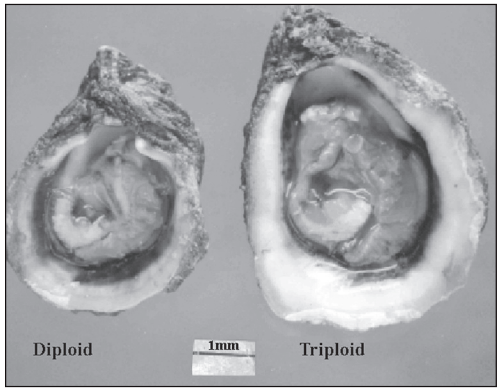

Fig.1. 12-month old diploid and triploid *C. madrasensis*

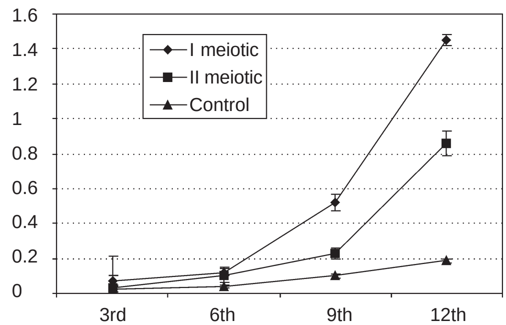

Fig. 2. Dry meat weight of triploid and diploid *C. madrasensis*

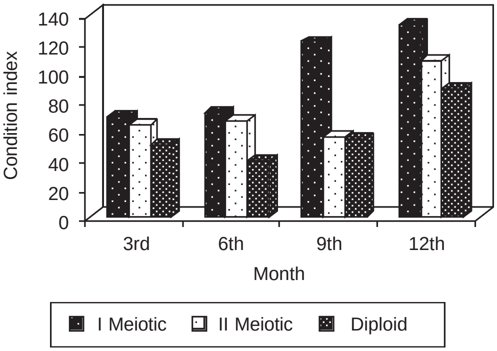

Fig. 3. Condition index of triploid and diploid *C. madrasensis*

rack and string method of culture is 80 t/ha. Considering the 126% higher dry meat weight of meiotic triploids, it shall be possible to effect a substantial increase in the meat production from oysters to 100 t/ha just by culturing triploids.

With regard to condition index, high value of 133.5 was registered for I meiotic triploid oysters as against 108.5 for II meiotic triploids and 88.7 for the diploids in this study (Fig. 3). High condition index in the triploids was observed by Nell *et al.*(1994). The higher meat condition may be due to reduced gonadal development as proposed by Beaumont and Fairbrother (1991). Condition Index plays an important role in marketability of oyster meat. High index values observed in triploids compared to the diploids throughout the year shall also add to higher economic returns.

As the growth rate of triploid *C. madrasensis* (12.9 mm/day) is more than that of diploids, use of triploids can facilitate higher production and bring about a reduction in the farming duration from 12 to 7 months resulting in lower production cost and higher economic returns.

6-DMAP is a safe chemical for inducing triploidy (Hand *et al.*, 2004). In a molluscan hatchery, 6-DMAP treatment process can be carried out without any additional facilities, even at room temperature. Hence, the additional cost for production of triploid seed will be

TABLE 1: *Comparison of growth in length, breadth and shell-on weight of diploid and triploid* C. madrasensis *(Mean ± SE)*

Months After treatment	Length (mm)			Breadth (mm)			Weight (g)		
	I meiotic	II meiotic	Diploid	I meiotic	II meiotic	Diploid	I meiotic	II meiotic	Diploid
2	9.3±0.61	9.6±0.57	7.1±0.41	7.1±0.49	7.6±0.51	5.8±0.30	0.1±0.028	0.4±0.02	0.05±0.005
3	27.1±1.83	24.2±2.58	18.7±1.76	23.8±1.82	21.6±2.9	16.6±1.52	2.5±0.33	2.3±0.53	1.1±0.21
4	35.1±2.21	31.8±2.24	31.3±2.51	29.7±2.31	27.4±2.65	24.4±2.13	5.3±0.70	4.9±0.90	3.3±0.64
5	37.4±2.39	34.5±2.38	33.2±2.78	33.2±2.47	29.5±2.70	25.6±2.09	6.0±0.80	5.6±1.03	4.1±0.77
6	37.9±2.49	35.5±2.27	34.6±2.83	34.0±2.36	29.0±2.36	27.5±2.09	6.5±0.88	5.6±1.04	4.2±0.80
7	39.1±2.67	39±1.54	38.8±3.68	32.6±2.77	28.1±2.23	32.1±2.84	7.0±1.05	5.5±1.02	6.0±1.26
8	41.7±3.17	32.5±2.23	40.2±3.56	33.5±2.91	26.6±2.32	31.1±2.53	8.0±1.40	4.5±0.89	6.7±1.31
9	41.9±3.32	32.8±2.27	40.1±3.38	32.5±2.82	25.9±2.03	31.3±2.71	8.1±1.41	4.6±0.88	6.6±1.40
10	45.0±3.51	29.9±2.47	40.8±4.0	35.5±2.81	25.1±2.67	32.1±2.74	10.6±1.85	4.1±0.81	7.6±1.57
11	51.7±4.05	38.3±2.28	46.2±4.30	45.5±4.01	33.1±3.71	38.5±4.47	15.5±2.72	6.9±2.01	10.4±2.58
12	55.7±5.01	49.0±1.82	43.0±3.33	50.3±3.85	34.8±4.45	36.3±3.31	18.3±2.87	8.6±2.76	9.4±1.84

very minimal. The additional cost for the production of 1 million triploid oyster seed will be Rs.1000/- (US $ 24) over that of diploid seed. This is towards the cost of 6-DMAP required for triploidy induction.

The protocols could be further refined for enhancing triploidy induction level. The findings from the present study could form the basis for further work in other bivalve species of the country. Any mollusc that normally shed both eggs and sperm into the surrounding water for external fertilization can be easily treated. Potential advantages of triploids vary with species, ranging from larger adductor muscles in scallops to increased survival in the Chinese pearl oyster, *Pinctada martensii*. The growers of triploid oysters in the North East Pacific, and increasingly in other parts of the world, benefit much because of their improved growth and marketability.

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