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Note

Induced triploidy in the edible oyster, *Crassostrea madrasensis* by temperature shock

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Abstract

Heat and cold shock were applied for triploid induction in the edible oyster *Crassostrea madrasensis*. Triploidy was confirmed by metaphase-spread preparation. The highest triploid induction by cold shock (5°C; 10 min) was 42.2% at larval stage and 33.3% at 'D' stage. With heat shock at 35°C (5 min) triploid percentage was 42% during larval stage and 39.1% at 'D' stage.

Triploidy, has been induced in a wide variety of marine bivalves. (Stanley *et al.*, 1981, 1984; Allen *et al.*, 1982; Chaiton and Allen, 1985; Quillet and Panelay, 1986; Beaumont and Contaris, 1988; Mason *et al.*, 1988; Supan *et al.*, 2000 ; Hand *et al.*, 2004; Mallia, 2004 and Thomas *et al.*, 2006). Induction of triploidy has been proposed as a means for enhancing growth rate and meat quality in bivalve molluscs. Triploidy is generally induced by treating fertilized eggs, either with chemicals or by applying thermal or high pressure shocks to eggs shortly after fertilization. The effect of temperature and pressure on developing eggs are that it disrupt the formation of the metaphase spindle, thereby preventing replicated chromosome sets from separating into daughter cells either at meiosis I or meiosis II. In contrast, subsequent to the removal of the shock, remaining processes of the meiosis proceed normally. The resulting eggs, now diploid rather than haploid, when fertilized by a haploid sperm give rise to a triploid zygote.

The commercially important edible oyster *Crassostrea madrasensis* enjoys wide distribution along Indian coasts. Considering the immense potential for large scale culture, the Central Fisheries Research Institute (CMFRI) has been conducting research on various aspects of this oyster. The Institute has already established a shellfish hatchery and developed techniques for mass production of edible oyster seed through hatchery systems (Nayar *et al.*, 1984; Muthiah *et al.*, 2000). The objective of this study is to optimize the production of triploid *C. madrasensis* by temperature shock and compare the survivability on 5th day.

Materials and methods

About 40 oysters which had been conditioned at 25°C for 15 days were induced to spawn by placing them in 32°C sea water. At the time of spawning, female and male oysters were taken out and kept separately in 5 litre glass beakers. After spawning, the individuals were removed from the spawning tank and separated sex wise in individual containers containing filtered seawater. The eggs were pooled and filtered through 100m sieves to remove debris. Eggs and sperms were mixed at the ratio of 10 ml: 1ml to facilitate fertilization and to avoid polyspermy. Time of the release of 50% of the first polar bodies in *C.madrasensis* was found to be 16 minutes after fertilization, at room temperature (29°C) (Mallia and Thomas, 2003). Two triploid inducing experiments were conducted separately using heat and cold shock.

Cold shock treatment: Fertilized eggs were exposed separately to low temperatures of 5°C and 12°C. In each, the duration of treatment was 10 and 20 minutes. The treatment was initiated 17 minutes after fertilization. To apply the cold shock fertilized eggs were collected in 20μm sieves and the sieves along with the eggs were immersed in seawater kept in 3 litre beaker maintained at low temperatures (5°C and 12°C) by adding ice.

Heat shock treatment: Fertilized eggs retained in a 20μm sieve were heat treated by immersing them in filtered seawater at 35°C for 5 min. Likewise treatments were given at temperatures 37°C and 39°C. Treatment was initiated 17min after fertilization. Duration of treatment was 10 and 5 minutes. Triplicates were maintained for each treatment and duration. The required temperature was maintained by using water baths.

Larval rearing: Larval rearing and ploidy determination was done following the method of (Thomas *et al.*, 2004). Initial counts of embryos in 1 ml sub samples

were taken by using Sedgwick Rafter cell counter (Nayar *et al.,* 1984). Survival to 'D' stage was determined by taking counts of 'D' shape larvae in 1ml aliquots 24 hr later. Survival to 'D' stage were calculated relative to control survival. In last experiment, mortality (M%) of larvae was also noted after 5 days, i.e. survival from straight hinge to umbo stage, was estimated as;

M% = (L_T -L_{T+N}/L_T) 100/N, where L_T = No. of larvae at time T, L_{T+N} = No. of larvae at T+N, and N = No. of days between measurements. All the statistical analyses were done using SYSTAT 7.0.1. Effect of treatment on survival and triploidy induction was tested using ANOVA (Two-way).

Results

Cold shock at 5°C for 10 min yielded 42.2% triploidy in day old embryos whereas 20 min exposure yielded 40.9 % only (Table 1). In the D- stage, the percentages were 33.33 and 30.33 for 10 and 20 min duration, respectively. Cold shock at 12°C for 10 min duration yielded 35.29% triploid embryos as against 36.6% for 20 minutes exposure. However in 'D' stage the percentage triploidy was similar for all treatments (32%). ANOVA of triploid percentages at larval stage gave significant differences between the two treatments and two different durations (Table 2), but the difference was not significant at the 'D' stage (P>0.1).

Highest triploid percentages (embryonic: 42 ± 1.24 and 'D' stage larvae: 39.13 ± 1.53 %) were obtained from heat shock at 37°C for 5 min duration (Table 3). Exposure for 10 minutes resulted in 41.85 ± 2.03% and 38.82±1.5 % respectively. At 35°C and 39°C, the percentages of triploid in embryos were 41.18 ± 0.78 and 40.75 ± 0.83, respectively for 10-minutes duration. At 39°C, 38 % triploidy was obtained with both durations. Lowest percentage of 30.8 and 34.4 was observed for the heat shock at 35°C for 5 and 10 minutes respectively. Triploid percentage realized from heat shock at 37°C for 5 and 10 minutes were 42 % and 39 %, respectively. Analysis of variance showed that the differential percentages of triploids among 'D' stage larvae produced at temperatures 35, 37 and 39°C were significant (Table 4), but the differences during larval stage were not significant (P>0.1). Effect of optimum temperature treatments are compared in Table 5.

Discussion

The efficiency of induction of triploidy may depend on synchrony of meiosis in the eggs relative to time of initiation and duration of treatments. At 29 °C the I PB of edible oyster is released at 10 min after fertilization and the II PB at 22 min (Mallia and Thomas, 2003). Experiments with temperature shock demonstrate that both heat and cold shock can be effective in inducing triploidy in edible oyster. The optimum cold shock was 5°C for 10 minutes. However, the percentage was lower (42.2%) compared to 66.7 % obtained for *C.gigas* at 0°C for 10 minutes (Yamamoto *et al.,* 1988) and 85.3% in *Mytilus edulis* at 1°C for 10 minutes (Yamamoto and Sugawara, 1988). The higher percentages obtained in these studies

Table 1. *Relative efficiency of different cold shock treatments for induction of triploidy in oysters*

Temperature	Percentage of triploids (first day larvae)		Percentage of triploids ('D' shaped larvae)	
	Duration of treatment		Duration of treatment	
	10'	20'	10'	20'
5°C	42.25±1.7	40.92±1.7	33.33±1.2	30.33±0.6
12°C	35.29±1.07	36.66±0.8	32.48±0.7	32.00±1.1

Table 2. *ANOVA on percentage of triploidy produced by cold shock (Larval stage)*

Source	Sum- of Squares	df	Mean-Square	F-ratio	P
Treatment	535.469	1	535.469	119.647	0.000
Duration	116.439	1	116.439	26.017	0.001
Treatment & duration	173.280	1	173.280	38.718	0.000
Error	35.803	8	4.475		

Table 3. *Relative efficiency of various temperatures and duration of heat shock for induction of triploidy in oysters*

Temperature	Percentage of triploids (first day larvae)		Percentage of triploids ('D' shaped larvae)	
	Duration of treatment		Duration of treatment	
	10'	5'	10'	5'
35°C	41.18±0.8	40.44±2.3	34.44±0.7	30.84±0.6
37°C	41.85±2.0	42.00±1.2	38.82±1.5	39.13±1.5
39°C	40.75±0.83	41.38±0.4	38.00±0.6	38.18±0.5

Table 4. *ANOVA on percentage of triploidy produced by heat shock (D stage)*

Source	Sum- of Squares	df	Mean-Square	F-ratio	P
Treatment	141.236	2	70.618	22.379	0.000
Duration	4.836	1	4.836	1.533	0.239
Treatment& duration	14.797	2	7.398	2.345	0.138
Error	37.867	12	3.156		

Table 5. *Effect of optimum temperature treatment for induction of triploidy in* C.madrasensis

Treatment	Triploid%		Mortality %
	Larval	'D' stage	(5th day)
Heat (37°C)	40.92	39.13	13.20
Cold (5°C)	42.25	33.33	16.08
Control	0	0	10.20

may be attributed to the lower treatment temperatures (0-1°C) used than 5°C attempted in this study.

Quillet and Panelay (1986) reported triploid yields of 25–45% after heat shock at 35 and 38°C in *C. gigas*. The wide range of triploidy rates may result from treatment being given 10-40 minutes after fertilization. Quillet and Panelay (1986) demonstrated that in *C.gigas* triploids could be induced by thermal shock without significant effects on early (24 hr) survival. Similarly, 24 hr survival was not significantly reduced by heat shock treatments below 38°C in *M.edulis* (Yamamoto and Sugawara, 1988).

Temperature shock will be very suitable for general commercial use because of the absence of the need for special chemicals and devices. Amongst other authors who have used temperature shock to induce triploidy in bivalves, Quilet and Panelay (1986) reported an average of around 20% triploidy using a short (10 minutes elevated temperature shock on the eggs of *C.gigas* whereas Gosling and Nolan (1989) reported 23-56% triploidy induction in *Tridacna semidecussatus* using 28-35°C. Such results are close to the data presented here and give some support to our low estimates for potential triploidy induction in *C.madrasensis*. According to Quilet and Panelay (1986) and Yamamoto and Sugawara (1988) more triploids might be produced by increasing duration of the temperature shock. But in our case increasing duration of thermal shock reduced the triploid percentage.

The survivability of the treated larvae was generally lower than the untreated controls but cold shock gave higher survivability of larvae than heat treatments. The reason for the increased survivability of cold shock treated larvae may be the lower percentage of triploids compared to the diploids that survive better.

It is very important to induce triploid animals at low cost and at high efficiency as near as 100% as possible for the commercial culture of marine bivalves. Thermal shock may affect the cytoskelton like microtubule as inferred from Inoue's dynamic equilibrium model (Inoue *et al.*, 1975). So regular factors of assembly-disassembly of microtubules (eg: Ca^{2+}) might be hopeful. Improvement of triploidy inducing efficiency by thermal shock may also be able to be accomplished by the synchronization of the development of zygotes depending on the incubation temperature. Inducing triploidy seems effective in preventing sexual maturation and such sterility effects in bivalves will be very useful in aquaculture.

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