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Performance of triploid edible oyster *Crassostrea madrasensis* (Preston): gonad development and biochemical composition

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Abstract

One of the most important parameters for successful commercial exploitation of bivalves is a need for fast growth. The potential for increased growth, meat yield and quality are the main advantages of triploid oysters. Triploid organisms have a limited capacity to develop gonads and thus are considered sterile or partially sterile and are useful and advantageous in shellfish aquaculture. Oysters occur all along the Indian coasts in backwaters, bays and estuaries. The edible oyster *Crassostrea madrasensis* is a commercially important bivalve distributed all along the east and west coasts of India and the edible oyster farming is becoming increasingly popular. *C. madrasensis* is a suitable species for culture because of faster growth rate and tolerance to wide range of salinity. The present study compares triploid and diploid edible oysters (*C. madrasensis)* of the same age, grown under similar conditions for one year to determine the accumulation of biochemical reserves at first maturation. Triploid edible oysters were produced by treating newly fertilized eggs with 6-Dimethyl amino purine (6-DMAP). Stunted growth of gonads was seen in triploids while in diploids both ovaries and testis were functional. Higher levels of total carbohydrate (including glycogen 20.25% in meiotic I triploids and 18.78% in meiotic II triploids over 12.95% in diploids) has a major role in increasing the superior performance of triploids. Meat quality is also expected to be higher in triploids.

Keywords: *Crassostrea madrasensis*, meiotic I and II triploids, gonad, protein, glycogen, lipid

Introduction

Induced triploidy is an effective tool to uncouple the related phenomena of reproductive effort, productivity and growth. Triploid organisms have limited capacity to mature and in most species where triploidy is attained individuals do not produce gametes or viable larvae and are considered sterile. An advantage of sterile organisms is that they transfer less biochemical energy to the gonad, so the other tissues can gain more weight over time.For this reason, triploidy has been induced in oysters (Tabarini, 1984; Beaumont, 1986; Komaru and Wada, 1989; Ruiz-verdugo *et al*., 2000; Yang *et al*., 2000; Maldonado-Amparo *et al*., 2004) and other molluscs of commercial interest (Guo and Allen 1994; Utting *et al*., 1996), caused by irregular synapses of chromosomes during early stages of meiosis. It has been postulated that beneficial effects of triploidy emanates from reproductive sterility. Since sterile animals do not utilize energy for reproductive activity (like diploids) it is conserved in the body. There is evidence that the reproductive cycle of triploid molluscs is affected in terms of glycogen utilization (Tabarini, 1984; Allen and Downing 1986, 1990; Komaru and Wada, 1989; Beaumont and Fairbrother, 1991; Ruiz-Verdugo *et al.,* 2000; Yang *et al.,* 2000).

Stanley *et al.,* (1984) and Allen and Downing (1986) observed inhibition of gametogenesis and significantly higher levels of the carbohydrates in triploid *Crassostrea gigas* compared to th diploids at the maturation peak, whereas triploids showed

significantly higher levels of carbohydrates. Akashige (1990) found twice the amount of glycogen in triploids than in diploids of *Crassostrea gigas.* For the clam *Tapes philippinarum*, higher carbohydrate content has been reported in the meat of triploids than of diploids (Utting *et al.,* 1996).

Nell *et al.,* (1994) compared the performance of triploids and diploids in Sydney rock oysters and recorded that triploid oysters had higher glycogen content than diploid siblings of *Saccostrea commercialis*. Tabarini (1984) established a link between partial sterility in triploid *Argopecten irradiens* and glycogen utilization and growth. Gonadal indices of triploid *A. irradiens* were consistently lower and correspondingly the glycogen content was consistently higher than in diploids.

Indian edible oyster *Crassostrea madrasensis* (Preston) being low in saturated lipids and high in glycogen and halogens are valuable as human food. Seasonal changes in the meat weight and proximate composition of an animal are associated with the reproductive cycle. Gametogenesis in bivalves represents a phase of particularly high demands of energy, when the transfer of proteins, lipids and carbohydrates from the adductor muscle and digestive gland to the gonad takes place. Energy is stored as lipid, protein and glycogen before the beginning of gametogenesis, to be used later for the maturation of gametes when energy demand is high (Gabbot, 1975; Barber and Blake, 1991; Shpigel *et al.,* 1992)

In this study gonadal condition, protein, fat and glycogen contents of two types of triploids (meiotic I and meiotic II) were analyzed and compared with diploids at quarterly interval for one year.

Material and methods

Oysters used in this study were produced as part of a study by us on inducing triploidy using the chemical 6-DMAP. The fertilized eggs were incubated with 6-DMAP (100 µM) for 8-min. duration for inducing meiotic I and meiotic II triploids. Untreated fertilized diploid eggs were reared as control. After hatchery phase of three weeks, the young oysters from three groups were reared in Tuticorin Bay for one year (November 2001 – October 2002) in the same condition. At three-month interval samples for biochemical analysis were taken from three groups of oysters after measuring the morphological traits (Mallia *et al.,* 2006). The triploidy was confirmed by chromosome counting in gill cells, by metaphase-spread preparation (Thomas *et al.,* 2004). Gonadal development was evaluated microscopically during the experiment. The samples were dried in an oven at 60ºC for 48 hrs. The dried samples were powdered in a mortar, transferred to a labeled polythene sachet and stored in a desiccator for further analysis.

Total carbohydrates: Phenol-sulphuric acid method of Dubois *et al.* (1956) was followed to estimate the total carbohydrate in the sample with D-glucose as the standard. The optical density of the colour developed was measured by UV/VS spectrophotometer GBC 110 with the samples taken in silica cuvette. The concentration (conc.) of glucose in the samples was calculated (in mg%) by comparing the optical density (OD) obtained for the sample with values using the formula:

$$\text{Concentration in mg}^{100\ mg}\ \text{dry tissue} = \left(\frac{\text{OD of the sample - O.D. of blank}}{\text{OD of the standard - OD of blank}}\right) \times \frac{\text{Conc. of standard}}{\text{OD of standard}}$$

Crude protein (protein): Nitrogen in the whole oyster was determined in 40 mg dried samples using micro-Kjeldhal technique and the values for nitrogen were multiplied by 6.25 to estimate the protein content.

Total lipids: Total lipid content was extracted following the method of Folch *et al.* (1956) using chloroform and methanol in the ratio 2:1and estimated gravimetrically.

All the values are expressed as percentage dry weight. Two-way ANOVA (SYSTAT, 7.0.1) was used to compare the biochemical composition of diploid and triploid groups at an interval of three months.

Results

Gonadal development: In January 2002 the triploids and diploids of *C. madrasensis* were inactive sexually and afterwards the diploids were identified

as male or female by the appearance of developing testis or ovaries. In July, the gonads of diploid oysters were densely packed. Female gonads were more easily discernible under microscope in a squash preparation. Ovaries showed numerous translucent and oval primary oocytes. By the end of the experiment (October 2002), the male gonads were cream-coloured and female gonads yellowish with several polygonal or suboval ripe oocytes and the gametes could be easily separated when punctured. On the contrary both meiotic I and meiotic II triploids never exhibited proper gonadal development. During June-July, traces of gonads were observed in the two triploid groups but the development never progressed and the squash preparation of gonad did not exhibit functional gametes. By the end of the experiment in the triploid groups, the gonads were fully shrunken and non-functional (Table 1). The triploid gonads were classified as immature or undifferentiated sex as only shrunken ovary was seen under microscope.

Total carbohydrates: Carbohydrate content showed wide differences between three groups in October. Higher levels of total carbohydrate (including glycogen; 20.25% in meiotic I triploids and 18.78% in meiotic II triploids over 12.95% in diploids) has a major role in increasing the performance of triploids (Table 2). ANOVA on the differences of carbohydrate content of diploid and triploids indicated high significance ($p<0.005$) (Table 3).

Table 1. Gonadal development of diploid and triploids of *C. madrasensis*

	January 02	April 02	July 02	October 02
Meiotic I (3n)	Not distinct	Not distinct	Rudimentary gonads	Non functional shrunken gonads
Meiotic II (3n)				
Diploid (2n)	Not distinct	Immature	Mature ovary & testis	Ripe gonads

Table 2. Biochemical composition (%) of diploid and triploids of *C.madrasensis* (Mean ± SE)

	January 02	April 02	July 02	October 02
Carbohydrate				
Meiotic I (3n)	13.25±0.23	15.65±0.21	16.50±0.07	20.25±0.63
Meiotic II (3n)	12.650.05	13.55±0.05	15.75±0.18	18.78±0.15
Diploid(2n)	12.65±0.07	12.76±0.11	14.28±0.18	12.95±0.03
Protein				
Meiotic I (3n)	78.50±0.09	77.1±0.12	76.00±0.10	72.15±0.10
Meiotic II (3n)	79.13±0.16	77.36±0.06	76.6±0.17	72.05±0.09
Diploid(2n)	78.82±0.09	78.51±0.04	77.85±0.04	78.42±0.05
Lipid				
Meiotic I (3n)	7.25±0.08	8.39±0.10	8.66±0.07	8.75±0.08
Meiotic II (3n)	7.24±0.08	8.36±0.22	8.46±0.19	8.55±0.21
Diploid(2n)	7.62±0.09	8.25±0.08	8.15±0.17	7.70±0.11

Table 3. ANOVA on biochemical composition

Parameters	Source of variation	Degree of freedom	Mean-square	F-ratio	Remarks
Carbohydrate	Meiotic I & Diploid	1	210.828	763.220	$p<0.005$
	Meiotic II & Diploid	1	70.538	314.944	$p<0.005$
	Meiotic I & Meiotic II	1	37.47	176.862	$p<0.005$
Protein	Meiotic I & Diploid	1	121.377	1617.27	$p<0.005$
	Meiotic II & Diploid	1	89.274	876.471	$p<0.005$
	Meiotic I & Meiotic II	1	2.461	17.525	$p<0.005$
Lipid	Meiotic I & Diploid	1	2.090	16.257	$p<0.005$
	Meiotic II & Diploid	1	0.918	3.420	$p<0.01$
	Meiotic I & Meiotic II	1	0.238	1.090	$p>0.01$

Crude protein: The protein content of diploid varied from 78.82% in January to 78.42% in October (Table 2). A high level of significance in protein was observed between meiotic I and diploid, meiotic II and diploid and between I and II meiotic triploid ($p< 0.005$) (Table 3).

***Total lipids*:** Higher values of lipid (8.75% in meiotic I triploid and 8.55% in meiotic II triploid over diploids (7.70%)) adds texture and flavour of triploids (Table 2). Statistical analysis gives a significant difference between meiotic I and diploid ($p<0.005$). But there is no significant difference between the two triploids ($p>0.01$) (Table 3).

Discussion

An advantage of producing triploid molluscs is that the individuals grow faster (Allen and Downing, 1986; Hawkins *et al.,* 1994; Hand *et al.,* 1998; Kesarcodi-Watson *et al.,* 2001). Several hypothesis have been proposed to explain the faster growth rate of triploids over diploids (Garnier-Gere *et al.,* 2002). The energy allocation hypothesis is based on the sterility or partial sterility of triploid molluscs and is characterized by triploids growing faster because energy is diverted from reproduction to growth (Stanley *et al.,* 1984; Allen and Downing, 1986; Akashige, 1990; Barber and Mann, 1991; Shpigel *et al.,* 1992; Hawkins *et al.,* 1994; Hand *et al.,* 1998). Differences in growth are not usually detected until the organisms reach first sexual maturity or until after first spawn (Stanley *et al.,* 1984; Tabarini, 1984; Barber and Mann, 1991; Beaumont and Fairbrother, 1991; Hand *et al.,* 1998; Ruiz-Verdugo *et al.,* 2001). Eversole *et al.* (1996) concluded that the amount of energy diverted into growth rather than into reproduction may account for the difference in size between diploids and triploids. In the present observation traces of gonads appeared in triploid individuals 6 months after culture but the development never proceeded as in other triploids that can be interpreted as energy loss for growth. In triploid scallop Ruiz-verdugo *et al*. (2000) indicated few or no eggs/sperms in a larger gonadal sac as in diploid individual. Allen *et al*. (1986) opined that varying DNA content and varying dosages of genetic information resulting from random assortment of one or more chromosomes as the reason for non-functional gonads in triploid fish. They also pointed out that even though triploid individuals initiate gametogenesis, it is not completed.

The decrease of carbohydrate in October in diploids might be due to secondary spawning during August-September. According to Ruiz-Verdugo *et al.* (2000), differences in carbohydrate between triploids and diploids are accompanied by mobilization of particular reserves to sustain reproduction. In the present study, the link between carbohydrate metabolism and gametogenesis is demonstrated by comparing the carbohydrate values of diploid and triploid in October 2002. Tabarini (1984) reported that triploid *Argopecten irradiens* had consistently lower gonadal indices than diploids and as a result they have higher carbohydrate content. Shpigel *et al.* (1992) attributed greater carbohydrate and protein contents in triploids because of less gamete production.

The lipid content differed significantly between meiotic I triploid and diploid. Ruiz-Verdugo *et al.* (2001) opined that higher values of lipid in the triploid *Argopecten ventricosus* might be due to failure of ovarian development and vitellogenesis. The higher lipid levels in meiotic I and meiotic II triploids in *C. madrasensis* can also be due to the failure of gonadal development. According to Barber and Blake (1991) during gametogenesis, several biochemical components are accumulated in the gonads, providing the structural and energetic material for oocyte development. However, in triploid individuals of most molluscs, gonad development is impaired as shown by reduced gonad indices (Komaru and Wada, 1989) or gonads exhibit total or partial sterility (Allen *et al.,* 1986; Allen and Downing, 1986; Komaru and Wada, 1989; Guo and Allen, 1994; Cox *et al.,* 1996; Eversole *et al.,* 1996). Hence mobilization of energetic components is also expected to be impaired thus showing high levels of biochemical parameters in the body. It was also pointed out that the environmental conditions in which organisms were reared before the estimation also influenced the lipid content (Ruiz-Verdugo *et al*., 2001). However, by rearing 3 groups in the same environment, greater lipid values observed in both meiotic I and meiotic II triploids than diploid in the

present study may be due to triploid effect *i.e*., mobilization of energetic components by partial or total sterility (Allen *et al.,* 1986; Allen and Downing, 1986; Komaru and Wada, 1989).

Comparison between two groups of triploids and diploid provides evidence that biochemical composition of diploid and triploid oysters is quite different. Beaumont and Fairbrother (1991) reported that faster growth of the triploid oyster in comparison to the diploid siblings was probably due to energy saving achieved by the reduced gonad development in the triploids. In normal diploids during oogenesis, primary oogonia undergo repeated mitosis to give secondary oogonia that enter meiosis I which is arrested at prophase I. This follows the growth phase during which oocytes undergo a period of vitellogenesis, which involves accumulation of mainly lipid globules and glycogen. Triploids are generally sterile and hence ovarian recrudescence does not take place normally. The metabolic energy otherwise utilized for gonadal development will be available for increased somatic growth, thereby resulting in larger animals.

The meiotic I and meiotic II triploids of *C. madrasensis* in the present study showed significant differences in biochemical composition with meiotic I triploids exhibiting higher values for carbohydrates and lipids indicating they are superior in meat content. Hawkins *et al.* (1994) showed that faster growth of meiotic I triploids compared to meiotic II triploids and diploids resulted from reduced energy expenditure associated with lower concentrations of RNA per unit of total tissue protein, indicating reduced rates of protein turnover and continuous degradation and renewal or replacement of cellular protein. The physiological basis to the relationship is that increased heterozygosity enables the triploid individual to sustain its basal metabolism with lower expenditure of energy (Hawkins *et al.,* 1994). The saving in energy may be redirected to fuel other functions such as somatic growth in *C. madrasensis* as evidenced by the fast growth rate in triploid individuals. In conclusion triploid sterility leading to increases in carbohydrates may result in lower energy allocation to gonad development in *C. madrasensis.*

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