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Performance of triploid Pacific Oysters *Crassostrea gigas* (Thunberg) reared in high carrying capacity ecosystem: survival, growth and proximate biochemical composition

Philippe GOULLETQUER [1], Jean-Pierre JOLY [1], André GÉRARD [2] Eric LE GAGNEUR [1], Jacques MORICEAU [1], Jean-Marie PEIGNON [2], Serge HEURTEBISE [2] and Pascal PHELIPOT [2]*

RÉSUMÉ

Performances de croissance d'huîtres creuses triploïdes *Crassostrea gigas* (Thunberg) élevées dans un système à capacité trophique élevée: taux de survie, croissance et composition biochimique

Des huîtres creuses triploïdes *Crassostrea gigas* ont été produites en 1990 au moyen d'un traitement des oeufs fertilisés à la cytochalasine B (CB). Ces lots d'huîtres triploïdes, ainsi que des huîtres traitées mais restées diploïdes, et des diploïdes témoins (non traités) de la même origine ont été mis en élevage dans la Baie des Veys qui représente un écosystème à capacité trophique élevée (Côte est Cotentin, Normandie). Un suivi mensuel des différents lots en 1992 a montré des performances de croissance et un engraissement supérieurs chez les triploïdes par rapport aux diploïdes avec cependant une hétérogénéité de croissance également supérieure. A la fin du cycle d'élevage de 26 mois, aucune fréquence de distribution bimodale, pouvant résulter d'une différence entre triploïdes obtenus en méiose I et II, des variables 'taille' et 'poids' n'a pu être observés. Les huîtres triploïdes ont présenté une gamétogénèse retardée. Les teneurs élevées en sucres chez les huîtres triploïdes ont présenté une faible variabilité autour de 40% de juin à septembre. Leurs taux de survie ont été inférieurs à ceux de la population témoin diploïde. Par ailleurs, les huîtres réfractaires au traitement à la cytochalasine B, et donc restées diploïdes, ont été plus sensibles aux facteurs de stress environnementaux indiquant ainsi un effet à long terme du traitement. Par conséquent, il apparaît important d'améliorer la méthode d'induction afin de maximiser le taux de triploïdie et de survie à long terme. A partir des informations obtenues, il apparait important de compléter les tests in-situ dans des écosystèmes pouvant présenter des facteurs de stress plus importants, en particulier dans des milieux à faible capacité trophique.

ABSTRACT

Triploid oysters *Crassostrea gigas* were produced in 1990 by treating fertilized eggs with cytochalasin B (CB). Triploids, treated diploids, and controls were deployed early 1991 in a high carrying capacity ecosystem on the Eastern Coast of Normandy (France). A monthly monitoring in 1992 showed that triploids yielded significantly higher growth rate and biochemical composition. However, growth was more heterogeneous. No evidence was found for a length or weight bimodal frequency distribution within triploid groups after a 26 months rearing cycle. Triploids showed retardation of gametogenesis. Carbohydrates content in triploids remained almost constant (40%) from June to September. Their survival rates were significantly lower than controls. Moreover treated diploids were found more sensitive to environmental conditions than controls, demonstrating long term CB effect on oysters. Therefore, the method of induction should be improved to maximize triploidy and long term survival rates. We recommend further field testings to assess triploid response to stressful environmental conditions, particularly in low carrying capacity ecosystems.

1 IFREMER RA, B.P. 32, F-14520 Port-en-Bessin (France).
2 IFREMER/GAP/URGE, B.P. 133, F-17390 La Tremblade.
* Present address : IFREMER/GAP/URAPC, B.P. 133, F-17390 La Tremblade.

INTRODUCTION

Extensive literature is now available regarding ploidy manipulation in molluscan shellfish (for review, see Beaumont and Fairbrother, 1991). Triploidy has been studied in several species including the Pacific oyster *C. gigas* and the Eastern oyster *C. virginica* (Stanley *et al.*, 1981). Ploidy manipulation is usually considered as a method for enhancing production. However, few studies analyzed oyster production over a full rearing cycle, and most of them have focused on yearlings (Allen and Downing, 1986; Akashige and Fushimi, 1992). Since oyster reproduction effort increases with age and is affected by triploidy, it seems of particular interest to study their production over the entire rearing cycle. Moreover, only few studies considered environmental constraints on genetically manipulated species (Shpigel *et al.*, 1992). Therefore, triploid oyster production is almost unpredictable prior to field testing.

Oyster culture is widely practised along the French coastline, from the English Channel (Normandy) to Mediterranean lagoons. Therefore, oysters face various environmental conditions affecting their physiological activity and growth rate. By way of example, natural spatfall occurs only in the southwest part of France. Before full-scale culture using genetically manipulated shellfish, it appears critical to test their physiological capacity and survival rate in various ecosystems. Besides survival rate, and from a management point of view, cohort homogeneity is also of particular interest for oyster farmers.

The present study was undertaken to determine (1) productivity of triploid oysters at two levels of stocking density in a high carrying capacity ecosystem, and (2) a possible length or weight bimodal frequency distribution resulting from triploids created during the meiosis I and meiosis II, and (3) the effect of the biochemical treatment on diploid growth. The overall objective is to assess the commercial feasibility based upon triploid oyster production.

MATERIALS AND METHODS

Oysters used in this field study were produced as part of a comprehensive cytogenetic research program aiming to develop new methodologies and improve three species, the Manila clam *Tapes philippinarum*, the flat oyster *Ostrea edulis* and the Pacific cupped oyster *Crassostrea gigas* (Dufy and Diter, 1990; Desrosiers *et al.*, 1994; Gérard *et al.*, 1993).

C. gigas broodstock was conditioned at IFREMER's research hatchery facility (URGE), Ronce-les-Bains (France). Triploids and diploids were produced from the same mass spawn on July 5, 1990. Triploidy was induced by treating eggs at 25°C with 0.5 $mg.l^{-1}$ cytochalasin B (CB) dissolved in DMSO (1 $mg.l^{-1}$) for 20mm, beginning 15mm after fertilization. Methodology was derived from the protocol of Downing and Allen (1987). After settlement (day 25th), spat from the CB treated groups were assayed by image cytometry (Gérard *et al.*, 1991) and found to contain 66% triploids. Oysters were maintained in nursery and fed with mixed phytoplankton culture.

On March 19, 1991, oysters (triploids averaging 2.33 g and diploids 2.63 g) were randomly selected and equally deployed in oyster bags within the intertidal area of two sites located on the Eastern Coast of Normandy: Ste Marie du Mont (site 1) and Bays of Veys (site 2), shellfish cultured areas characterized by a low (1,500 metric tons) and high (10,000 tons) stocking density respectively (Kopp *et al.*, 1991; Jeanneret *et al.*, 1992) (Fig. 1). Moreover, this area is characterized by low summer temperature (T°=17°- 20°C) impeding intensive natural spatfall.

In 1992, experimental populations were sampled on a monthly basis. Length and total weight were measured individually on 30 animals to the nearest mm and 0.01 g respectively. Relative survival rate was estimated by counting dead animals. Oysters were shucked and a piece of gill removed for ploidy control by image cytometry (Gérard *et al.*, 1991). The remainder of each oyster was stored at -60°C, then freeze-dried 36 hours before weighing whole dry meat. Proximate biochemical composition was estimated using 10 mg of freeze dried homogenate. Lipids were extracted, purified and then analyzed according to Marsh and Weinstein (1966), and Bligh and Dyer (1959). Carbohydrates and glycogen analysis followed the procedure of Dubois *et al.* (1956).

Three groups were defined for data treatment based upon ploidy levels: triploids (3N), CB treated diploids (2N), and diploids (control).

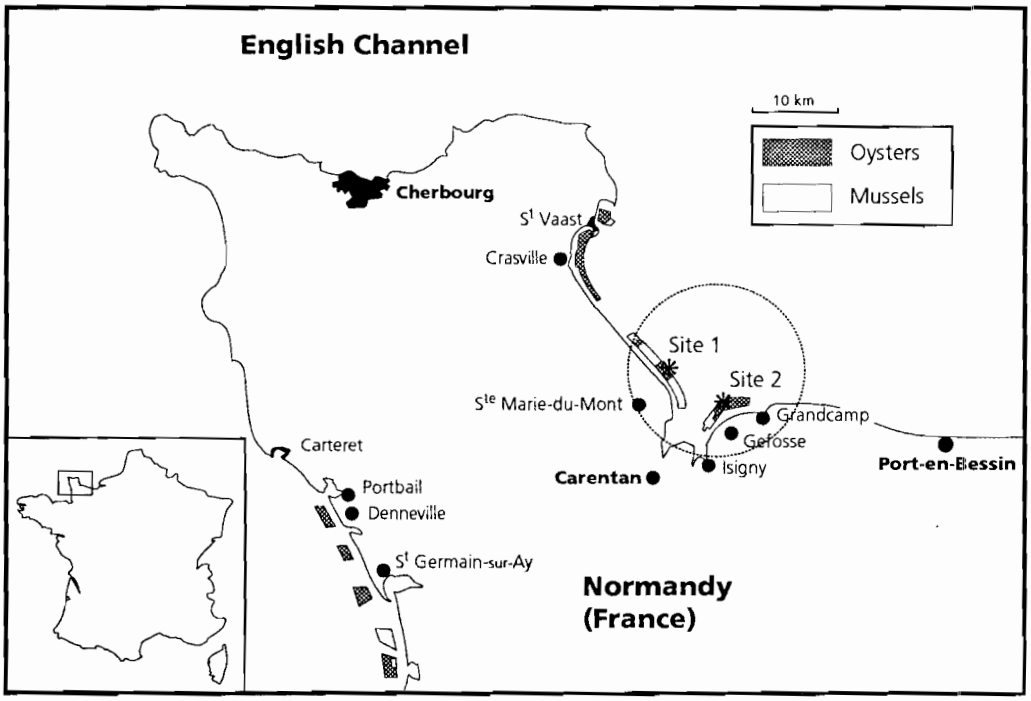

Figure 1. Geographic distribution of the experimental sites.

RESULTS

Survival

We estimated the proportion of triploids in the spat prior to field deployment; 66% of the sampled spat were triploid in 1990. After a 26 month rearing cycle, a sub-sample showed that 53.6% and 70% of treated animals were triploids on site 1 and 2 respectively. A Chi-square test was performed on data resulting from the 1992 monthly monitoring on site 1. There was no significant difference in triploid proportion over the course of the experiment (χ^2=3.42, d.f.=5, P>0.05). None of the 62 controls were triploid. Similar tests on the survival rate of the treated and controlled populations estimated by field counting showed significant differences (2 x 2 contingency χ^2=31.41, P<0.0001, site 1; χ^2=9.07, P<0.001, site 2). Survival rates of controls were significantly higher than treated populations (i.e., site 1: 68.9% vs 48.6%, site 2: 87.1% vs 73.8%).

Growth

Distribution fitting

Analysis of total weight frequencies did not reveal a bimodal distribution within the triploid group, in spite of the 26 months life span. Cumulative frequencies revealed distribution patterns among treated, controlled diploids and triploids (Fig. 2). The lowest slope coefficients were observed for both triploid groups, demonstrating growth heterogeneity. Negative Skewness and Kurtosis coefficients characterized treated diploids on both sites (i.e., site 1: -0.15, -1.41; site 2: -0.32, -1.35) and indicated a distribution trend toward a flat curve with short tails, and the lower tail longer than the upper. Controlled populations showed positive values for both coefficients (i.e., site 1: 0.45, 0.31; site 2: 0.39, 0.12) describing a steep distribution at the center and upper tail longer than the lower. Regarding triploids, the distribution tended to a flat curve with the upper

longer than the lower tail (i.e., site 1: 0.69, -0.41; site 2: 0.52, -0.30). However, assumption of normal distribution was still valid in all cases to allow parametric testing since standardized coefficients were within the range -2.0 to +2.0.

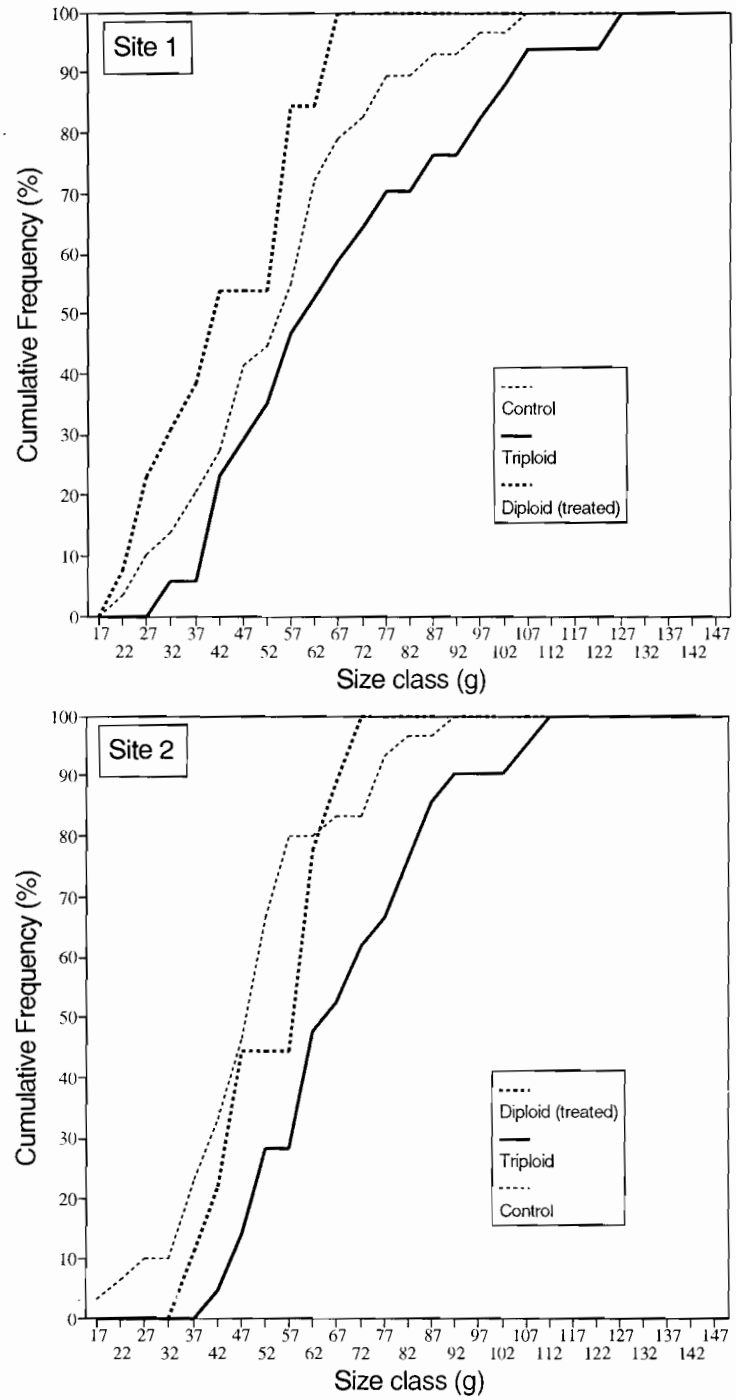

Figure 2. Cumulative oyster total weight frequency (%) for control, diploids, and triploids.

Site effect

No significant difference in length or total weight was noted between sites within each group. In contrast, ANCOVA for the dry meat weight with the oyster length as a covariate estimated significant difference for each group (i.e., triploids, $F=73.7$, $P<0.0001$; treated diploids, $F=5.7$, $P<0.05$; controls, $F=67.2$, $P<0.0001$). Overall growth performance on site 1 was significantly greater than those on site 2.

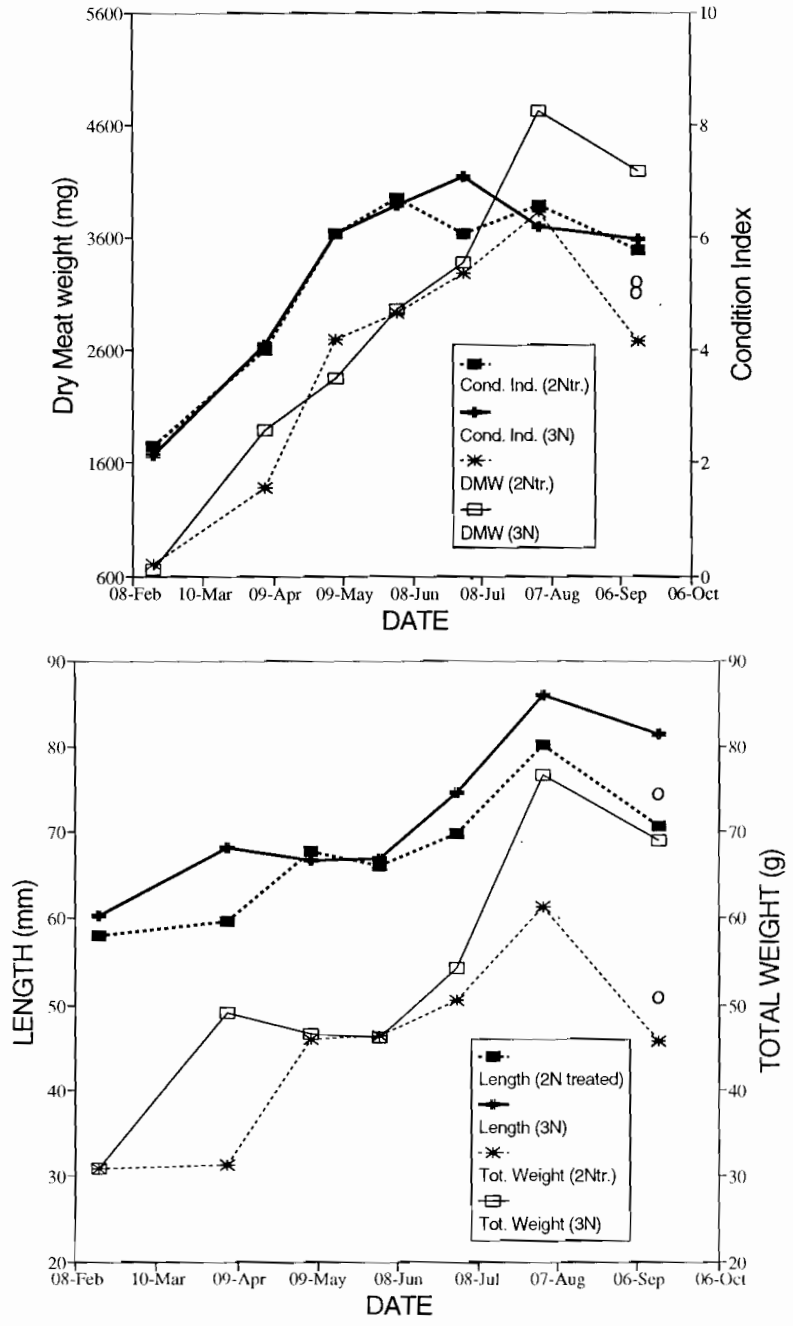

Figure 3. Comparison of mean oyster length (mm), total weight (g), dry meat weight (mg) (3A), and condition index (3B) between control (O), 2N treated, and 3N over the sampling period on site 1.

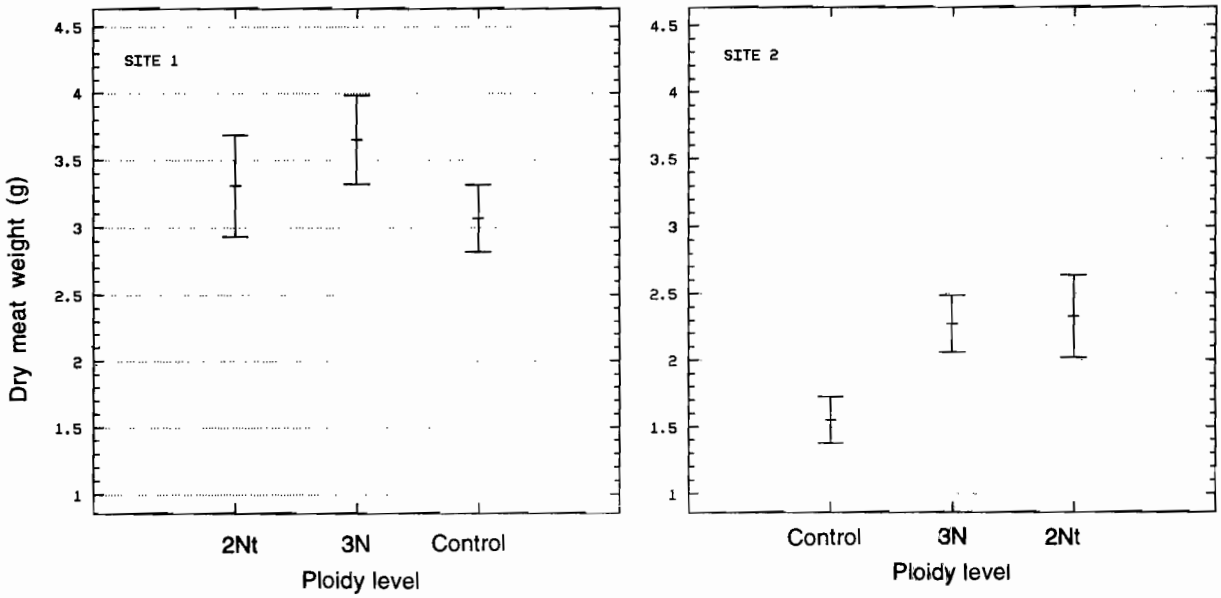

Figure 4. Oyster dry meat weight (g) comparison (ANCOVA results, mean ± SE) on september data on both sites. Ploidy level: 2Nt = treated diploids; 3N = triploids, and control). Oyster length is the covariate.

Ploidy effect

After completion of the rearing cycle, total oyster weight and length were significantly different among triploids, treated diploids and diploids for both sites (ANOVA: site 1, length, $F=3.5$, $P<0.05$; total weight, $F=4.4$, $P<0.02$; site 2, length, $F=6.35$, $P<0.01$; total weight, $F=7.57$, $P<0.002$). Monthly growth data on site 1 demonstrated that the main difference, favoring the triploids, occurred during the reproductive period in summer (Fig. 3).

From 18 February to 15 September 1992, overall increase in dry meat weight was 637% in triploids, and only 381% in treated diploids. Following spawning, which occurred at site 1 between 3 August and 15 September, dry meat weight decreased by 9.1%, and 14.4% of pre-spawn weight for treated diploids and diploids respectively. The reproductive effort for triploids reached 3.9% of pre-spawn weight for a standardized oyster (65 g total weight). Although initiated, triploid spawning was probably still in progress at the completion of our study. On site 2, spawning was retarded for triploids and treated diploids. Dry meat weight for controls declined by 42.3%. We should also note for both sites in September the slight length and weight decrease which might result from a selective mortality on large oysters during the reproductive activity.

Ploidy effect was analyzed on September dry meat weight data using ANCOVA with the oyster length as a covariate (Fig. 4). Significant difference was observed among ploidy level on site 2 ($F=8.3$, $P<0.001$). Since the spawning was delayed, multiple range analysis, based upon the Newman-Keuls method, classified treated diploids and triploids as a homogeneous group. In contrast, ploidy effect was not significant on site 1 ($F=2.0$, $P=0.14$). This resulted from the high post-spawning variability (mean±SD: triploid, 4.18±2.07; treated diploid 2.68 ±1.03, diploid 3.04 ±1.27g).

Proximate biochemical composition

Triploids, and diploids with treated diploids, showed different patterns of utilization of carbohydrates, glycogen and to a lower extent, lipids. Carbohydrate storage occurred until early July when it reached a record high of 42% for diploids and triploids (Fig. 5). Thereafter a steadily decline was noted for controls and treated diploids until pre-spawning (29.7%). In contrast, carbohydrates content for triploids remained constant around 40%. A similar pattern was noted for

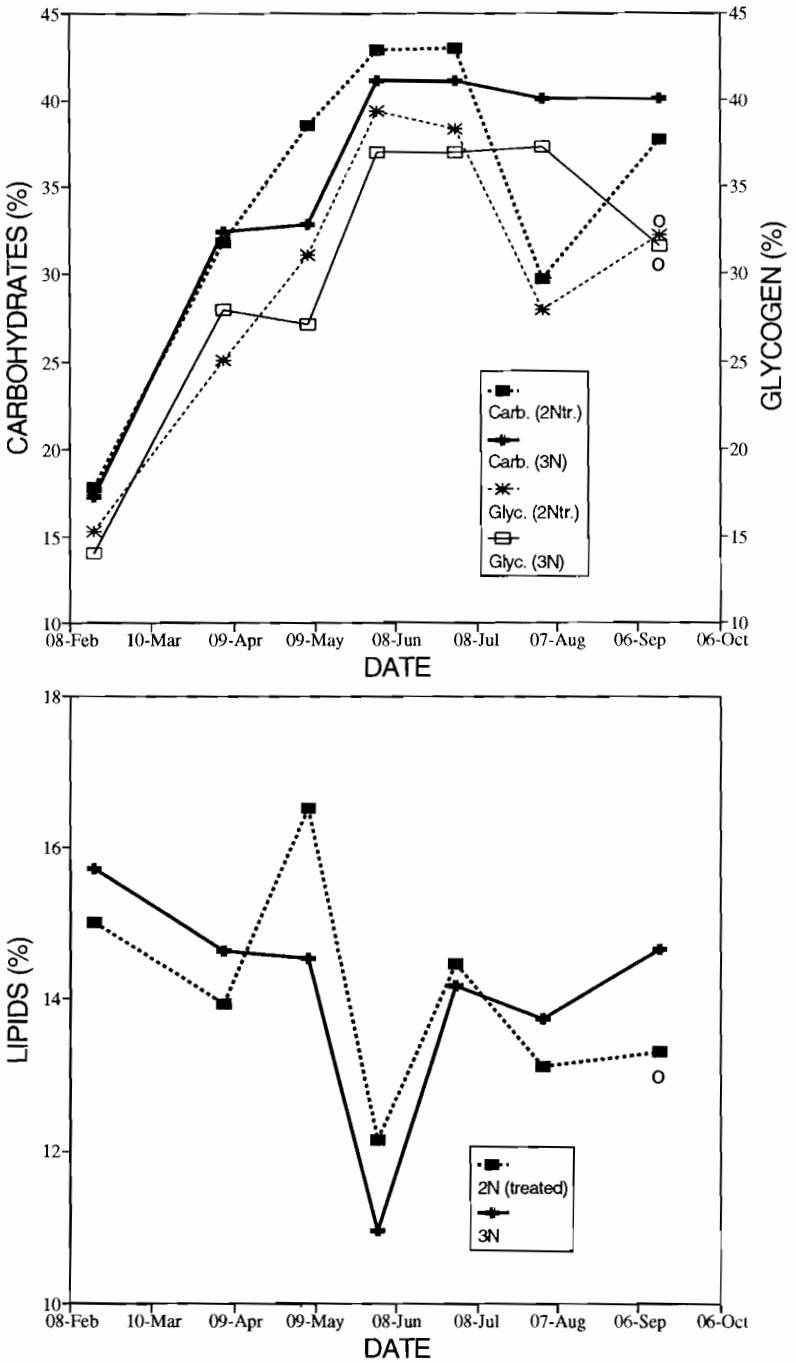

Figure 5. Comparison of oyster proximate biochemical composition: carbohydrates (%), glycogen (%) (5A), and lipids (%) (5B) over the sampling period (site 1). Control (O), 2N treated, and 3N.

glycogen until August. Then, glycogen declined slightly in September with a concomitant lipid increase, indicating gametogenesis activity. Carbohydrates and glycogen increased in diploids after spawning while lipids increased and carbohydrates decreased in triploids (Fig. 5).

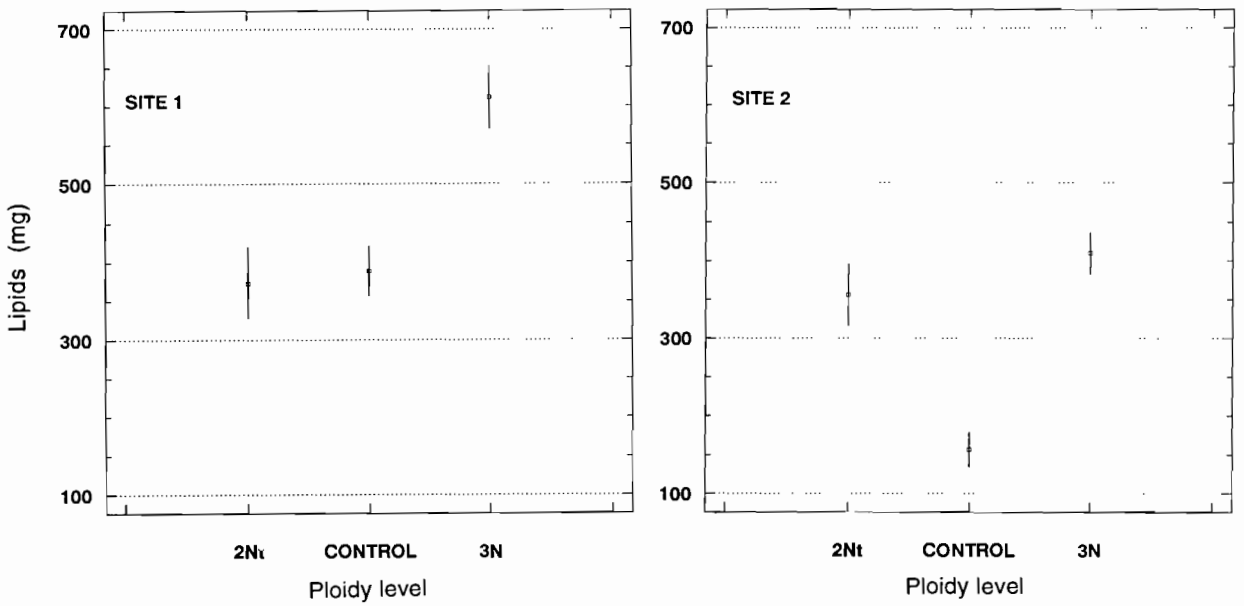

Figure 6. Comparison of lipids content (mg) (ANCOVA results, mean 95% confidence intervals) on september data on both sites. Ploidy level: 2Nt = treated diploids; 3N = triploids, and control. Oyster dry meat weight is the covariate.

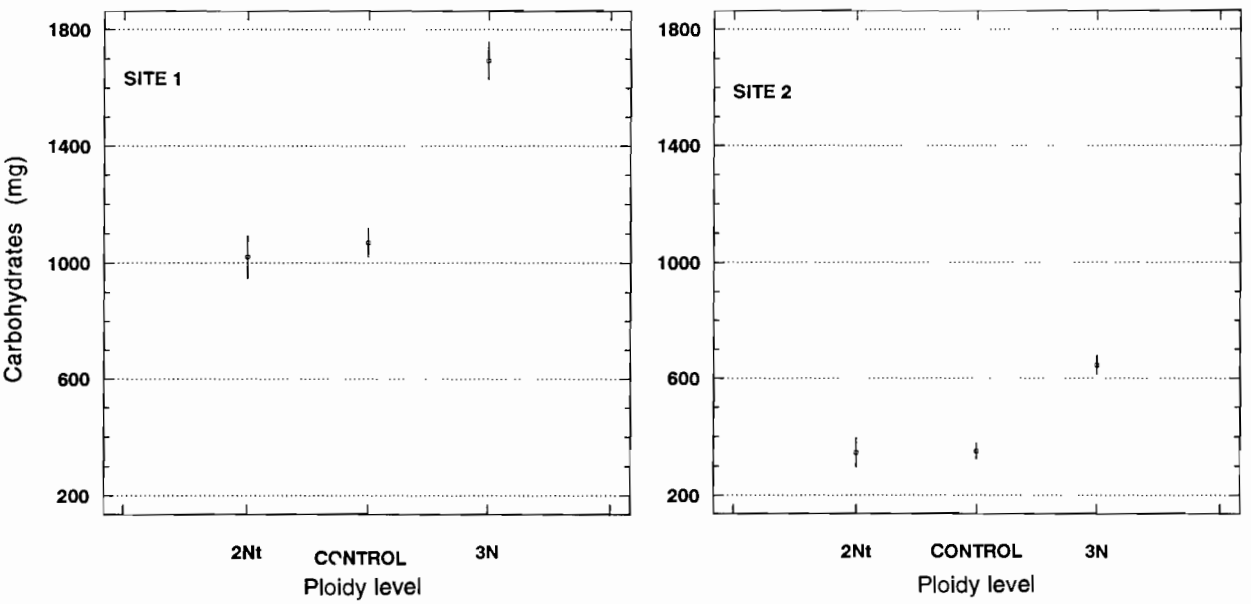

Figure 7. Comparison of carbohydrates content (mg) (ANCOVA results, mean 95% confidence intervals) on september data on both sites. Ploidy level: 2Nt = treated diploids; 3N = triploids, and control. Oyster dry meat weight is the covariate.

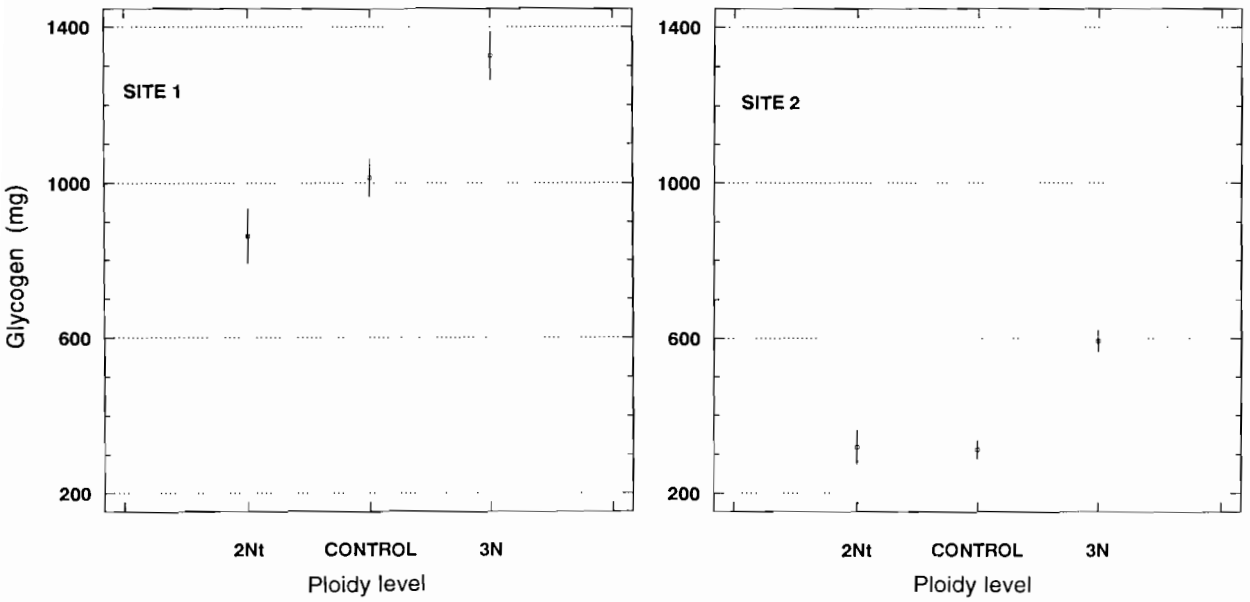

Figure 8. Comparison of glycogen content (mg) (ANCOVA results, mean 95% confidence intervals) on september data on both sites. Ploidy level: 2Nt = treated diploids; 3N = triploids, and control. Oyster dry meat weight is the covariate.

ANCOVAs with the dry meat weight as a covariate were performed on September data (Fig. 6,7,8). On site 1, significant differences were observed among ploidy groups regarding lipids ($F=3.25$, $P<0.05$) (Fig. 6) and carbohydrates ($F=8.1$, $P<0.001$) (Fig. 7). For both variables, treated diploids and controls were significantly lower than triploids. Since glycogen concentration increased in the post-spawning period in diploids and treated diploids, and triploids had the opposite trend, no significant difference was noted among groups ($F=1.75$, $P=0.18$). Similar ANCOVAs on site 2 were significant in all cases (lipids, $F=4.03$, $P<0.005$; carbohydrates, $F=38.1$, $P<0.0001$; glycogen, $F=40.5$, $P<0.0001$) (Fig. 6,7,8). However, since treated diploid spawning was delayed, average lipid content was homogeneous between treated diploids and triploids (i.e., 356.2 mg, 416.2 mg) and significantly higher than control mean (i.e., 156.7 mg). Similarly, carbohydrates and glycogen contents were significantly lower in diploids and treated diploids than triploids (351.5 mg, 346.5 mg, 645.2 mg; 311.6 mg, 318.2 mg, 593.1 mg respectively).

DISCUSSION

Allen and Downing (1986) have demonstrated an effective gametogenesis and spawning in triploids. Gonadal development in triploids was retarded and resulted in substantial differences in glycogen content during gametogenesis. In our study, triploids showed also a reproductive activity but neither carbohydrate and glycogen decline during summer. Gametogenesis for treated diploids and controls induced catabolism of energetic reserves, resulting in carbohydrates decline and concomitant lipids increase. The reduced reproductive activity in triploids limited this biochemical pathway (Mathieu and Lubet, 1993). Only a slight decline was noted in September. The particularly high food availability might be responsible for this dissimilarity since glycogen content is regulated by environmental conditions including temperature and food level (Gabbott, 1975). Temperature effects neurosecretory hormones, which control storage tissues and germinal cells (Gabbott, 1975; Lubet and Mathieu, 1978). Moreover, summer temperature in this ecosystem

varies around 17°-20°C, the critical lower limit for *C. gigas* successful spawning (Héral and Deslous-Paoli, 1991). However diploids did spawn. Therefore, we can suggest that triploids are either (1) under the same regulatory control as diploids but their physiological responses might be tied to a different temperature threshold, or (2) a feedback action by the maturing gonad effects the neuroendocrine control of storage tissues (Mathieu and Lubet, 1993).

We have shown that triploids were significantly larger than diploids and treated diploids by the end of the monitoring. This might be explained by the reduced energetic spending for gametogenesis, energy being preferentially used for growth (Allen and Downing, 1986). Reference to heterozygosity has also often been used to explain growth discrepancies (Koehn *et al.*, 1988). Triploid oysters created during the release of the first polar body (Meiosis I) are characterized by higher heterozygosity and would yield enhanced growth, even though this theoretical advantage has not yet convincingly been demonstrated (Stanley *et al.*, 1984, Yamamoto *et al.*, 1988). Li *et al.* (1992) and Jiang *et al.* (1993) on the pearl oyster *Pinctada martensii* and Mason *et al.* (1988) on *Mya arenaria* were unable to establish a positive correlation between triploid heterozygosity and growth.

In our study, triploids were created at both first and second polar releases (Meiosis I & II). CB treatments at first polar body formation may effect larval survival (Downing and Allen, 1987). Since no heterozygosity control was carried out on triploids, it seems difficult to conclude on any relationship. However no expected growth difference within the triploid groups was observed by the end of the experiment. Individual variability, selective mortality, or lacking effect of heterozygosity might be either responsible for this final distribution.

However, we have shown that greater size heterogeneity is a triploid characteristic. Hooker and Morse (1985) and Morse (1984) have suggested that extreme heterogeneity of sizes and growth rates observed in haliotids is not necessarily the result of genetic or nutritional variation, but is likely to reflect inadequate satisfied physiological requirements. By way of example, provision of exogenous peptide hormones reduced size heterogeneity (Morse, 1984). Therefore, overall triploidy results including glycogen metabolism, abnormal reproductivity and size heterogeneity, might be explained by abnormal functioning neurosecretory system, affected by the CB treatment .

Several studies have shown comparable survival rate between diploid controls and triploids (Stanley *et al.*, 1984; Allen and Downing, 1986; Chaiton and Allen, 1985). Allen and Downing (1986) observed higher survival during reproductive activity, and Akashige and Fushimi (1992) reported twice as much survival as control during the resting period. This study is an exception to these findings since treated oyster survivorship is significantly lower. Moreover, no long-term negative effects from cytochalasin B were found on shellfish growth (Downing and Allen, 1987). Since triploidy percentage remained constant during the experiment and survivorship of treated animals was significantly lower, we conclude that treated diploids are more sensitive to environmental conditions than controls. This demonstrates a long term CB effect on oysters. Even the recent evidence suggesting there may be some spontaneous chromosome loss in triploids as they age does not counteract these results (Allen, 1993). This is also of particular interest for hatchery production and prompts us to recommend optimization of the methodology to maximize the triploidy rate. Recently, new inductions yielded 95% of triploid using CB (Gérard *et al.*, 1994). Besides, the new chemical inducer 6-DMAP has been already successfully tested, yielding to higher larval survivorship and 85% of triploid (Desrosiers *et al.*, 1993).

The unexpected results of survivorship as well as the higher growth variability in triploids demonstrate the need for further testing in various ecosystems including low carrying capacity environments. This would allow better assessment of triploid sensitivity and performance to stressful environmental conditions, therefore allowing enhancement of oyster production.

REFERENCES

Akashige S. & Fushimi T., 1992. Growth, survival, and glycogen content of triploid Pacific oyster *Crassostrea gigas* in the waters of Hiroshima, Japan. *Nippon Suisan Gakkaishi* 58(6):1063-1071.

Allen S.K., 1993. Triploids for field tests? The good, the bad, and the ugly. *Journal of Shellfish Research* **12**(1): Abstract only.

Allen S.K. & Downing S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content, and sexual maturation in yearlings. *Journal of Experimental Marine Biology and Ecology*, **102**:197-208.

Beaumont A.R. & Fairbrother J.E., 1991. Ploidy manipulation in molluscan shellfish: a review. *Journal of Shellfish Research*, **10**(1):1-18.

Bligh E.G. & Dyer W.F., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**: 911-917.

Chaiton J.A. & Allen S.K., 1985. Early detection of triploidy in the larvae of the Pacific oyster *Crassostrea gigas* by flow cytometry. *Aquaculture*, **48**: 35-43.

Desrosiers R.R., Gérard A., Peignon J.M., Naciri Y., Dufresne L., Morasse J., Ledu C., Phelipot P., Guerrier P. & Dubé F., 1994. A novel method to produce triploids in bivalve molluscs by the use of 6-Dimethylaminopurine. *Journal of Experimental Marine Biology and Ecology*, **170**: 29-43.

Downing S.L. & Allen S.K., 1987. Induced triploidy in the Pacific oyster *Crassostrea gigas*: optimal treatments with cytochalasin B depend on temperature. *Aquaculture*, **61**:1-15.

Dubois F., Gilles A, Hamilton J.K., Rebecs P.A. & Smith F., 1956. Colorimetric method for determinaion of sugar and related substances. *Analytical Chemistry*, **28**: 350-356.

Dufy C. & Diter A., 1990. Polyploidy in the Manila clam *Ruditapes philippinarum*. 1. Chemical induction and larvae performance of triploids. *Aquatic Living Resources*, **3**: 55-60.

Gabbott P.A., 1975. Storage cycles in marine bivalves molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. *In*: Ninth European Marine Biology Symposium (H. Barnes ed.), Aberdeen, pp. 191-211.

Gérard A., Peignon J.M. & Chagot D., 1991. Contrôle de la ploïdie par imagerie numérique dans les expériences d'induction de la triploïdie chez les mollusques bivalves. ICES, Mariculture Committee\F:12,11p.

Gérard A. & 20 collaborators, 1993. Obtention de souches conchylicoles performantes par polyploïdisation (4ème partie). IFREMER RIDRV-93-022-RA\ La Tremblade, 55p.

Gérard A. & 25 collaborators, 1994. Obtention de souches conchylicoles performantes par polyploidisation (5ème partie). IFREMER RIDRV-94-17-RA\ La Tremblade, 78p.

Héral M. & Deslous-Paoli J.M., 1991. Oyster culture in European countries. *In*: "The culture of estuarine and marine bivalve mollusks". Edit. W. Menzel, CRC Press, New-York, pp. 153-190.

Hooker N. & Morse D.E., 1985. Abalone: The emerging development of commercial cultivation in the United States. *In*: Crustacean and mollusc aquaculture in the United States (J.V. Huner and E. Brown (Editors). AVI Publishers, Westport, pp. 365-413.

Jeanneret H., Kopp J., Joly J.P., Moriceau J. & Le Gagneur E., 1992. L'ostréiculture sur la côte Est-Cotentin. IFREMER RIDRV-92.010-RA\ Port en Bessin, 64p.

Jiang W.G., Li G., Xu G., Lin Y. & Wing N., 1993. Growth of the induced triploid pearl oyster, *Pinctada martensii* (D.). *Aquaculture*, **111**: 245-253.

Koehn R.K., Diehl W.J. & Scott T.M., 1988. The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulinia lateralis*. *Genetics*, **118**:121-130.

Kopp J., Joly J.P., Moriceau J., Le Gagneur E. & Jacqueline E., 1991. La conchyliculture en Baie des Veys. IFREMER DRV\RA:91p.

Li G., Jiang Z.G. & Shen W., 1992. Comparison of heterozygosity at three loci and growth between triploid and diploid pearl oysters, *Pinctada martensii* (D.). *Tropic Oceanology* (China), **11**(4): 84-88.

Lubet P.E. & Mathieu M., 1978. Experimental studies on the control of the annual reproductive cycle in the pelecypod molluscs *Mytilus edulis* (L.) and *Crassostrea gigas* (Th.). *General and Comparative Endocrinology*, **34**: 109p.

Marsh J.B. & Weinstein D.B., 1966. Simple charring method for determination of lipid. *Journal of Lipid Research*, **7**: 574-576.

Mason K.M., Shumway S.E., Allen S.K. & Hidu H., 1988. Induced triploidy in the soft-shelled clam *Mya arenaria*: energetic considerations. *Marine Biology*, **98**: 519-528.

Mathieu M. & Lubet P., 1993. Storage metabolism and reproduction in marine bivalves - a brief review. *Invertebrate Reproduction and Development*, **23** (2-3):123-129.

Morse D.E., 1984. Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. *Aquaculture*, **39**:263-282.

Shpigel M., Barber B.J. & Mann R., 1992. Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology*, **161**:15-25.

Stanley J.G., Allen S.K. & Hidu H., 1981. Ploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. *Aquaculture*, **23**:1-10.

Stanley J.G., Hidu H. & Allen S.K., 1984. Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. *Aquaculture* **37**:147-155.

Yamamoto S., Sugawara Y., Nomura T. & Oshino A., 1988. Induced triploidy in Pacific oyster *Crassostrea gigas*, and performance of triploid larvae. *Tohoku Journal of Agricultural Research*, **39**(1):47-59.