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PERFORMANCE OF TRIPLOID PACIFIC OYSTERS *CRASSOSTREA GIGAS* (Thunberg) REARED IN HIGH CARRYING CAPACITY ECOSYSTEM: SURVIVAL, GROWTH, AND PROXIMATE BIOCHEMICAL COMPOSITION.

by

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

Keywords: C. gigas, triploidy, survival rate, biochemical composition.

RÉSUMÉ: Une production d'huîtres japonaises triploïdes *C. gigas* a été réalisée en 1990 par induction à la cytochalasine B d'oeufs fertilisés. Triploïdes, diploïdes réfractaires et une population témoin ont été mis en élevage au début 1991 sur la côte Est de Normandie (France). Le suivi mensuel a montré une meilleure croissance et composition biochimique des triploïdes avec cependant une plus forte hétérogénéité en taille. Un seul mode a été observé après 26 mois d'élevage. Les triploïdes montrent un retard de gamétogénèse et une teneur constante en sucres de juin à septembre. Les taux de survie sont plus faibles chez les triploïdes, et les diploïdes traités sont plus sensibles que les témoins aux conditions environnementales. La technique d'induction doit être améliorée et la résistance des triploïdes testée en conditions stressantes.

Mots-clés: C. gigas, triploidie, survie, composition biochimique.

Introduction

Extensive literature is now available regarding ploidy manipulation in molluscan shellfish (for review, see Beaumont and Fairbrother, 1991). Triploidy has been studied on 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; Arashige 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 before field testing.

Oyster culture is widely distributed 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) the triploid oyster production at two levels of stocking density in a high carrying capacity ecosystem, and (2) a possible bimodal distribution resulting from triploids created during the meiosis I and meiosis II, and (3) the effect of the biochemical treatment on diploid. 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 characteristics, the Manila clam *Tapes philippinarum*, the flat oyster *Ostrea edulis* and the Pacific cupped oyster *Crassostrea gigas* (Dufy and Diter, 1990; Desrosiers et al., 1993; 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 0.5mg.l-1 cytochalasin B (CB) dissolved in DMSO (1mg.l-1) for 20mm, beginning 15mm after fertilization. Methodology was derived from Downing and Allen (1987) protocol. After settlement (day 25th), spat from the CB treated groups were assayed and found to contain 66% triploids. Oysters were maintained in nursery and fed with mixed phytoplankton culture.

On 19 March 1991, oysters (triploids averaging 2.33g and diploids 2.63g) were randomly selected and equally deployed on two sites located on the Eastern Coast of Normandy: Ste Marie du Mont (site 1) and Bay 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 0.01g 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 10mg 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).

Results

Survival

We estimated the proportion of triploids in the spat prior to field deployment; 60% of the spat sampled 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

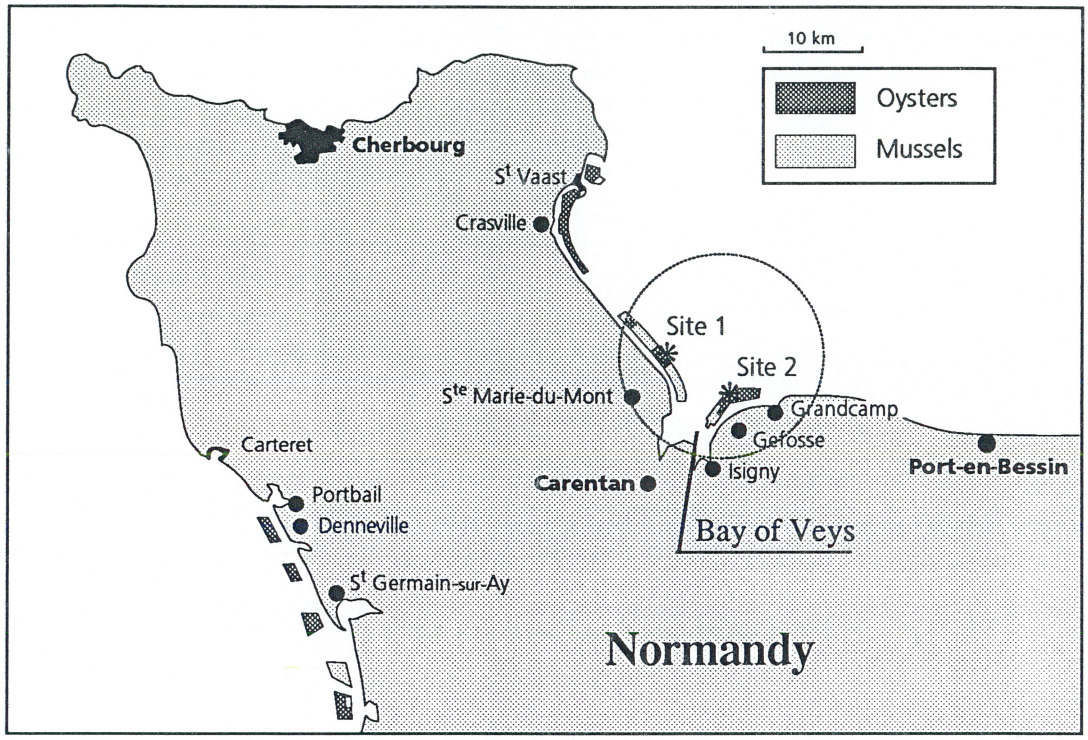

Fig. 1 : Geographic distribution.

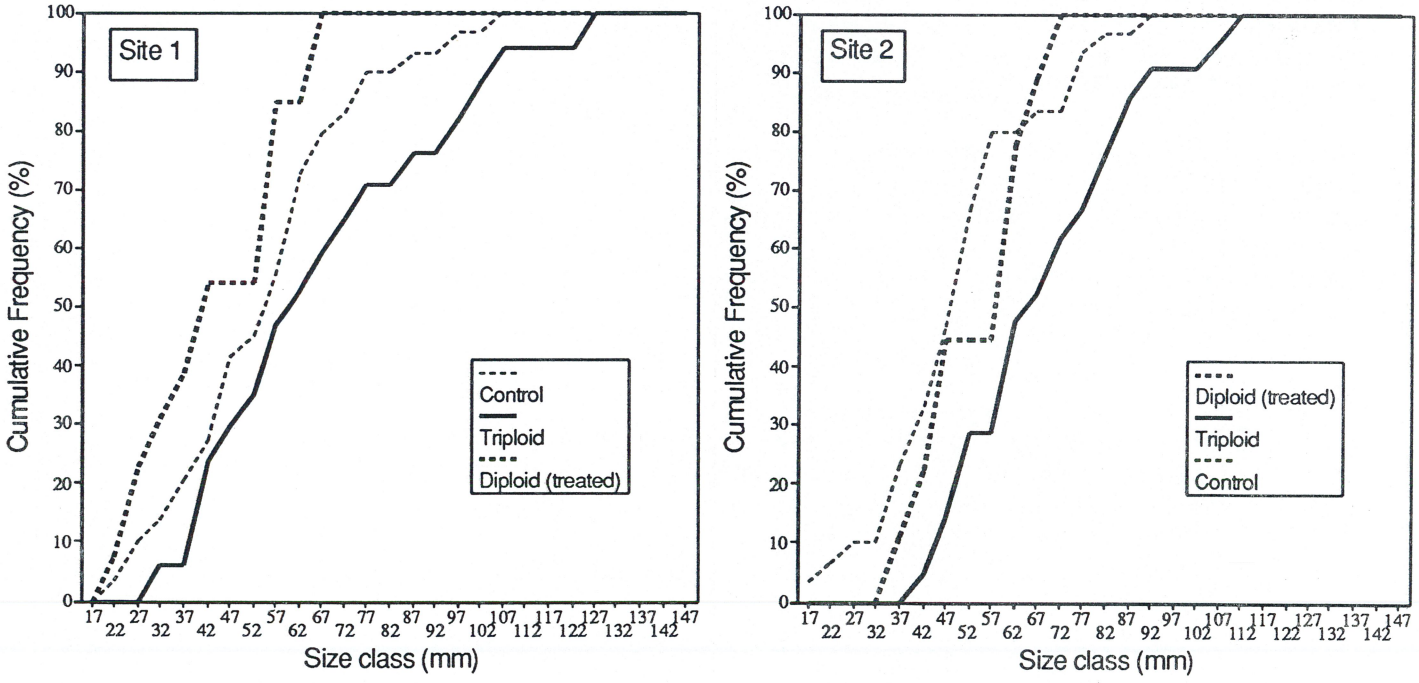

Fig. 2 : Cumulative frequency (sites 1 & 2).

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 allow describing a bimodal distribution within the triploid group, in spite of the 26 months life span. Cumulative frequencies described 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.

Site effect

No significant difference in length and total weight was noted between sites within each group. In contrast, ANCOVA for the dry meat weight with the total weight as a covariate estimated significant difference for each group (i.e., triploids, F=181.4, P<0.0001; treated diploids, F=26.9, P<0.0001; controls, F=165.0, P<0.0001). Overall growth performance on site 1 was significantly greater than those on site 2 (Fig. 5).

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, F=3.5, P<0.05; F=4.4, P<0.02; site 2, F=6.35, P<0.01, 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 on 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 (65g 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%.

Ploidy effect was analyzed on September dry meat weight data using ANCOVA with the total weight as a covariate. Significant difference was observed among ploidy level on site 2 (F=10.7, P<0.0001). Since the spawning was delayed, multiple range analysis classified treated diploids and triploids as a homogeneous group. In contrast, ploidy effect was not significant on site 1 (F=2.76, P=0.07). This was probably due to 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 and treated diploids showed different patterns of utilization of carbohydrates, glycogen and to a lower extent, lipids. Carbohydrates storage occurred until June when reaching a record high of 42% for diploids and triploids (Fig,4). 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 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 .

ANCOVAs with the dry meat weight as a covariate were performed on September data. On site 1, significant differences were observed among ploidy groups regarding lipids ($F=3.25$, $P<0.05$) and carbohydrates ($F=8.1$, $P<0.001$). For both variables, treated diploids and controls were significantly lower than triploids (Fig. 6 and 7). Since glycogen concentration increased in 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, $F=40.5$, $P<0.0001$). However, since treated diploid spawning was delayed, average lipid content was homogeneous between treated diploids and triploids (i.e., 356.2mg, 410.2mg) and significantly higher than control mean (i.e., 156.7mg). Similarly, carbohydrates and glycogen (Fig. 8) contents were significantly lower in diploids and treated diploids than triploids (351.5mg, 346.5mg, 645.2mg; 311.6mg, 318.2mg, 593.1mg 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. 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 assume that triploids are under the same regulatory control as diploids but their physiological responses might be tied to various thresholds.

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 preferentially used for growth (Allen and Downing, 1986). Reference to heterozygosity has also often been used to explain growth discrepancies. 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), 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 heterozygosity and growth. In our study, triploids were created at both first and second polar releases (Meiosis I & II). CB treatments at first 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.

Several studies have shown comparable survival rate between diploid controls and triploids (Stanley et al., 1984; Allen et al., 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 triploid survivorship is significantly lower. Moreover, no long-term negative effects from cytochalasin B were found on shellfish testing (Downing and Allen, 1987). Since triploidy percentage remained constant during the experiment and survivorship of treated animals lower, we conclude that treated diploids are more sensitive to environmental conditions. 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 prompt us to recommend to optimize the methodology to maximize the triploidy rate. Recently,

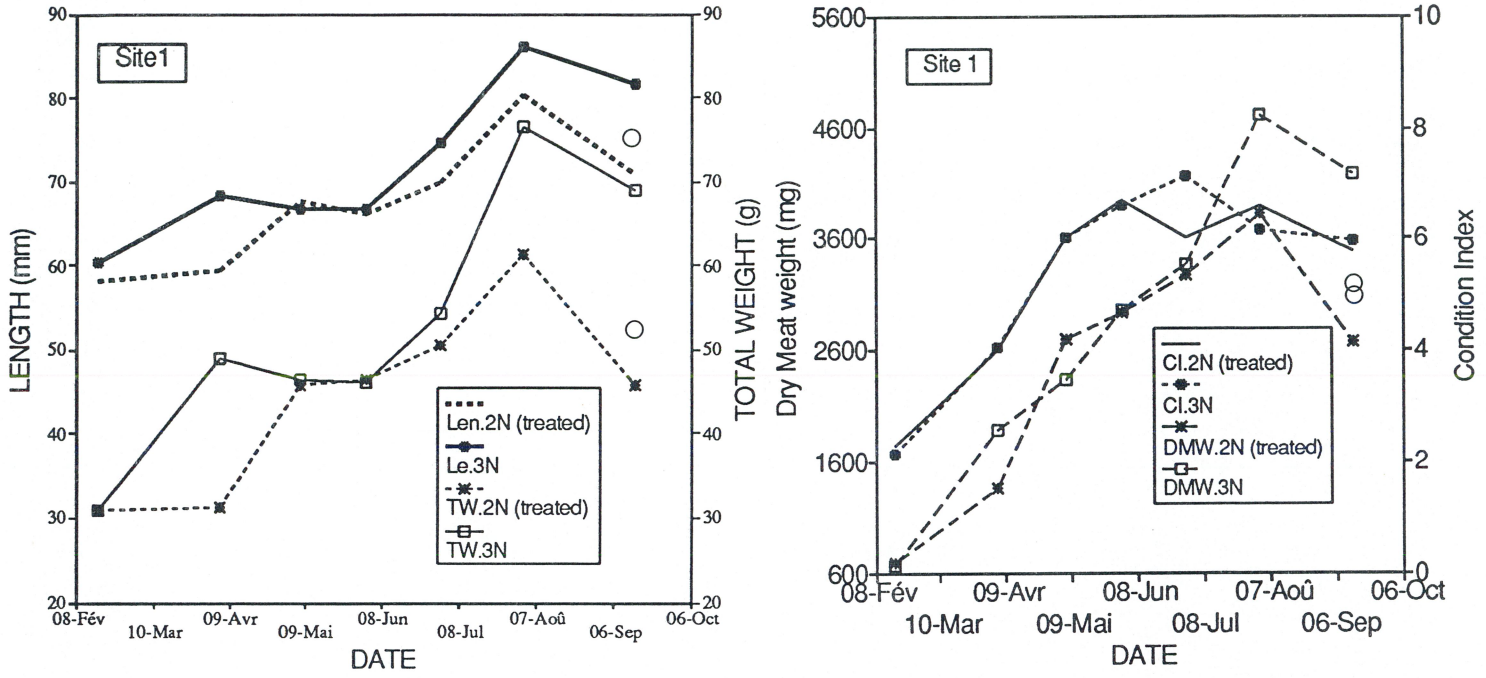

Fig. 3 : comparison of length, total weight, dry meat weight and condition index between control (o) and treated 2N and 3N oysters from site 1.

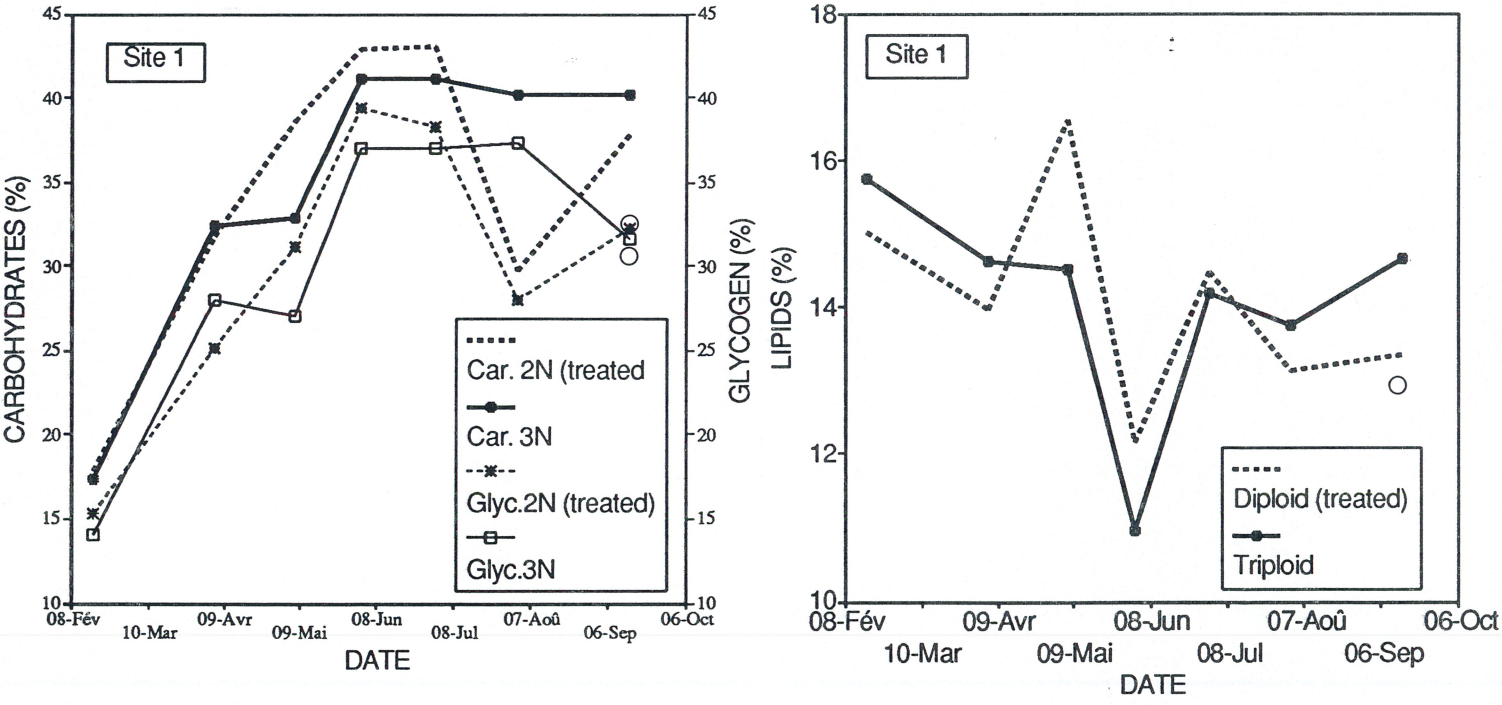

Fig. 4 : Comparison of carbohydrates, glycogen and lipidsbetween control (o) and treated 2N and 3N oysters from site 1.

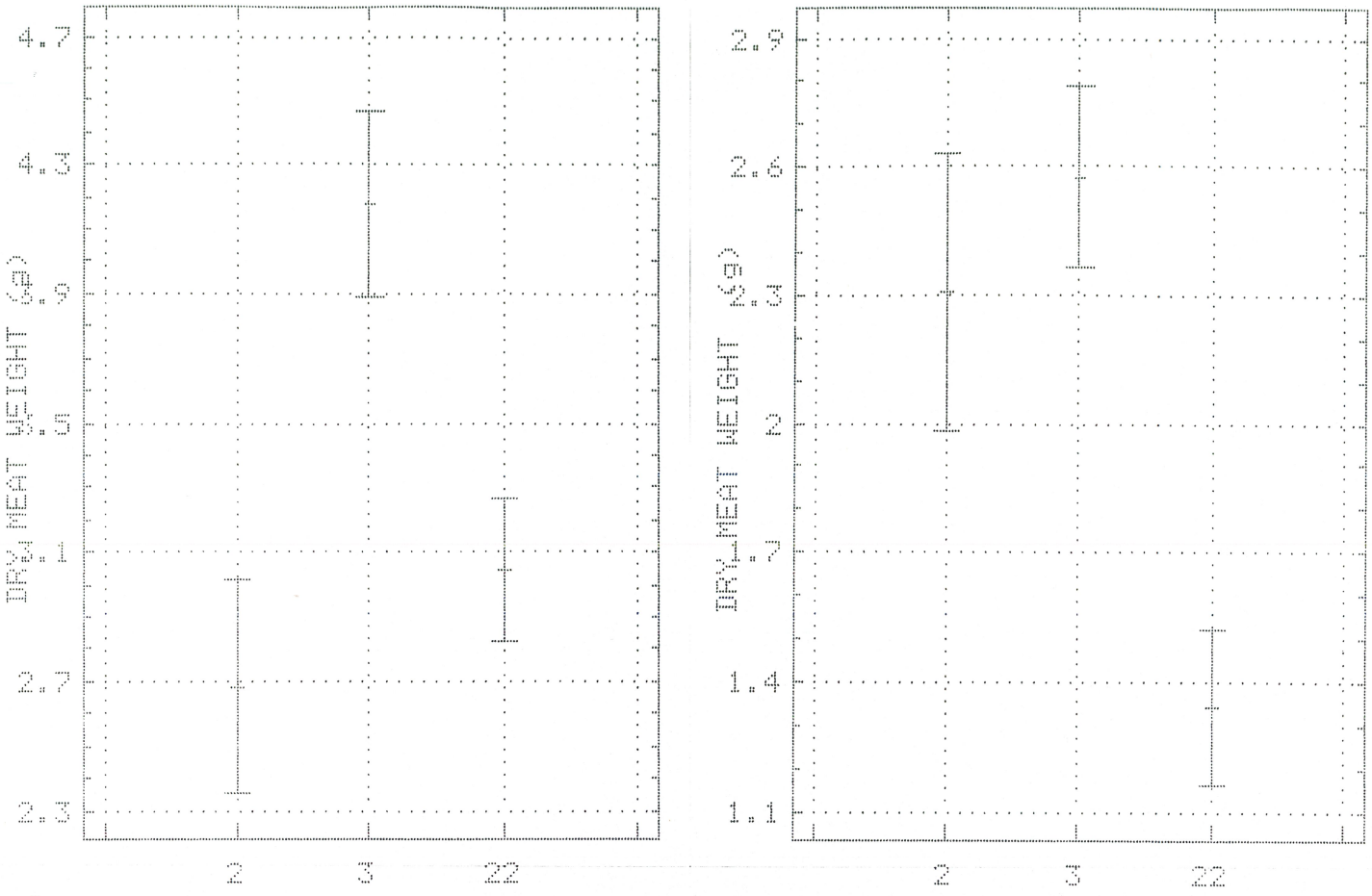

Fig 5 : Dry meat weight ANCOVA on septembre data from site 1(left) and site 2 (right). Ploidy level : 2 = 2N treated, 3 = 3N treated, 22 = control. (mean ± SE).

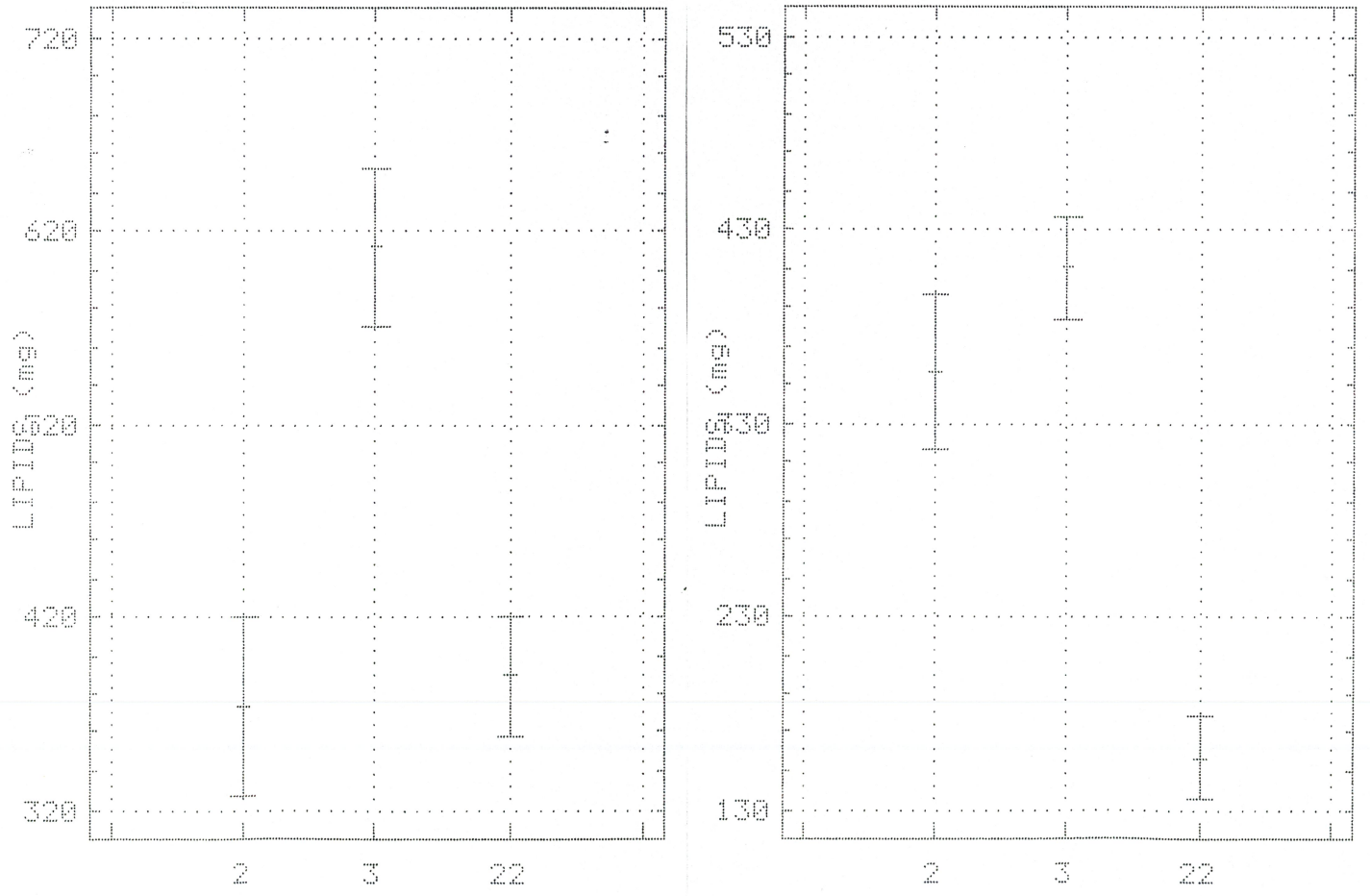

Fig. 6 :Lipids ANCOVA on septembre data from site 1 and 2 (mean ± SE).

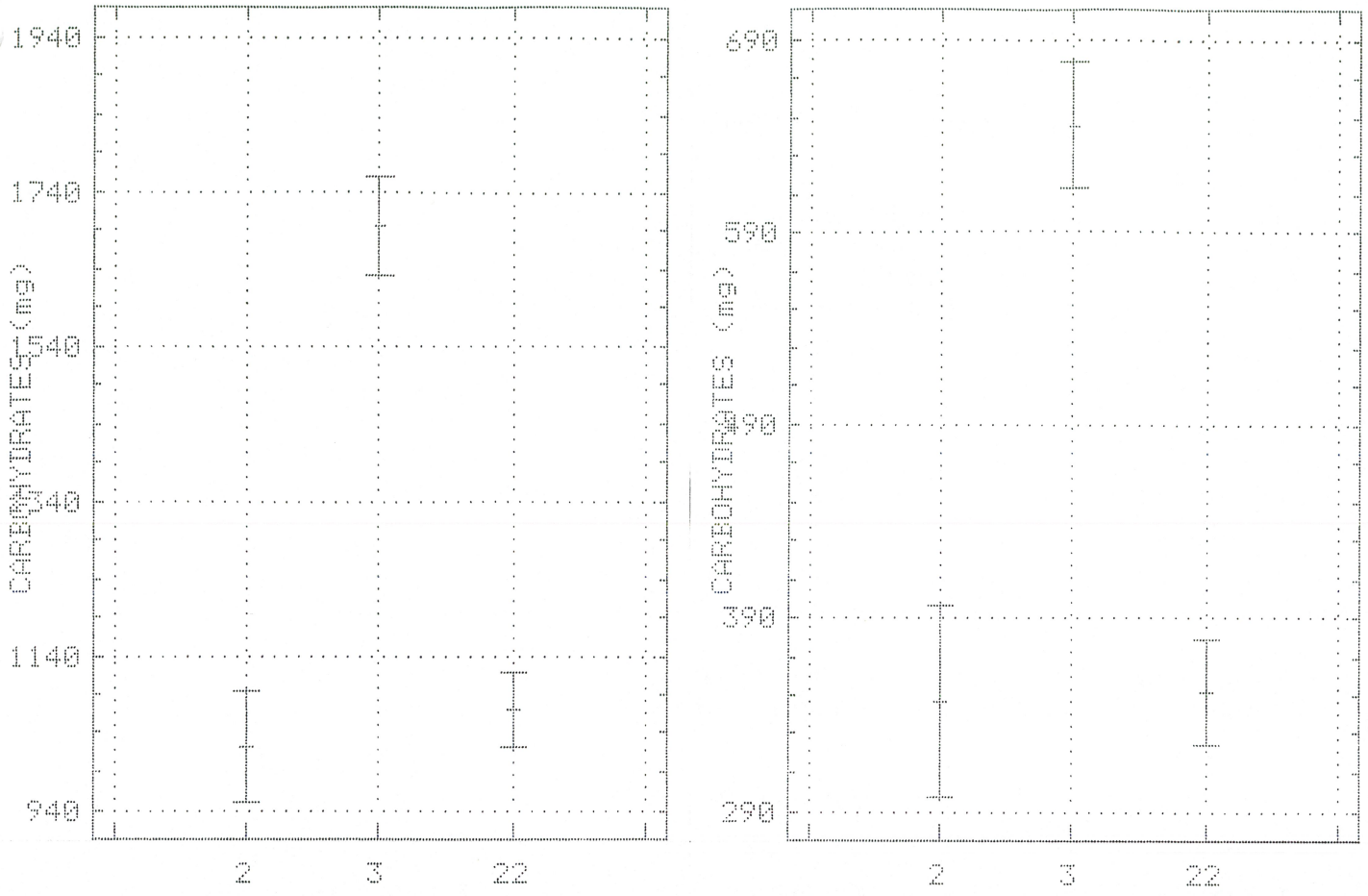

Fig. 7 ; Carbohydrates ANCOVA on septembre data from site 1 and 2 (mean ± SE).

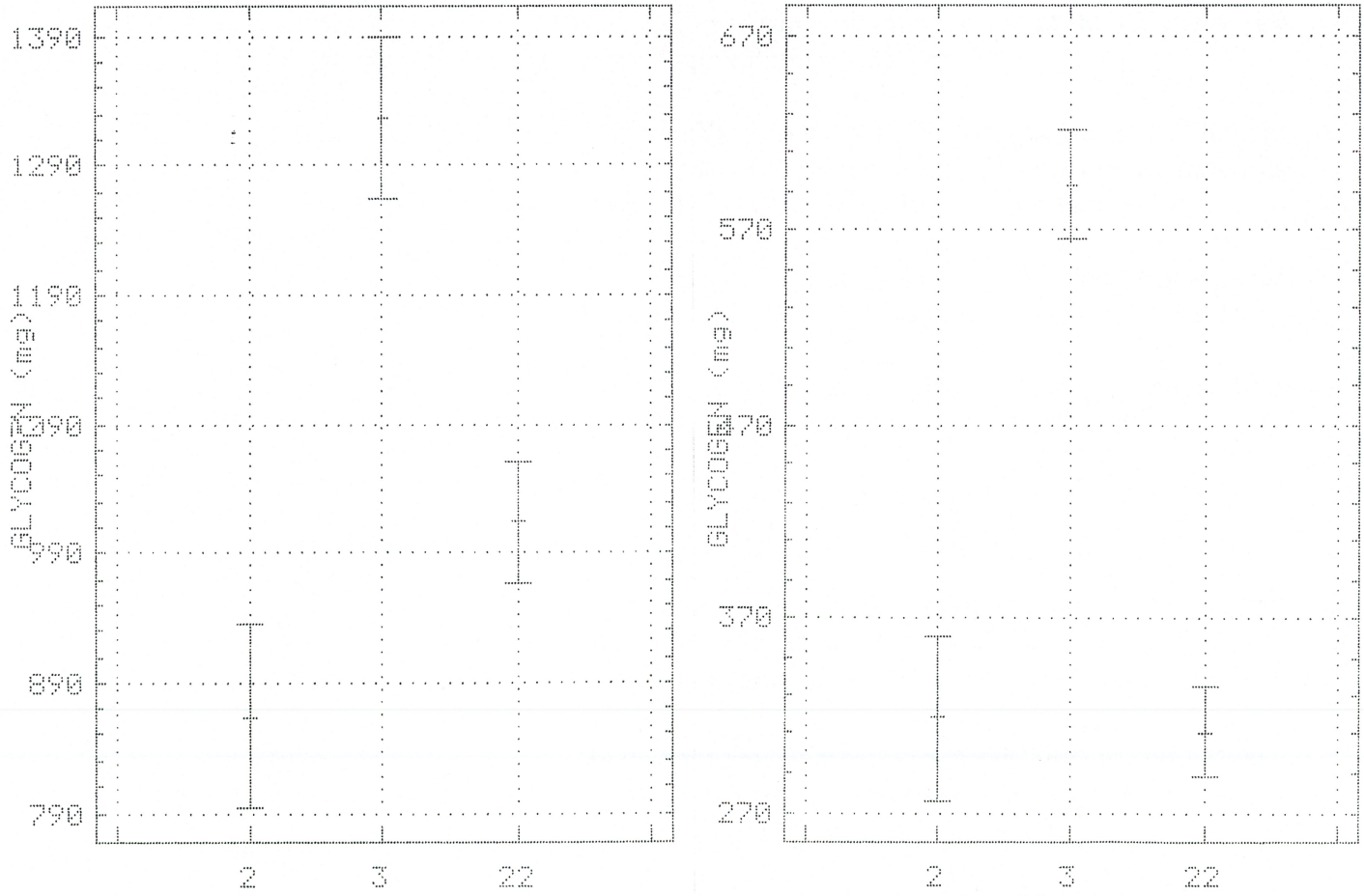

Fig. 8 ; Glycogen ANCOVA on septembre data fromsite 1 and 2 (mean ± SE).

new inductions yielded 95% of triploid using CB. 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; Gérard et al. 1993).

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

References

Akashige S. and Fushimi T., 1991. 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. J. Shellfish Res. 12(1): Abstract only.

Allen S.K. and Downing S.L., 1986. Performance of triploid Pacific oysters, Crassostrea gigas (Thunberg).I. Survival, growth, glycogen content, and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol., 102:197-208.

Beaumont A.R. and Fairbrother J.E., 1991. Ploidy manipulation in molluscan shellfish: a review. J. Shellfish Res. 10(1):1-18.

Bligh E.G. and Dyer W.F.,1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Chaiton J.A. and 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., and Dubé F., 1993. A novel method to produce triploids in bivalve molluscs by the use of 6-Dimethylaminopurine. J. Exp. Mar. Biol. Ecol. (In press.)

Downing S.L. and 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., and Smith F., 1956. Colorimetric method for determinaion of sugar and related substances. Anal. Chem., 28: 350-356.

Dufy C and Diter A., 1990. Polyploidy in the Manila clam Ruditapes philippinarum. I. Chemical induction and larvae performance of triploids. Aquat. Living Res., 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, eds. H. Barnes, Aberdeen:191-211.

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

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

Héral M. and 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, 50p.

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., and Wing N., 1993. Growth of the induced triploid pearl oyster, Pinctada martensii (D.). Aquaculture, 111:245-253.

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., and Shen W., 1992. Comparison of heterozygosity at three loci and growth between triploid and diploid pearl oysters, Pinctada martensii (D.). Tropic Oceanol. (China), 11(4):84-88.

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

Marsh J.B.and Weinstein D.B., 1966. Simple charring method for determination of lipid. J. Lip. Res., 7: 574-576.

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

Shpigel M., Barber B.J., and Mann R., 1992. Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, Crassostrea gigas (Thunberg). J. Exp. Mar. Biol. Ecol., 161:15-25.

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

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

Yamamoto S., Sugawara Y., Nomura T., and Oshino A, 1988. Induced triploidy in Pacific oyster Crassostrea gigas, and performance of triploid larvae. Tohoku J. of Agric. Res., 39(1);47-59.