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Comparative growth of triploid and diploid juvenile hard clams *Mercenaria mercenaria notata* under controlled laboratory conditions

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A B S T R A C T

Induced triploidy has been used in oyster culture to improve growth, but has not been fully explored for the hard clam *Mercenaria mercenaria notata*. Therefore, growth was examined in approximately 14 week-old (Exp I) and 15–18 week-old (Exp II) triploid juvenile hard clams in two 3-week experiments. Triploidy was induced chemically (cytochalasin B, 1.0 mg/l) by inhibiting polar body I (PBI) or polar body II (PBII). Growth, as a percentage change in live weight (LW), of triploids was significantly ($P<0.001$) less compared to diploids in both experiments. In Experiment I, LW increased 250% and 269% for PBI and PBII triploids (initial average LW 93.6 ± 19.0 and 59.5 ± 11.7 mg/clam), respectively, and 341% for diploids (initial average LW 72.0 ± 16.7). Additionally, diploids within triploid groups of Experiment I had lower LW increase (218–296%) as compared to untreated control diploids (341%). In Experiment II, LW increased 422% for PBII triploids (initial avg. LW 11.8 ± 1.6 mg/clam) and 549% for diploids (initial average LW 11.7 ± 1.9 mg/clam). Juvenile triploid clams did not exhibit better growth than diploids in these laboratory trials, but triploid clams may have a growth advantage during stressful conditions or as adults during reproduction as triploids are virtually sterile, which would allow for somatic growth during a time when diploids are spawning and losing mass. Additionally, the use of untreated control diploids is recommended for ploidy experiments rather than diploids found within triploid groups.

1. Introduction

Hard clam, *Mercenaria spp*., aquaculture in Florida has shown a dramatic increase during the last two decades with sales increasing progressively from less than $3 million in 1991 to $18.3 million in 2001 (FASS, 2004). The production of hard clams has increased through expansion of cultivation area. However, hard clam culturists in southwest and west Florida report below average survival during the prolonged hot summers (Leslie Sturmer, Department of Fisheries and Aquatic Sciences, University of Florida, Multi-County Aquaculture Extension, personal communication).

Higher mortality may be caused in clams that are already exhausted from spring spawning and lose more body mass (Nell, 1993) due to the shortage of phytoplankton and high temperatures associated with prolonged hot summers (Ohgai et al., 1982; Weiss et al., 2007). An earlier study with oysters found that low glycogen levels following spawning may be associated with high mortality during high summer temperatures (Perdue et al., 1981). As triploid bivalves generally have reduced gametogenesis (Shpigel et al., 1992; Eversole et al., 1996; Utting et al., 1996; Brake et al., 2004; Maldonado-Amparo et al., 2004), triploid clams may have a higher survival rate as they would have higher body mass because glycogen has not been used for gametogenesis (Perdue et al., 1981).

Differences in growth between diploid and triploid bivalves have been contradictory, with some authors observing better performance of triploids over diploids (e.g., Allen and Downing, 1986) and others observing the opposite (e.g., Stanley et al., 1984). For example, triploid Sydney rock oysters, *Saccostrea commercialis*, were found to be 41% heavier than diploids after 2.5 years of growth (Nell et al., 1994). Utting et al. (1996) detected heavier triploid Manila clams, *Tapes philippinarum*, which had higher condition index and carbohydrate content than diploids of the same age (2, 3 and 4 years). Conversely, growth of triploid lion-paw scallop, *Nodipecten subnodosus*, did not exceed that of diploids during a grow-out period of 21 months (Maldonado-Amparo et al., 2004). Similarly, growth of diploid and triploid larvae and juveniles of Manila clam, *T. philippinarum* (Laing and Utting, 1994; Shpigel and Spencer, 1996) and Venerid clam, *Tapes dorsatus* (Nell et al., 1995), were not different.

Triploid hard clam *Mercenaria mercenaria* have shown similar contradictory differences in performance. Hidu et al. (1988) reported that the dry tissue weight and shell measurements of triploid hard clams were smaller than those of diploids after three growing seasons. Eversole et al. (1996) found no difference in size of triploid hard clams

compared to diploids at six and 27 months of age. However, during the same study, they found that triploids had better growth (27% versus 14% increase in shell length for triploids and diploids, respectively) from the period of 27 to 47 months (Eversole et al., 1996). During this last 20-month period, diploid clams experienced at least two spawning periods reflecting the possible difference in energy allocation between triploids and diploids (Eversole et al., 1996).

The conflicting published data on growth of triploid hard clams, especially at different ages, indicate the need for further evaluation of triploid hard clams. Most studies for growth comparisons were performed in natural environments where other factors may interfere with the evaluation. Therefore, the present study was undertaken to examine if differences in growth could be detected in juvenile triploid clams when exposed to similar controlled laboratory conditions.

2. Materials and methods

2.1. Production and culture

Triploid and diploid juvenile hard clams were produced from broodstock *Mercenaria mercenaria notata* obtained from a commercial hatchery (HB Clams, Inc., Ft. Pierce, Florida). Separate spawns of single or double parent crosses were performed to produce different families of triploid and diploid sibling clams. Triploidy was induced by inhibiting the formation of either the first polar body (PBI) or the second polar body (PBII) of fertilized eggs using 1.0 mg cytochalasin B (CB)/l seawater (Scarpa et al., 1994).

Each spawn was divided to produce diploids (untreated control) or triploids (treated groups, either PBI or PBII). The different groups were cultured separately using standard protocols for hatchery and nursery practices (Hadley et al., 1997). Initial triploidy proportions in larvae were measured by flow cytometry following the method of Allen and Bushek (1992) as described in the next section.

2.2. Assessment of triploidy

Ploidy was measured by flow-cytometry following the procedure of Allen and Bushek (1992). Briefly, larvae or whole clam samples were taken and placed in a fluorochrome (DAPI) freezing solution and stored at −80 °C. Samples were thawed, processed to produce a cell suspension, and analyzed on a Partec PA flow-cytometer. Known diploid samples were used as standards before analyzing experimental samples. The modal value of triploid DNA fluorescence value should be 1.5-fold that of the diploid value (Allen, 1983).

2.3. Growth experiments

Two three-week growth experiments were performed using juvenile clams approximately 14 weeks old (99 days, Exp I) or 15–18 weeks old (107–129 days, Exp II). In Experiment I, the effect of triploid type (i.e., polar body I or II inhibition) was examined. In Experiment II, the effect of only triploidy (PBII inhibition) was examined.

In Experiment I, three replicate beakers (4 l) with 20 clams each were used for each ploidy treatment (PBI triploid, PBII triploid and diploid control). In Experiment II, four replicate beakers (4 l) with 12 clams each from three different families (K, M, and O, $n=36$ total clams/beaker) were used for each ploidy treatment (PBII triploids and diploid control). Individual clams were placed in separate openings of a plastic grid (1.2×1.2 cm openings) with screen (1.4 mm openings) on bottom. The trays were suspended approximately 8 cm from the bottom in 3 l seawater in the beakers. Clams were fed with the microalgae *Isochrysis sp.* (clone T-ISO), which is the recommended algal species for supporting growth in both larval (Tiu et al., 1989) and post-set hard clams (Wikfors et al., 1992). Algae were provided at a density of 100,000 cells/ml as recommended by Hadley et al. (1997) for the clam sizes used in the present study.

Clams were fed twice a day (~12 h apart) in Experiment I. In Experiment II, clams were initially fed twice a day (~12 h apart) and increased to three times per day (~6 h apart in a 12 h period) at the beginning of week 2 and four times per day (~4 h apart in a 12 h period) at the beginning of week 3. Culture water was aerated gently to keep microalgae in suspension and water was changed daily. Temperature and salinity were measured daily for Experiment I and averaged 28 °C±1.5 ($n=38$) and 34±0.7 ppt ($n=23$). In Experiment II, only temperature was measured daily and averaged 29 °C±0.6 ($n=57$).

Clams of similar initial size within a group were selected and initial shell length (SL, anterior-posterior) and live weight (LW) were measured using a Vernier caliper and a balance, respectively. Individual clams were measured weekly after being removed from water and blotted dry. Weekly and cumulative growth (G) in SL and LW was calculated as follows:

$$G_{SL\ or\ LW} = [(\text{Final}_{SL\ or\ LW} - \text{Initial}_{SL\ or\ LW}) / \text{Initial}_{SL\ or\ LW}] \times 100.$$

At the end of each experiment, individual clams were sacrificed, whole clam sample taken and ploidy measured by flow cytometry as described above.

2.4. Statistical analysis

Only clams that were confirmed to be triploids were used for growth (SL and LW) comparisons with diploids and are referred to as PBI triploids or PBII triploids. Any clam that died during the experiment was omitted (0 clams in Experiment I and 9 clams in Experiment II). In Experiment I, SL and LW data were analyzed using one-way analysis of co-variance (ANCOVA) using initial mean shell length or live weight as the covariate (Underwood, 2001) as these measurements were initially significantly different. The low and unbalanced numbers of clams in replicate tanks prevented statistical comparison of growth using nested ANOVA. Therefore, growth (% increase in SL and LW) data from the three replicates for each treatment (PBI, PBII, diploids) were combined and compared using one-way ANOVA. In Experiment II, two statistical tests were conducted to test the effect of replicate tanks, ploidy, and family: nested ANOVA, then after pooling the nested term (replicate tanks) with the error term, two-way mixed model ANOVA was used to test the effect of ploidy as a fixed factor and family as a random factor on clam growth (Underwood, 2001). Differences were considered significant at $P<0.05$. If ANOVA indicated a significant effect, then a post-hoc analysis of data was performed using Bonferroni test for multiple comparisons of means (Underwood, 2001).

3. Results

3.1. Triploidy

In Experiment I, post-experiment measurement of ploidy in the experimental clams revealed a lower than expected number of triploids in both triploid treatments. Initial triploid proportions in larvae were 77% and 86% for PBI and PBII triploid groups, respectively. However, post-experiment analysis of the clams revealed only 60% and 18% for PBI and PBII treatments, respectively.

In Experiment II, post-experiment measurement of ploidy in triploid groups revealed triploid proportions that were adequate for statistical comparison: Family K was 65% triploid, Family M was 92% triploid, and Family O was 94% triploid.

3.2. Growth

The two experiments showed similar results. Shell length and live weight increased with time in all treatments (Tables 1 and 2). Growth was always highest during the first week of each experiment and was lower for the subsequent weeks (Figs. 1–5). In general, growth of

Table 1
Mean (±SD) shell length (A) and live weight (B) of triploid and diploid juvenile hard clams *Mercenaria mercenaria notata* at weekly intervals during a three-week growth study

(A)	Mean Shell Length (mm)±SD				# of clams
Time (weeks) Experimental Group	0$^{\#}$	1	2	3	
Control (Diploids)	6.5±0.5$^{a,\#\#}$	8.5±0.7	10.1±0.8	11.2±0.8	60
PB I Triploids*	7.4±0.6^{b}	9.0±0.6	10.3±0.6	11.4±0.8	36
PB II Triploids**	6.4±0.4^{a}	8.0±4.0	9.2±0.7	9.9±0.7	11
PB I Diploids***	7.9±0.5^{c}	9.4±0.6	10.7±0.7	11.6±0.9	24
PB II Diploids****	6.9±0.6^{b}	8.5±0.8	9.9±0.8	11.2±1.0	49

(B)	Mean Live Weight (mg)±SD				# of clams
Time (weeks) Experimental Group	0$^{\#}$	1	2	3	
Control (Diploids)	72.5±16.7$^{a,\#\#}$	140.1±30.3	229.2±47.9	317.3±68.4	60
PB I Triploids*	93.6±19.0^{b}	155.9±28.4	240.7±43.1	323.9±61.9	36
PB II Triploids**	59.5±11.7^{a}	111.1±20.1	171.8±36.0	219.2±49.3	11
PB I Diploids***	114.4±22.0^{c}	182.5±34.1	272.1±50.5	363.4±74.8	24
PB II Diploids****	81.4±23.1^{b}	146.8±39.0	232.2±59.2	322.1±83.3	49

ANCOVA showed significant effect ($P<0.05$) of initial shell length and live weight on these parameters measured at different times (week 1, 2 and 3). Post-hoc tests were considered only for initial size (time=0) because shell length and live weight at later times were affected by the initial values (Exp I).
Statistical comparisons are between control diploids and each of the other four groups. Within column means with different letters differ significantly ($P<0.05$ One-Way Analysis of Variance).
*Clams confirmed to be triploids by flow cytometry in the PBI triploid group.
**Clams confirmed to be triploids by flow cytometry in the PBII triploid group.
***Clams confirmed to be diploids by flow cytometry in the PBI triploid group.
****Clams confirmed to be diploids by flow cytometry in the PBII triploid group.

triploid clams was similar or significantly slower than control diploids (Figs. 1–5).

3.2.1. Experiment I

3.2.1.1. Shell Length. At the beginning of Experiment I, mean initial shell length (SL) of the clams differed (ANOVA $P=0.000$). Post hoc tests indicated that the mean SL of PBI triploids was significantly larger than the other two groups ($P<0.001$; Table 1A). ANCOVA revealed that initial shell length had a significant effect on final SL ($P<0.001$). When comparing mean shell length among the three treatments (Table 1A) after the first, second, and third week and using mean initial SL as covariate, ploidy had an effect on SL ($P<0.001$) during each of the 3 weeks.

Weekly percentage increase in shell length (%SL) was significantly less for triploids compared to diploids only after the first 2 weeks, but not after the third week (Fig. 1a). There was no difference in weekly %SL

Table 2
Mean (±SD) live weight of triploid and diploid juvenile hard clams *Mercenaria mercenaria notata* from different families at weekly intervals during a three-week growth study (Exp II)

Family	Time (weeks) Experimental Group	Mean Live Weight (mg)±SD				# of clams
		0	1	2	3	
K	Control Diploids	11.7±1.5^{a,*}	26.6±4.2^{a,*}	49.7±11.1^{a,*}	76.3±22.8^{a,*}	48
K	PB II Triploids**	11.0±1.4^{a}	25.1±4.1^{a}	40.8±10.3^{b}	57.0±26.5^{b}	31
M	Control Diploids	11.2±2.0^{a}	24.0±5.2^{a}	42.7±12.2^{a}	63.0±18.8^{a}	47
M	PB II Triploids**	12.3±1.6^{a}	26.1±4.8^{a}	40.8±10.4^{b}	58.2±20.3^{b}	44
O	Control Diploids	12.2±1.7^{a}	28.5±4.3^{a}	55.6±9.6^{a}	86.9±21.6^{a}	48
O	PB II Triploids**	11.7±1.6^{a}	26.2±4.6^{a}	45.7±12.6^{b}	66.7±26.5^{b}	45
All	Control Diploids	11.7±1.9^{a}	26.4±4.9^{a}	49.4±12.1^{a}	75.5±23.2^{a}	143
All	PB II Triploids**	11.8±1.6^{a}	25.8±4.5^{a}	42.6±11.4^{b}	61.1±24.6^{b}	120

*Means with different letters in two consecutive rows in a column differ significantly ($P<0.05$ One-Way Analysis of Variance).
** Only clams confirmed to be triploids by flow cytometry.

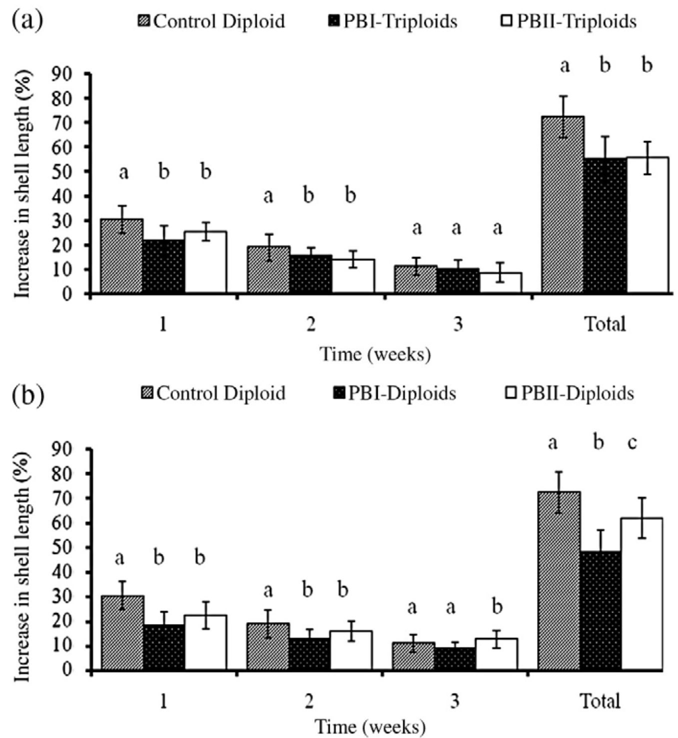

Fig. 1. Weekly and total growth (% increase) in shell length of *Mercenaria mercenaria notata* from different ploidy types: a) Control diploids, PB I triploids (produced by inhibiting first polar body) and PB II triploids (produced by inhibiting second polar body); b) Control diploids, PBI Diploids (confirmed diploids in the PBI treatment) and PBII Diploids (confirmed diploids in the PBII treatment). Different letters represent significant differences ($P\leq0.05$).

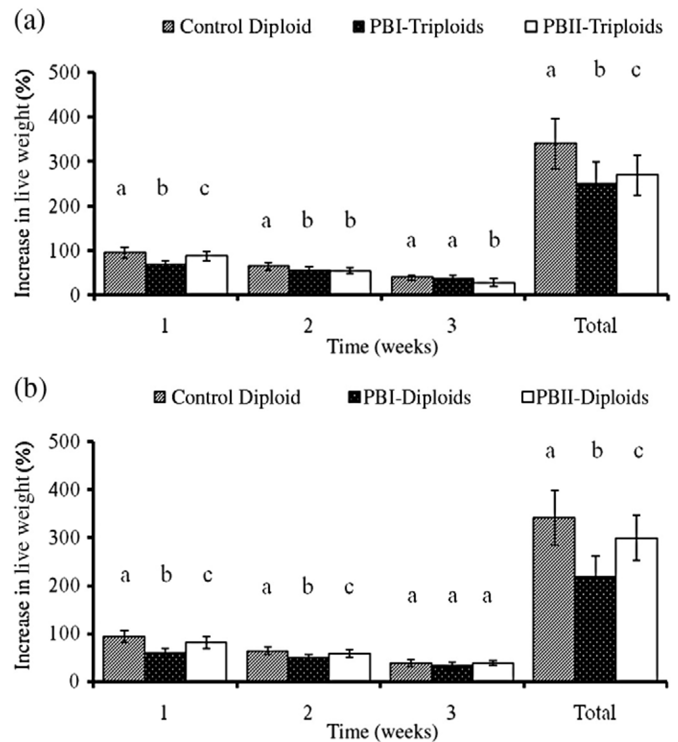

Fig. 2. Weekly and total growth (% increase) in live weight of *Mercenaria mercenaria notata* from different ploidy types: a) Control diploids, PB I triploids (produced by inhibiting first polar body) and PB II triploids (produced by inhibiting second polar body); b) Control diploids, PBI Diploids (confirmed diploids in the PBI treatment) and PBII Diploids (confirmed diploids in the PBII treatment). Different letters represent significant differences ($P\leq0.05$).

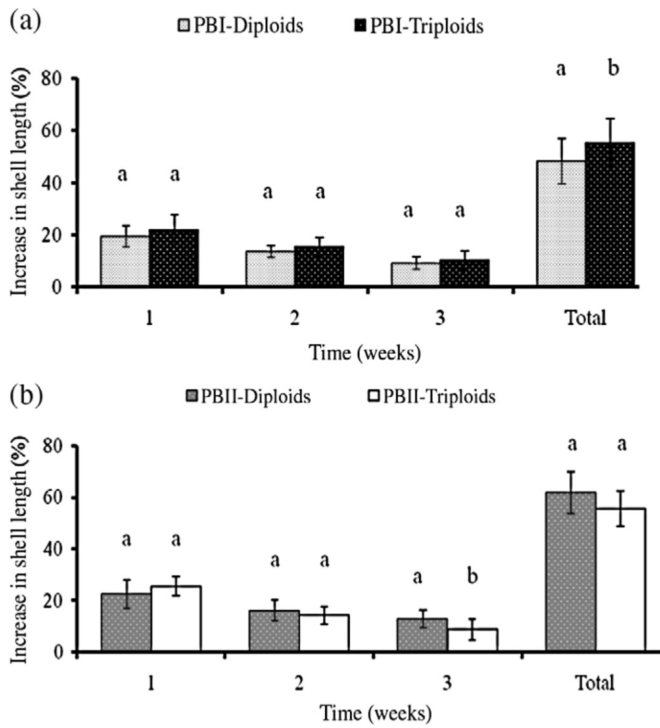

Fig. 3. Weekly and total growth (% increase) in shell length of *Mercenaria mercenaria notata* from confirmed triploids versus confirmed diploids within PBI and PBII treatments, respectively: a) PB I triploids (produced by inhibiting first polar body) versus PBI diploids (confirmed diploids within the PBI treatment); and b) PB II triploids (produced by inhibiting second polar body) versus PBII diploids (confirmed diploids within the PBII treatment). Different letters represent significant differences ($P \leq 0.05$).

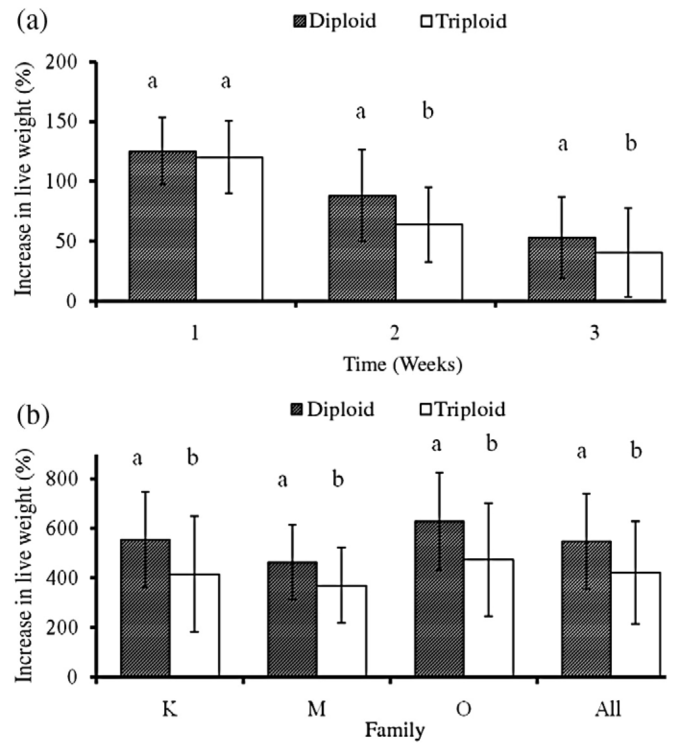

Fig. 5. Weekly and total growth (% increase) in live weight over 3 weeks of *Mercenaria mercenaria notata* from PBII triploids (produced by inhibiting second polar body during meiosis) and diploids (control): (a) weekly growth of all families pooled together, b) cumulative growth over the 3 weeks for each family and for all families pooled together. Different letters represent significant differences ($P \leq 0.05$).

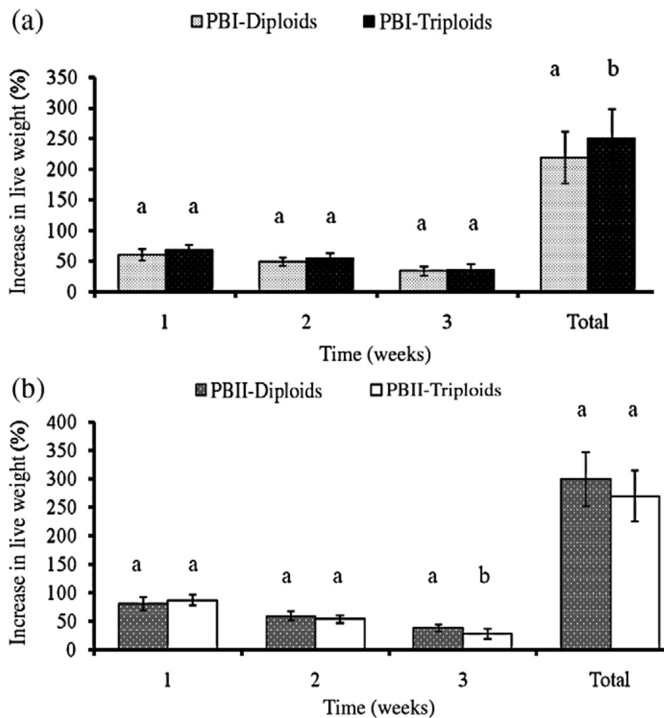

Fig. 4. Weekly and total growth (% increase) in live weight of *Mercenaria mercenaria notata* from confirmed triploids versus confirmed diploids within PBI and PBII treatments, respectively: a) PB I triploids (produced by inhibiting first polar body) versus PBI diploids (confirmed diploids within the PBI treatment); and b) PB II triploids (produced by inhibiting second polar body) versus PBII diploids (confirmed diploids within the PBII treatment). Different letters represent significant differences ($P \leq 0.05$).

increase between triploid types (P=0.197; Fig. 1a), although weekly %SL increase decreased over time for all groups (Fig. 1a). Total cumulative %SL increase over the 3 weeks was significantly less (P<0.001) for PBI and PBII triploids than in control diploids (Fig. 1a). The cumulative %SL increase between the two triploid groups was not significantly different (P=1.000; Fig. 1a).

3.2.1.2. Live weight. As with shell length, at the beginning of Experiment I, initial live weight (LW) among the three groups was significantly different (P<0.001; Table 1B). PBI triploids were initially heavier than both PBII triploids and diploids (Table 1B). PBII triploids and control diploids were not significantly different (P=0.066; Table 1B).

Weekly percentage increase in live weight (%LW) showed a trend similar to that of SL for the different ploidy types (Fig. 2a). After 1, 2 and 3 weeks, weekly %LW increase was significantly different between groups (P<0.001; Fig. 2a), even after adjustment for initial variation in LW that was used as covariate. Ploidy had a significant (ANCOVA P<0.001) effect on weekly %LW increase as well as total %LW increase over the 3 weeks (Fig. 2a). In contrast to SL that showed differences only during the first 2 weeks, %LW increase was also significantly different for week three (P<0.001; Fig. 2a).

After 1 week of growth, average %LW increase in both PBI and PBII triploids was significantly smaller (P<0.001) than diploids (Fig. 2a). The two triploid groups were also different (P<0.001); PBI being the slowest. In contrast to %SL increase, %LW increase of PBI triploids was significantly slower than PBII triploids (Fig. 2a). During the second week, %LW increase in both triploid groups was significantly slower than diploids, but %LW increase was not significantly different (P=1.000) between the two triploid groups. During the third week, %LW increase of both PBI triploids and diploids was similar (P=0.091) and greater (P<0.001 and 0.039, respectively) than PBII triploids. Over the course of the experiment, weekly %LW increase decreased for all groups (Fig. 2a). Total cumulative %LW increase over the 3 weeks was

significantly different among the three groups ($P<0.001$). PBI and PBII triploids had lower %LW increase than diploids (Fig. 2a) and the two triploid groups were also different ($P<0.001$; Fig. 2a); PBI triploids exhibited the least growth (Fig. 2a).

3.2.1.3. Diploids within triploid groups. As triploid proportions in both PBI and PBII experimental treatments were low (60% and 18% triploids in PBI and PBII, respectively), there was an adequate number of confirmed diploid clams within each triploid group (PBI=24 and PBII=49 clams; Table 1) to conduct additional statistical tests to check if diploids within the triploid groups were similar to the control diploids. If diploids within triploid groups were similar, they could be used as controls. Clams confirmed to be diploids within the PBI and PBII experimental treatments (herein referred to as PBI diploids and PBII diploids, Table 1) were exposed to similar conditions during triploidy induction treatment and culture. The only difference between these diploids and triploids would be their ploidy.

The initial average sizes (SL and LW) of PBI diploids and PBII diploids were significantly larger than those of the control diploids (Table 1A and B). PBI diploids were also significantly larger than PBI and PBII triploids (Table 1A and B). PBII diploids were not significantly larger than PBI triploids, but were larger than PBII triploids (Table 1A and B).

Both initial size and ploidy had an effect on final size and growth (% increase in SL or LW, ANCOVA $P<0.001$). After 1 week of growth, average %SL increase in both PBI diploids and PBII diploids was significantly smaller ($P<0.001$) than control diploids (Fig. 1b). During the second week of growth, the three groups showed the same trend as the first week ($P<0.001$ and 0.013, for PBI diploids and PBII diploids, respectively; Fig. 1b). During the third week, %SL increase of PBI diploids was not different ($P=0.182$) from control diploids, but %SL increase of PBII diploids was significantly faster than control diploids ($P=0.040$). Over the course of the experiment, weekly %SL increase decreased for all groups (Fig. 1b), but total %SL increase was least for PBI diploids, followed by PBII diploids (Fig. 1b).

Weekly %LW increase exhibited a similar trend to %SL increase. After 1 week of growth, average %LW increase for PBI diploids and PBII diploids was significantly lower ($P<0.001$) than control diploids (Fig. 2b). During the second week of growth, the three groups showed the same trend as the first week ($P<0.001$ and 0.012, in PBI diploids and PBII diploids, respectively; Fig. 2b). During the third week, %LW increase of both PBI diploids and PBII diploids were not different from control diploids ($P=0.055$ and 1.000, respectively). Over the course of the experiment, weekly %LW increase decreased with time for all groups (Fig. 2b), but total %LW increase was least for PBI diploids, followed by PBII diploids (Fig. 2b).

Comparing the growth of confirmed triploids with their respective confirmed diploids, within each treatment showed that weekly %SL increase was not different, but total %SL increase of PBI triploids (55±9%) was significantly greater than PBI diploids (48±9%; $P=0.018$; Fig. 3a). However, total %SL increase of PBII triploids (56±7%) was not significantly different from PBII diploids (62±8%; $P=0.291$; Fig. 3b).

Similarly, total growth as measured by %LW increase of PBI triploids (250±23%) was significantly greater than PBI diploids (219±42%; $P=0.042$; Fig. 4a), but total %LW increase of PBII triploids (269±45%) was not different from the PBII diploids (299±48%; $P=0.551$; Fig. 4b).

These results show that in contrast to control diploids that exhibited significantly faster growth than triploids of both PBI and PBII experimental groups, diploids within triploid groups exhibited growth rates that were either slower than (PBI triploid group) or similar to (PBII triploid group) confirmed triploids.

3.2.2. Experiment II

In Experiment II, only live weight was measured as clams were smaller in size than those used in Experiment I. Only PBII triploids were tested in comparison to diploids (control), but the PBII triploids originated from three distinct sets of parents for true replication. Although families were of different age (maximum 22 days), initial live weight of PBII triploids and control diploids was not significantly different ($P=0.988$; Table 2).

Weekly %LW increase was not significantly different between triploids and diploids after week 1 ($P=0.282$; Fig. 5a), but was significantly less in triploids after the second ($P<0.001$) and third ($P=0.028$) week (Fig. 5a).

Ploidy had a significant effect on total %LW increase (2-way ANOVA; $P=0.020$; Fig. 5b), but there were no differences between families ($P=0.051$; Fig. 5b). Within siblings of the same genetic background (same family), growth of triploid clams were always less than diploids (Fig. 5b).

4. Discussion

Comparison of growth of the hard clams in the present study with other studies is complicated by the fact that clams used were of different age, size or exposed to different environmental conditions. Also, no standardized growth rate was used. Therefore, transformations were made to the original data from other studies to make comparisons easier.

Growth of triploids under the experimental conditions used in the present study was still significant and promising. Grown from an initial size of 6–8 mm to 9.2–12.2 mm in 3 weeks, the growth rates observed in both triploid and diploid clams (0.17, 0.20, 0.22 mm/day in PBI triploids, PBII triploids and control diploids, respectively) were either similar or exceeded that observed in some previous studies of growth for diploid clams (Menzel, 1963; Hadley and Manzi, 1984; Buzzi and Manzi, 1988; Craig et al., 1988; Buzzi, 1990) and those summarized by Ansell (1968). This may be because most of the previous studies performed on hard clams were either in the field, at cooler temperatures, or used different feeding regimes or different clam sizes than those used in the present study.

In the study of Eversole et al. (1996), although the hard clams were 6 months of age, mean shell length was 1–2 mm smaller than the initial sizes used in the present study (Experiment I) where juvenile clams were 3.5 months old. Even though clams were bigger in the present study, their growth rate was twice as high (~0.2 vs. ~0.1 mm/day) as that in Eversole et al. (1996).

In New York, hard clams of 8.8 mm SL fed 5.0×10^4 algal cells/mL (*Pseudoisochrysis paradoxa*) for 3 weeks grew 0.09 mm/day at 21 °C (Bricelj and Malouf, 1984). In South Carolina, hard clams of similar sizes grew at a rate of about 0.07 mm/day during the rapid growing season (Hadley et al., 1997). Summerson et al. (1995) reported that Virginia seed clams kept in an up-weller nursery system in North Carolina grew about 0.03 mm/day, however, the stocking density during that study was much higher (6500–9790 clam/19 l) as compared to 20–36 clams/3 l in the present study. Clam seed of 3 mm length planted in Alligator Harbor, Florida, grew ~0.08 mm/day at the end of the first year of growth (Menzel, 1963).

Other studies reported growth rates similar or only slightly lower than the present study. In Delaware, Turner and Miller (1991) found that the average growth of 13–15 month old hard clams (SL 20 mm) was 0.19 mm/day when clams were fed 48,000 algal cells/ml (C-ISO). A lower daily increment of approximately 0.10 mm/day was observed when clams (SL 20 mm) were planted in natural sediments (Hibbert, 1977; Eldridge et al., 1979). Craig et al. (1988) found a maximum growth of 0.17 mm/day for *M. mercenaria* seed (SL 8.7 mm) cultured in Christmas Bay, Texas for one year. Bock and Miller (1994) deployed clams in an intertidal sandflat and reported growth rates of 0.11, 0.04 and 0.001 mm/day at 24, 15 and 8 °C, respectively. Nursery seed clams (SL 6.0 mm) planted in the Indian River Lagoon (IRL), FL grew 0.095 mm/day, whereas during the same study, grow-out seed clams (SL 21.2 mm) grew ~0.05 mm/day (Fernandez et al., 1999).

These results indicate that the conditions used in the present study were beneficial and maximized clam growth in both triploids and

diploids. The daily increment of shell growth in triploids was similar to or faster than most reported growth rates for clams. No live weight data at a similar age (size) could be obtained from the previous studies for comparison. However, considering the faster live weight increase in Experiment II (422% for PBII triploids and 549% for diploids) compared to Experiment I (269% for PBII triploids and 341% for diploids), daily increment of SL in clams used in Experiment II would be expected to be higher than the range observed in Experiment I as per Haskin's data (1950). The observed faster growth rates in the present study may be attributed to a higher abundance of food, higher temperature, lower stocking density, or less stressful environmental conditions under the laboratory conditions.

The observed decline in weekly growth rates of clams in all groups of both experiments can be explained by the larger initial size at the beginning of each week than the previous week. Similarly, Pratt and Campbell (1956) observed an inverse relationship between growth increment and initial size of *Venus mercenaria* when growth was expressed in terms of shell length in the range of 35–70 mm. Animals of 35–39 mm SL grew three times as fast as animals of 65–69 mm (Pratt and Campbell, 1956). In contrast, when the increase in SL was expressed in volumetric terms, the increment was nearly constant over the range from 35 mm to 70 mm (Pratt and Campbell, 1956). Another possible explanation may be the increased food requirements of fast growing clams might not have been supported by the amount of food added, although feeding (# times/day) was increased in the second experiment, growth rate still decreased; similar to that found in Experiment I. Clearance rate studies might be required throughout the experimental duration to adjust feed availability.

Triploid bivalves in many cases exhibit better growth compared to diploids (e.g., Allen and Downing, 1986; Beaumont and Fairbrother, 1991; Garnier-Géré et al., 2002). Although many studies have been published on examining growth in triploid bivalves (e.g., Beaumont and Fairbrother, 1991; Brake et al., 2004; Hand et al., 2004), only a few studies examined seed before planting (e.g., Toro and Sastre, 1995). Clams (6–8 mm) tested in the present study were similar to field nursery and grow-out sizes (Manzi et al., 1980; Kraeuter and Castagna, 1985; Marelli and Arnold, 1996; Fernandez et al., 1999). Nursery planting size of 6 mm and grow–out planting size of >10–11 mm are used in Florida (Fernandez et al., 1999). Comparison of triploid-diploid performance in the laboratory at these sizes might give an idea about possible differential growth in the field without the complications of other environmental conditions.

In both experiments, the weekly and total cumulative growth of triploids was either slower or similar to that of control diploids. The proportion of the increase of SL to that of LW was constant (SL% / LW%=0.22, 0.21, and 0.21 for PBI, PBII and diploid control, respectively) reflecting the similar growth trend in the two indices. This is in agreement with Haskin (1950) who found that the relation between the cube root of LW and SL is linear and that there is no change in proportion between the two parameters with clam growth.

In Experiment I, the observed lower triploid proportions in the two triploid groups from that expected, based on the initial larvae triploid proportion, was perhaps due to differential survival of triploids and diploids within each group. However, the differential reduction of % triploids in PBI and PBII groups (only 17% decrease for PBI and 68% for PBII) could not be explained. Another possibility is a biased selection of larger sized clams for use in the experiment. Since slower growth rates of triploid clams was confirmed in the present study, selection of the larger sized clams may have led to selecting more of the fast growing diploids within the triploid groups. The smaller sized clams within the same batch may have had higher triploid proportions. However, no ploidy data were collected from the smaller clams within these groups to confirm this assumption. Conversely, in Experiment II when more caution was taken in selecting clams that were representative of the population, triploid levels were higher; hence, these “older” clams in Experiment II were smaller in size.

Different initial size among the tested groups was another problem in Experiment I. Because PBI triploids were initially larger than those of PBII triploids and control diploids, it was not known whether the reason for their slower growth was because of larger starting size or due to triploidy. Pratt and Campbell (1956) observed an inverse relation between quahog growth and initial SL over the range of 35–70 mm, but no similar studies were found for the size range used in the present study. To account for this problem, data were statistically re-tested using ANCOVA with initial size (SL or LW) as covariate to correct for the initial differences.

Although ANCOVA showed an effect of the initial size on final size, ANCOVA indicated that ploidy also significantly affected final size and %growth. Initial size differences from ploidy occurred even within the same treatment. The initial larger size of confirmed diploids as compared to triploids within the two triploid treatments (PBI and PBII) may confirm the possible faster growth of control diploids than their triploid siblings.

As ANCOVA showed significant effect of both initial size and ploidy on growth, the effect of ploidy on growth rate was only partial in Experiment I. However, Experiment II confirmed the slower growth of triploids as compared to control diploids at the young age used. In contrast, growth of diploid clams within triploid treatment groups was usually slower than the PBI and PBII triploids. This is in contrast to control diploid growth that was always superior to all. Therefore, control diploids are necessary for comparison; diploids within triploid groups are not good controls for triploid evaluation (e.g., Brake et al., 2004).

The observed lower growth of the CB-treated (triploidy induction procedure) diploids compared to untreated control diploids cannot be explained, but should be taken into consideration when evaluating polyploidy. Earlier studies found a negative effect of CB on bivalves through decreased survival during early development (e.g., Stanley et al., 1981; Baron et al., 1989), but no evidence of continued negative impact on larval or juvenile growth (e.g., Baron et al., 1989), as observed in the present study.

Differential growth of diploids within triploid and control groups was seen with the mussel *Mytilus edulis* deployed for eleven months at low-growth sites; mean SL of diploids in the control group was significantly different, but no measurements reported, from confirmed diploids in the experimental triploid group (Brake et al., 2004). The control diploids were then excluded from the comparison and triploids were compared with the experimental diploids only (Brake et al., 2004). They found no differences in mean SL (Brake et al., 2004). It is possible that comparison to control diploids would have given different results.

According to previous studies (Allen et al., 1982; Mason et al., 1988; Hawkins et al., 1994), higher heterozygosity and, as a consequence, faster growth was expected from PBI triploids rather than PBII triploids. Control diploids were expected to show the least values. For example, Stanley et al. (1984) found that triploid American oysters, *C. virginica*, exhibited faster growth only in the PBI triploids, which had the highest heterozygosity among the three groups. Shell height was 12% greater than both diploids and PBII triploids during their first two years of growth. PBII triploids were not significantly larger than diploids.

In the present study, results contrast with that found in other bivalve mollusks, especially triploid oysters (Allen and Downing, 1986; Shpigel et al., 1992; Nell et al., 1994). Growth differences among experimental groups did not support the heterozygosity hypothesis (Stanley et al., 1984) as the growth of PBI triploids was slower than PBII triploids. The control diploids had the greatest growth followed by PBII triploids, and the least being the PBI triploids. This may be caused partially by the initial larger size of PBI triploids, but ploidy still had a significant effect as indicated by ANCOVA. Furthermore, in Experiment II, PBII triploids again did not exhibit a growth advantage over control diploids in any of the tested families. There was no evidence of family effect on triploid-diploid growth. Clams at this age, and under the laboratory conditions

used, were not influenced by family genetics, but by triploidy. Growth rates might be different at different ages or at different environmental conditions. It is possible that superior growth of triploid hard clams will be associated with maturation and spawning as occurs in other bivalves (Guo and Allen, 1994; Eversole et al., 1996; Ruiz-Verdugo et al., 2000; Brake et al., 2004).

Eversole et al. (1996) found no difference in size of triploid hard clams compared to diploids at six and 27 months of age. However, during the same study, triploids showed better growth over the period of 27 to 47 months (Eversole et al., 1996), following at least two spawning periods in diploids. This reflects the possible difference in energy allocation between triploids and diploids (Eversole et al., 1996) during maturation and spawning. Guo and Allen (1994) found that triploid dwarf clam *Mulinia lateralis* were significantly larger than diploids at three months post-fertilization. Similarly, triploid catarina scallop, *Argopecten ventricosus,* started to show growth advantage over diploids after three months of growth, corresponding with maturation (Ruiz-Verdugo et al., 2000). Furthermore, triploid mussels showed better growth during the second year when they were sexually mature (Brake et al., 2004).

5. Conclusion

The size range used in the present study may assist in projections of the expected performance of triploid and diploid clams in grow-out conditions at the time of transplantation. Enhanced growth (shell length and live weight) of triploid hard clams was not shown under our experimental conditions and at the young age of the clams used (3–4 month-old). Triploids grew at similar or slower rates than their control diploid siblings in all cases. However, it is not yet known whether the same superior growth by diploids would occur under stressful conditions, such as lack of food or high temperature. Further research is required to investigate the performance of triploids in the field, especially in view of survival and stress resistance that can be translated into higher production. Additionally, the use of diploids within triploid groups as controls for ploidy comparison is not recommended.

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