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Influence of Triploidy on the Biochemical Composition and Fiber Size of Bay Scallop (*Argopecten irradians*; Lamarck) Adductor Muscle

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ABSTRACT

The adductor muscles of triploid and diploid bay scallops, *Argopecten irradians* (Lamarck 1819) were examined to investigate mechanisms that may be responsible for the greater size often observed in triploid bivalves. Tissue dry weight and biochemical composition of the adductor muscle, as well as number and size of adductor muscle fibers were compared between triploid and diploid bay scallops. Analysis of protein, carbohydrate, and lipid content of bay scallop adductor muscles showed no significant difference between the triploids and diploids. Measurements of adductor muscle fiber size and number of fibers per unit area indicate that the triploids have larger fibers than diploids (17% larger) but do not have a significantly different number of fibers per unit area. Our results suggest that triploids may compensate for larger fiber size by decreasing connective tissue.

Keywords: bivalve, energy, gigantism, polyploidy, reallocation

INTRODUCTION

Triploidy induction in bivalve mollusks is widely used by aquaculturists to achieve better growth rates (Beaumont and Fairbrother 1991). In oysters, triploids are also more marketable due to their sterility or partial sterility (Allen and Downing 1986; Guo *et al.* 1996). It is believed that sterility leads to less biochemical energy being transferred to the gonad, so other tissues gain more weight over time. This is particularly advantageous for pectinids in the US, where the gonad has no commercial value and is discarded while the adductor muscle is highly valued (Gosling 2003). Several studies have already been conducted on pectinid triploid induction (Tabarini 1984; Beaumont 1986; Ruíz-Verdugo *et al.* 2000) and have shown increased growth in triploids.

Triploid induction and performance has been well studied, however, the mechanism causing greater size in triploid bivalves is not yet clear. Several hypotheses have been offered. A genetic hypothesis suggests heterosis or increased fitness of triploids due to higher heterozygosity (Allendorf and Leary 1984; Guo *et al.* 1992), influencing feeding rate, absorption efficiency, and growth efficiency (Hawkins and Day 1999; Hawkins *et al.* 2000). Another genetic hypothesis suggests that triploids will have greater growth due to faster transcription (Magoulas *et al*. 2000; Garnier-Géré *et al.* 2002). An energy reallocation hypothesis states that sterile triploids do not use energy for gametogenesis, thus more energy is directed towards somatic growth. This hypothesis was supported in work on pacific oysters, *Crassostrea gigas*, where triploids were only larger than diploids after reaching sexual maturity (Allen and Downing 1986). Also, Tabarini (1984) found that triploid bay scallops *Argopecten irradians*, grow faster than diploids during gametogenesis. Lastly, the polyploid gigantism hypothesis suggests that increased body size in triploid bivalves is a function of an increase in cell volume but a lack of cell number compensation (Guo and Allen 1994). A nucleus containing an extra set of chromosomes would require a larger cytoplasm so that nutrients and organelles are adequately proportioned during the cell cycle (Guo and Allen 1994). In triploid finfish, there is an increase in cell volume accompanied by a reduction in cell number in most tissues and organs (Benfey 1999). Some studies on triploid bivalves do not show a decrease in cell number, particularly in the adductor muscle (Guo and Allen 1994; Palacios *et al.* 2004). Guo and Allen (1994) suggested further studies to substantiate the polyploid gigantism hypothesis should be directed at adductor muscle size, cell size, and cell number.

In the present study, the adductor muscles of triploid and diploid bay scallops, *Argopecten irradians* (Lamarck 1819), are investigated to examine the energy reallocation and polyploid gigantism hypotheses. Tissue dry weight and biochemical composition of the adductor muscle, as well as number and size of adductor muscle fibers were compared between triploid and diploid organisms.

MATERIALS AND METHODS

Sexually mature (gamete producing) bay scallop broodstock, collected from Lagoon Pond (Oak Bluffs, MA, USA) and Meneshma Pond (Aquinnah, MA, USA), were induced to spawn via thermal shock at the Martha's Vineyard Shellfish Group (MVSG) hatchery (Vineyard Haven, MA). Triploid induction was achieved by chemical treatment. Eggs were placed in a 400 µM solution of 6-dimethylaminopurine (6-DMAP) 15-25 min after fertilization for 11 min. They were then rinsed with seawater and transferred to aerated conicals. An untreated control was simultaneously cultured throughout the experimental period. Ploidy determinations were conducted on samples one month post-spawn (larvae) and then again on samples nine months post-spawn. All samples were shipped to the Virginia Institute of Marine Science (VA, USA) where percent triploid was determined using flow cytometry (Allen and Bushek 1992; Guo *et al.* 1993).

Seed scallops (2-4 mm) were held in a tidal upweller for three

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weeks, and then transferred to bottom cages in the highly productive Katama Bay in Edgartown, MA. Scallops were maintained in these cages at similar densities for two growing seasons (2003-2004). Comparative growth and yield of triploid versus diploid bay scallops is described in Surier *et al.* (2007).

In December 2004, 25 samples each of triploid and diploid adductor muscles were collected from those being held in Katama Bay and were sent on ice to Brooklyn College (New York, USA). The wet weight of each adductor muscle was obtained and the samples were dried at 60°C for at least 48 h. The dried tissue samples were ground to a fine powder. Three replicate subsamples from each individual scallop (n = 25) were used to determine gross carbohydrate, protein, and lipid content. Total carbohydrates were determined using the phenol-sulfuric acid method of Dubois *et al*. (1956). A calibration curve was created using glucose as a standard. The Coomassie blue method (Bradford 1976) was used to determine protein content. Bovine serum albumin was used as a standard in the calibration curve. Total lipid content was quantified using the gravimetric, chloroform-methanol extraction of Folch *et al.* (1957). Specific procedural details of the biochemical analyses are described in Surier *et al.* (2007).

Additional triploid (n = 6) and diploid (n = 10) adductor muscles were processed for histological analysis. A portion of each adductor muscle was fixed in Bouin's solution, embedded in Polyfin™, sectioned transversely (7 µm), and stained with Masson trichrome stain (Presnell and Schreibman 1997). The number of fibers in a fixed grid of smooth muscle in triploid and diploid scallops was enumerated. Smooth muscle fibers (n = 30) were photographed at 240X magnification and their diameter measured using NIH ImageJ software (Palacios *et al*. 2004).

Measurements of diploid and triploid adductor muscle biomass were compared by one-way analysis of variance (SPSS Version 11.5). The Mann-Whitney test (Zar 1999) was employed to test for differences in biochemical content, fiber number, and fiber size following appropriate transformations. All data is presented as mean ± standard error (SE). The level of significance was set at $p < 0.05$.

RESULTS

Ploidy determinations showed the one month post-spawn sample was 80% triploid and the nine-month post-spawn sample was 96% triploid. This suggests that triploid induction was highly successful and it is likely that the individuals used in our study were true triploids.

Adductor muscle size did not differ statistically between ploidy groups. The diploid samples had a mean wet weight of 3.9 ± 0.25 g and a dry weight of 0.90 ± 0.07 g. Triploid samples had a mean wet weight of 4.11 ± 0.19 g and a dry weight of 0.96 ± 0.05 g.

Analysis of protein, carbohydrate, and lipid content of bay scallop adductor muscles yielded no statistically significant difference between the means of the two ploidy groups (**Fig. 1**). Protein content of triploid adductor muscle was 509.71 ± 14.01 µg $mgDW^{-1}$ and the diploids had 483.65 ± 17.11 µg $mgDW^{-1}$ protein. The triploid group had a carbohydrate content of 183.9 ± 8.41 µg $mgDW^{-1}$ that was slightly higher than the carbohydrate content of the diploid group, which was 164.38 ± 10.45 µg $mgDW^{-1}$. The total lipid content of the triploid bay scallops was 39.28 ± 1.3 µg $mgDW^{-1}$ compared to 40.62 ± 1.76 µg $mgDW^{-1}$ for the diploids.

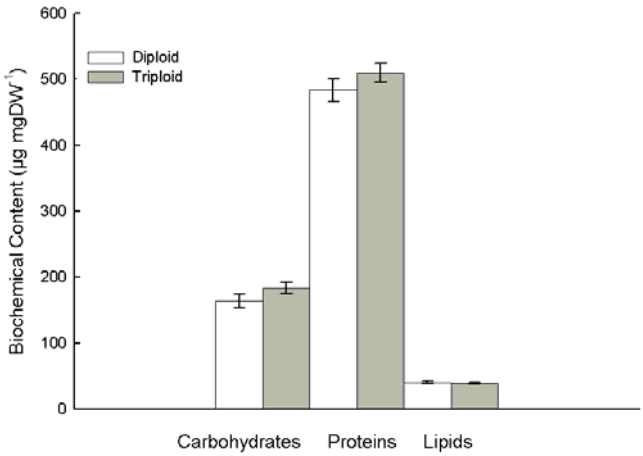

Fig. 1 Gross carbohydrates, proteins, and lipids in the adductor muscles of diploid and triploid *Argopecten irradians* sampled in December 2004. For each ploidy group, n = 25 organisms. The Mann-Whitney test was used to test for differences between groups.

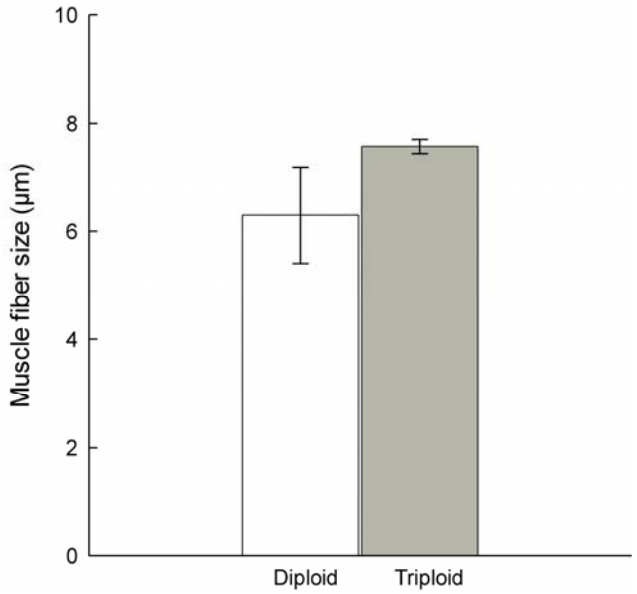

Fig. 2 Adductor muscle fiber size in diploid and triploid *Argopecten irradians* sampled in December 2004. For the diploid group, n = 10 organisms and for the triploid group, n = 6 organisms. The Mann-Whitney test was used to test for differences between groups.

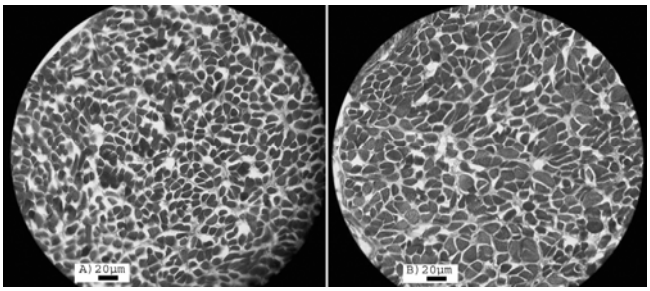

Fig. 3 Histological sections (240X) showing the number and size of adductor muscle fibers of diploid (A) and triploid (B) *Argopecten irradians* sampled in December 2004. For the diploid group, n = 10 organisms and for the triploid group, n = 6 organisms. The Mann-Whitney test was used to test for differences between groups.

Measurements of muscle fiber diameter demonstrated a significant difference (Mann-Whitney; $p < 0.001$) between the ploidy groups (**Figs. 2, 3**). The triploid bay scallops had a mean diameter of 7.58 ± 0.13 µm whereas the diploid muscle fibers measured 6.30 ± 0.09 µm. In contrast, there was no significant difference in the number of muscle fibers per unit area between ploidy groups (**Figs. 3, 4**). The triploid bay scallops had a mean count of 56.65 ± 4.55 fibers and the diploid fiber count was 63.14 ± 4.14.

DISCUSSION

No difference in adductor muscle wet weight and dry weight was observed between the triploid and diploid groups. However, measurements of samples from the same cohort through the summer of 2004 indicated that the triploids' adductor muscles were 52% and 54% larger in wet weight and dry weight, respectively (Surier *et al.* 2007). In the fall of 2004 the triploid adductor muscles were 17 and 9% larger in wet weight and dry weight, respectively (Su-

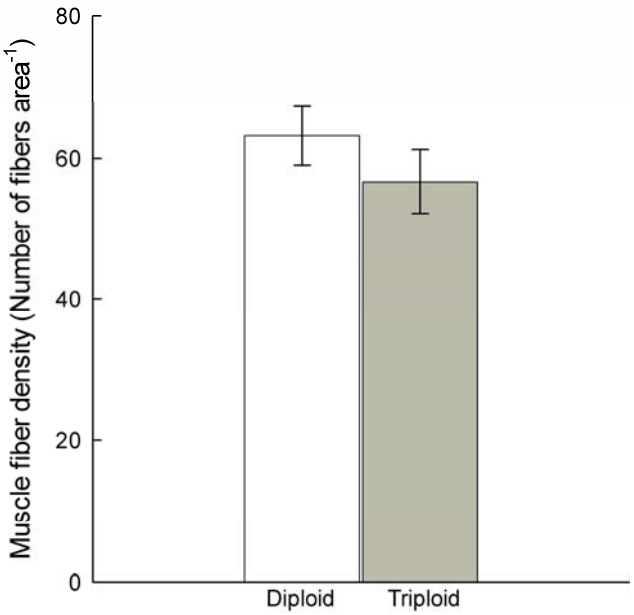

Fig. 4 The number of adductor muscle fibers in diploid and triploid *Argopecten irradians* sampled in December 2004. For the diploid group, $n = 10$ organisms and for the triploid group, $n = 6$ organisms. The Mann-Whitney test was used to test for differences between groups.

rier *et al.* 2007). In addition, measurements on bay scallops of the same cohort at other sites located near MVSG through December 2004 indicated a significantly greater adductor muscle size in triploids as compared to their diploid controls (Surier *et al.* 2007). The lack of triploid adductor muscle size advantage in the samples taken in the present study may be related to the late sampling date. The late sampling date may also partially explain why differences were not observed between triploid and diploid bay scallops in biochemical content. The scallops were collected long after the spawning season, which typically occurs in August and September in the Northeast US (Rhodes 1991). Any disparity between ploidy groups due to resource reallocation may have been expressed prior to spawning as observed by Tabarini (1984). A lack of triploid size advantage along with no differences in biochemical composition was observed by Racotta *et al.* (2008) in lion-paw scallops, *Nodipecten subnodosus*, grown in the high-food environment of Magdalena Bay (Baja California Sur, Mexico). These authors suggested that in a highly productive environment there is no need for energy reserve mobilization since ingested food can directly support gonad development. Although our scallops were grown in the food-rich environment of Katama Bay (Barber and Blake 1983), the model proposed by Racottta *et al.* (2008) could not be applied to our results since differences in adductor muscle size were identified in samples taken earlier in the season as described in Surier *et al.* (2007). Alternatively, feeding efficiencies may play a role in the lack of triploid size advantage. Kesarcodi-Watson *et al.* (2001) found that triploid rock oysters, *Saccostrea glomerata* formerly *S. commercialis*, have a lower scope for growth due to greater energy usage for respiration and reduced clearance rates under high food conditions. Racottta *et al.* (2008) suggested that this may also explain their results with *N. subonodosus* in Magdalena Bay. The lack of triploid size advantage along with no differences in biochemical composition observed in our study may also be explained by this model if there was significant seasonal variation in food concentration (i.e. low food concentration in summer and high food concentration in winter). However, food quantity was not measured in our study.

Measurements of adductor muscle fiber size and number of fibers per unit area indicate that the triploids have larger fibers than diploids (17% larger) but do not have a significantly different number of fibers per unit area. Results seem to show that, although triploids have larger fibers, the volume of a fiber bundle (represented by the number of fibers per unit area) remains constant. This could be explained by a decrease in connective tissue in triploids compensating for a larger fiber size. Although the triploids sampled in this study have larger fibers they cannot explain an increase in somatic tissue volume, thus partially negating the polyploid gigantism hypothesis. An increase in muscle fiber size could however explain an increase in density and weight of triploid tissue in bay scallops sampled by Surier *et al.* (2007) earlier in the year. An increase in fiber size and a lack of fiber reduction was found in triploid catarina scallops (Palacios *et al.* 2004). These authors observed similar results while their triploid muscles were larger than diploids and showed significant difference in carbohydrate content. Our results may be explained by a decrease in connective tissue in triploids compensating for a larger fiber size. In summary, our results demonstrate that triploid bay scallops have 17% larger adductor muscle fibers than diploids, even when there is little difference in adductor muscle size.

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