Aquaculture 499 (2019) 9–16

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Review

A meta-analysis of growth rate in diploid and triploid oysters

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ABSTRACT

Around 34 years ago, the first reports on the performance of triploid oysters were published. Since then, triploid oysters have offered many benefits to the oyster industry, such as faster growth, improved meat quality, partial sterility, and increased survival due to disease resistance. However, the extent of a triploid growth advantage, in particular, can vary across studies, measurement parameters, environmental conditions and husbandry practices. To quantitatively compare diploid and triploid oyster growth rates, a meta-analysis was performed with 29 published studies using triploid oysters produced by chemical induction or by crossing diploid and tetraploid oysters (i.e., "mated triploids"). The difference in growth rate between ploidy was evaluated using natural log transformed response ratios (ln [3n/2n]) in a random-effects model weighted by sample size. The positive response ratios in 126 of the 148 independent experiments showed a significant growth advantage of the triploid over the diploid. On average, mated triploids grew 20% faster than diploids in shell height and 49% faster in whole wet weight. While chemically induced triploids had marginally less growth advantage than mated triploids, growing on average 8% faster than diploids in shell height and 31% faster in whole wet weight. Response ratios for experiments using mated triploids and measuring whole wet weight was significantly affected by species and length of study, while response ratios for experiments using chemically induced triploids and measuring whole wet weight was significantly affected by initial size at deployment. Conversely, response ratios for experiments using mated triploids or chemically induced triploids and measuring shell height were not affected by any tested moderator. The lack of a triploid growth advantage in 15% of the experiments (22/148) could have been influenced by a variety of factors including intraspecific variation, differences in sampling, husbandry practices, and environmental conditions.

1. Introduction

Research into genetic improvements (e.g., polyploidy) correlates with the rise in hatchery produced oysters and a dependence on hatchery produced seed in areas such as, the Pacific Northwest of the U.S. (Clark and Langmo, 1979). Groundbreaking experiments by Stanley et al. (1984) with *Crassostrea virginica* and Allen and Downing (1986) with *C. gigas*, were some of the first to quantify the performance of triploids relative to diploids. By the 1999–2000 season, triploid *C. gigas* accounted for one-third of aquaculture production in Washington and Oregon (Nell, 2002). Soon after, areas on the east coast of the U.S. (Chesapeake Bay) adopted triploid aquaculture for *C. virginica* in response to the collapse of wild oyster stocks. Since 2008, triploids consistently make up around 80 to 95% of total oysters grown in Virginia (Murray and Hudson, 2015; Callam et al., 2016). Nowadays, triploid production for the half-shell market has been adopted worldwide (S. Allen, pers. comm.).

There are two primary techniques to produce triploids: chemical induction or through the mating of a diploid and a tetraploid (i.e., mated triploids). Chemical induction involves either 6-dimethyl-aminopurine (6-DMAP) or cytochalasin B (CB) to retain the first or second polar body thereby blocking meiosis I or meiosis II respectively. CB is a known carcinogen and a more dangerous chemical to work with than 6-DMAP (Gérard et al., 1999). However, CB has been found to produce a higher percentage of triploids than 6-DMAP (Gérard et al., 1999) and is often the most effective chemical used. Treatment of the eggs at meiosis I must be done within the first 15 min after fertilization as to block the first chromosome division and retain 2 N chromosomes. Treatment at meiosis II is done between 15 and 30 min after fertilization to block the second chromosome division and retain 1 N chromosomes that are genetically identical, except in cases where there has been recombination. In this way, meiosis I treatment can result in increased heterozygosity (genetic variation) compared to oysters treated at meiosis II. However, treatment at meiosis I is not common commercially as the triploids are harder to induce, have lower survival and the treatment is more likely to produce aneuploids (Gérard et al., 1999; Hand et al., 1999; Guo et al., 1992).

Chemical induction is not reliable in producing a 100% triploid population, while crossing a tetraploid with a diploid achieves very close to pure triploid (Guo and Allen, 1994b). Since its inception in 1994, tetraploids are now produced in North America (e.g. Stone et al., 2013; Dégremont et al., 2012; Ibarra et al., 2017), Europe (e.g. Buestel et al., 2009), Australia (e.g. Nell and Perkins, 2005), Chile (Cultimar; Tongoy, Chile), China (Guo, 2004) and Korea (Guo et al., 2008).

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https://doi.org/10.1016/j.aquaculture.2018.09.018
Received 15 January 2018; Received in revised form 7 September 2018; Accepted 10 September 2018
Available online 12 September 2018

Tetraploid production is achieved by finding the very small percentage of fecund female triploids, and fertilizing these eggs with sperm from a diploid and blocking the first polar body (Guo and Allen, 1994b). Mated triploids, in turn, are produced by fertilizing female diploid eggs with sperm from male tetraploids. These mated triploids, therefore, receive two sets of chromosomes from the male tetraploid, while chemically induced triploids receive two sets of chromosomes from the female diploid and the two chromosomes are either genetically different (retaining the first polar body) or identical (retaining the second polar body). The distinction in the origin of the extra set of chromosomes is important to note as it could influence differences in triploid performance (Callam et al., 2016; Wang et al., 2002).

Depending on the species and culture conditions, the advantages of triploids can vary from faster growth (Walton et al., 2013; Nell and Perkins 2005), improved meat condition (Garnier-Géré et al., 2002; Barber and Mann, 1991), greater disease resistance (Dégremont et al., 2015) and population control (Guo, 2009). The enhanced performance in triploids can be explained by their partial sterility while faster growth, in particular, is also related to energy reallocation, polypoid gigantism, and increased heterozygosity. Energy reallocation is apparent once oysters reach sexual maturation, where triploids reallocate energy from gametogenesis to somatic growth (Allen and Downing, 1986). Polypoid gigantism helps to explain increased growth prior to sexual maturity, where faster growth in triploids is a result of the increase in cell volume and lack of cell-number compensation (Guo and Allen, 1994a). Only heterozygosity can explain differences in growth between chemically induced and mated triploids. Increased heterozygosity is commonly correlated with faster growth among diploid individuals (Zouros et al., 1988; Alvarez et al., 1989) and is thought to contribute to faster growth in meiosis I triploids compared to meiosis II triploids (Stanley et al., 1984; Hawkins et al., 1994; Mallia et al., 2006) and faster growth in mated triploids compared to meiosis II triploids (Wang et al., 2002). However, studies have found no correlation between increased heterozygosity and triploid growth in other bivalves; *Pinctada martensii* (Jiang et al., 1993), *Mytilus edulis* (Beaumont et al., 1995) and *Mya arenaria* (Allen et al., 1982).

The comparison between triploid and diploid growth and mortality has been found to depend on environmental conditions. Under unfavorable growth conditions (low salinity, low dissolved oxygen, high disease pressure, poor food quality and availability) several studies have cited faster growth in triploids and similar survival to diploids (Garnier-Géré et al., 2002; Smith et al., 2000), faster growth in triploids and lower survival than diploids (Goulletquer et al., 1996; Stanley et al., 1984), or similar growth and lower survival than diploids (Callam, 2013; Cheney et al., 2000). Part of the variation in triploid growth and mortality comes from the difficulty in comparing vastly different waterbodies with "poor" water quality given the complexities of acute (e.g., disease and parasites) and chronic stressors (e.g., low dissolved oxygen, high temperature, low salinity, and harmful algal blooms).

Additionally, measuring oyster growth through morphology and biomass is highly influenced by environmental conditions and poses challenges unlike isodiametric shellfish, such as clams or scallops. Shell morphometry (i.e., shell height, length and width) is influenced by habitat, how the oyster settles on a substrate, how densely packed the oysters are, or, in aquaculture, how they are handled (Harding, 2007; Stone et al., 2013). Whereas biomass (i.e., whole, tissue, and shell weight) is an indicator of food quality, food availability, oyster filtration rate, and fecundity (Chávez-Villalba et al., 2010; Davis, 1994; Li et al., 2009; Cox and Mann, 1992). When determining growth, it is therefore important to use both shell morphometry and biomass to account for effects of the environmental conditions. This study uses meta-analysis to determine whether there is a significant growth advantage of triploid oysters over diploid oysters across a wide range of studies, species, and environmental and physical conditions.

2. Methods

2.1. Literature search

Studies were obtained from several literature databases, including Web of Science, Google Scholar, and Aquatic Sciences and Fisheries Abstracts (ASFA), using combinations of the following relevant keywords: "diploid", "triploid", "oysters" and "growth". Out of 100 results in Web of Science, 2540 results in Google Scholar, and 214 results in ASFA, the list was reduced to 29 independent studies that directly reported growth rates of both diploids and triploids or reported initial and final measurements in shell height, whole wet weight, or both to allow the calculation of an average oyster growth rate. Studies were separated by whether triploids were chemically induced (blocking either polar body I or polar body II during fertilization) or produced through mating a tetraploid and a diploid. When calculating response ratios (described in detail below in Section 2.2), only diploids and triploids of the same species were compared. Comparisons were not made across species, such as between diploid *Crassostrea virginica* and triploid *C. ariakensis*. Six species were included in the dataset: *Crassostrea gigas*, *Crassostrea hongkongensis, Crassostrea madrasensis, Crassostrea virginica*, *Ostrea edulis*, and *Sassostrea glomerata* (formerly *S. commercialis*).

For the meta-analysis, more than one response ratio was calculated from a single publication if the experiments took place in a unique body of water with different environmental parameters, such as temperature and salinity, given their effects on growth. Initial and final shell height, whole wet weight, or both measurements of diploid and triploid oysters were extracted from each study to calculate the growth rate per day (Eq. (1)). Shell height was defined as the length from the hinge to growing edge. When data were presented in figures, the relevant information was extracted using ImageJ software (Rasband, 2014). In studies with significantly different growth between selectively bred diploid or triploid lines, the selected lines were compared separately. At a minimum, data associated with sample size and species were collected. When possible, other parameters, such as temperature, salinity, tidal height, and grow out gear (e.g., cage, floating bag, and lantern net) were noted.

$$\text{Growth rate} = \frac{\text{Final size} - \text{Initial size}}{\text{Days deployed}} \quad (1)$$

where final and initial size were in grams (whole wet weight) or millimeters (shell height).

2.2. Effect size calculations

All calculations were conducted using the *metafor* package (Viechtbauer, 2010) in the statistical software program, R (R Core Team, 2014). The natural log-transformed ratio of means, also called the response ratio (Eq. 2; Hedges et al., 1999), was chosen to quantify the magnitude of the difference between triploid and diploid growth rates. Response ratios compare the mean difference between an experimental treatment (triploid) and a control treatment (diploid) in a unitless ratio and are commonly used in ecology due to the ease of interpretation and strong statistical properties. A natural log-transformed response ratio of zero would indicate no difference in growth between triploids and diploids. Response ratios greater than zero (lower 95% confidence interval is greater than zero) would indicate that triploids grow faster than diploids.

$$\text{Response ratio} = \ln\left(\frac{X_T}{X_D}\right) \quad (2)$$

where X_T and X_D are the growth rate means of the triploid and diploid oysters, respectively.

As most studies included only initial and final measurements, error estimates for growth rate were not available. Instead, studies were