Aquaculture and Fisheries 6 (2021) 75–83

Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries

Use of natural trophic resources by Eastern oysters and Pacific oysters of different ploidy

Darien D. Mizuta [a], Gary H. Wikfors [a,*], Shannon L. Meseck [a], Yaqin Li [a], Mark S. Dixon [a], Hyun Jeong Lim [b], In Joon Hwang [c], Magalí Bazzano [a], Steven Pitchford [a]

[a] Northeast Fisheries Science Center (NEFSC), NOAA, 212 Rogers Ave, Milford, CT, 06460, USA
[b] East Sea Fisheries Research Institute, National Institute of Fisheries Science, Gangneung, 25435, South Korea
[c] West Sea Fisheries Institute, National Institute of Fisheries Science, Incheon, 22383, South Korea

ARTICLE INFO

Keywords:
Ploidy
Farmed oysters
Feeding
Biodeposition
Seston
Metabolism

ABSTRACT

Ploidy manipulation, such as triploidy, in farmed oysters has been used as a tool to enhance oyster quality throughout all seasons because triploid oysters allocate less energy to gametogenesis, and therefore are deemed better performers than fertile diploids. Nevertheless, scientific reports describing no differences between ploidies and, in certain conditions, disadvantages of triploids are not uncommon. As the use of triploid oyster seed increases culture cost for growers, consistency in performance of triploids is considered to be an important goal. Thus, research to assess how ploidy affects physiological processes underlying oyster performance is fundamental for the aquaculture industry. This work was undertaken to assess if ploidy-based differences in performance in the two most commonly cultivated commercial species of oyster in the United States, *Crassostrea virginica* and *Crassostrea gigas*, are associated with filtration, feeding, and metabolism. To test this hypothesis, biodeposition measurements were made with oysters exposed to ambient water conditions at locations and seasons providing a variety of environmental conditions. Oysters did not show differences in filtration and feeding associated with ploidy, but physiological feeding variables fluctuated with environmental characteristics associated with spatial and seasonal differences. A preliminarily test of the hypothesis that differences in energy metabolism may account for differences in performance among ploidy levels indicates that basal metabolic rates of diploid, triploid, and tetraploid Eastern oysters are not different.

1. Introduction

Manipulation of ploidy, or the number of chromosome sets, in oysters has been targeted as one means to improve production and quality of cultured products (Walton et al., 2013). Triploid oysters originally were produced experimentally on the US East Coast in the late 20th century (Stanley, Allen, & Hidu, 1981) and later, for practical purpose of preventing spawning of non-native Pacific oysters (Allen, Downing, & Chew, 1989). Triploids became possible on a large scale because of the development of tetraploid oysters (Guo & Allen, 1996). Commercial triploid oyster seed are created in hatcheries principally by crossing tetraploid males with diploid females and are referred to as "biological triploids" (Guo & Allen, 1996), as opposed to chemically-induced triploids. Triploidy reduces reproductive effort; triploid individuals produce minimal amounts of gonadal tissue and tend not to spawn, instead allocating energy mostly to growth (Hawkins et al., 2000). Thus, faster-growing triploids have higher meat quality during the normal spawning season in summer, coincident with high market demand and when diploid oyster quality suffers from spawning condition (Nell, 2002). Triploids were additionally reported as having better shape (Walton et al., 2013) and higher condition indices (Matthiessen & Davis, 1992; Nell, 2002; Shpigel, Barber, & Mann, 1992; Wadsworth, 2018) regardless whether it was of induced or biological origin.

A further incentive to grow triploid oysters is that triploid oysters tend to reach market size faster, reducing exposure time to diseases that could cause mortality (Barber & Mann, 1991), which is especially important in disease-affected areas where shellfish farming is performed. In relation to resistance to diseases, triploidy has provided different results depending upon species, site, and method of triploidy induction, with a general neutral result for survival, when compared to diploids (*cf.* Dégremont, Garcia, & Allen, 2015).

Literature is inconclusive on survivorship of triploid versus diploid,

* Corresponding author. Northeast Fisheries Science Center (NEFSC), NOAA, 212 Rogers Ave, Milford, CT, 06460, USA.
E-mail address: gary.wikfors@noaa.gov (G.H. Wikfors).

https://doi.org/10.1016/j.aaf.2020.03.011
Received 27 August 2019; Received in revised form 3 February 2020; Accepted 23 March 2020
Available online 18 April 2020

regardless of method of triploid production. Some reports describe superior survival of triploids (Dégremont, Garcia, Frank-Lawale, & Allen, 2012), which seems to vary with site and latitude (Ibarra, Ascencion-Michel, Ramirez, Manzano-Sarabia, & Rodriguez-Jaramilo, 2017); whereas, others report similar survival between ploidies (Walton et al., 2013). Cheney, MacDonald, & Elston (2000) studied summer mortality and reported that Pacific oyster triploid mortality began earlier and spiked higher than diploids under high temperature and low dissolved oxygen conditions typical of the season. In Incheon, South Korea, however, farmed triploids seem to resist mortalities that affect diploids during the summer (Lim et al., 2018; *unpublished data)*. Recently there have been indications that triploid oysters have some disadvantages in some growing areas. Several commercial growers reported higher rates of mortality in triploids, sometimes up to 90% of a crop during summer, indicating a failure to thrive when compared to diploids grown at the same location (Guévélou et al., 2019; Wadsworth, 2018). Thus, overall advantages and disadvantages of triploid oyster performance are inconsistent in the scientific literature.

In farming operations in USA, notably in Chesapeake Bay, triploids are used widely (Murray & Hudson, 2012). The technology required to produce hatchery-bred triploid oysters imposes a cost on growers that must be justified by increased product quality and value or higher crop productivity. Accordingly, comparing differences in physiology between oyster ploidies provides valuable commercial information to guide the shellfish industry, especially when current knowledge is scarce and conflicting.

As illustrated by the studies cited above, research on the performance of diploid and triploid shellfish has concentrated in growth, survival, reproduction, and resistance to pathogens (see for example in Nell, 2002; Walton et al., 2013; Dégremont et al., 2015; Ibarra et al., 2017). Food acquisition and assimilation underlie performance of bivalves. Yet, feeding studies comparing bivalves of different ploidy are rare at present. Feeding behavior and metabolic efficiency in Pacific oyster diploids and chemical triploids was compared by Hawkins et al. (2000) under laboratory conditions and found to be higher in triploids. McCarthy, Crawford, Eriksen, & Ross (2016) found no difference in stomach contents of triploids and diploids at the same area but discussed how triploid advantage may depend upon environmental conditions. Building upon these findings, a reasonable hypothesis is that oysters of different ploidy have contrasting performance because they may assimilate food differently when subjected to the same environmental conditions, as diploid and triploid oysters may have different capacities to exploit natural phytoplankton food sources that vary with season. Indeed, in one of the few studies on the topic, juvenile and adult Sydney rock oyster (*Saccostrea commercialis*) triploids were unable to utilize food as efficiently as diploid counterparts in environments with high food availability (Kesarcodi-Watson, Klumpp, & Lucas, 2001a).

As metabolism also plays a role in energy acquisition through food conversion, studies focusing on differences in metabolism between ploidies also are foundational to ploidy performance. Measurements of metabolic rates in whole-animals using sealed chambers and oxygen measurements are used often, but are expensive and labor-intensive. For screening of relatively large numbers of oysters for relative metabolic rate, a flow-cytometric method based upon a chemical probe, DCFH-DA that is oxidized to a fluorescent compound, DCF, can be used within oyster hemocytes (Lambert, Soudant, Choquet, & Paillard, 2003).

Researchers familiar with ploidy manipulation in oysters are aware of flow-cytometry as a technology used to quantify the DNA content of individual cells to ascertain ploidy (Allen, 1983). A cell-permeable, fluorescent compound that binds to nucleic acids, propidium iodide, is used in this application. Beyond this kind of quantitative assay of cell contents, many flow-cytometric methods have been developed to measure metabolic processes in cells (Shapiro, 2005), often in circulating cells such as blood cells in vertebrates and equivalent cells, termed hemocytes, in oysters and other invertebrates. DCFH-DA oxidation within cells has been used widely as an indicator of "oxidative stress" in oysters and other invertebrates (Bass et al., 1983). Cellular processes leading to the oxidation of non-fluorescent DCFH-DA to the fluorescent compound DCF have been shown to be complex and to differ between mammalian blood-cell types, for example (Kalyanaraman et al., 2012). Nevertheless, for a given cell type, the rate of oxidation of DCFH-DA to DCF is dependent upon the rate of oxidative metabolism – essentially basal metabolic rate – unless perturbed by a physiological stress that stimulates additional oxidative processes (Bass et al., 1983). In the present study, we took advantage of the general relationship between oxidative metabolism and DCFH-DA oxidation as a metabolic proxy to compare metabolic rates of diploid, triploid, and tetraploid oysters maintained under identical conditions and sampled without induction of stress-stimulated ROS.

Characterization of filtration and feeding rates and metabolism of diploid and triploid oysters in natural field conditions may help elucidate oyster performance and resulting production differences obtained in previous studies. To verify if differences in performance of different ploidies could have a nutritional basis, the biodeposition method of Iglesias, Urrutia, Navarro, & Ibarrola (1998) was used with the two most widely-farmed oyster species in the world (FAO, 2018), *C. virginica and C. gigas,* to assess possible ploidy differences in filtration and feeding variables. Additionally, we investigated possible differences in catabolic metabolism through measurements of hemocyte oxidative metabolism as a first investigation of metabolic-rate variance among oysters of different ploidy. Biological triploids were used to be consistent with the triploids used in most commercial farming enterprises. To our knowledge, this is the first attempt to identify performance differences in both Eastern oysters and Pacific oysters of different ploidy using the same methodology.

2. Material and methods

2.1. Site descriptions

Experiments were performed with *C. virginica* in two locations in the USA during three seasons (spring in May, summer in August, and autumn in November) and with *C. gigas* at one location in South Korea (in summer in June) in 2017.

In Virginia, within the Chesapeake Bay estuary, biodeposition measurements were made at two locations: Virginia Institute of Marine Science (VIMS), Gloucester Point, York River (37.247° N and 76.504° W, referred to as 'GP'), and the Kaufman Aquaculture Center (also part of VIMS) located in Topping on Locklies Creek near the mouth of the Rappahannock River (37.588° N and 76.436° W; referred as 'RH'). The York River can be considered a weakly-eutrophic system compared to others in Chesapeake Bay (Sin, Wetzel, & Anderson, 1999). Total freshwater discharge in the York River, that includes two tributaries, is 70 m^3 s^{-1}, and salinity varies with tide, with saltwater intrusion to 31 km upriver during low flow (Bender, 1986; Hyer, 1977). The Rappahannock River has been seasonally hypoxic in the lower portion of the river; whereas, the mid-reach is characterized by high chlorophyll, and the upper reach is dominated by sewage discharges (Park, Kuo, & Neilson, 1996). When water temperature exceeds 20 °C, the dissolved-oxygen concentrations in the rivers reach less than 50% saturation, with hypoxic conditions appearing earlier and lasting longer in the Rappahannock River than the York River (Kuo & Neilson, 1987). Biodeposition measurements were conducted on May 22, August 14, and November 14 of 2017 at Gloucester Point, and on May 23, August 15, and November 15 of 2017 at the Rappahannock site.

In South Korea the measurement was conducted at the West Sea Fisheries Research Institute in Incheon (37.449° N and 126.375° W; referred as 'IN'), using water pumped from the adjacent coastal area. A single measurement was conducted during summer, June 16 of 2017.

2.2. Water characterization

To characterize the water during the biodeposition measurements, a handheld YSI 85 (YSI Incorporated, Yellow Springs, OH, USA) was used to collect salinity, temperature, and oxygen values. Samples also were collected for chlorophyll *a* and particle size determination on a flow cytometer. Water samples collected during the filtration measurements were collected for assessment of organic and inorganic particulate matter.

The two USA sites are known to have seasonal harmful algal species that may affect shellfish feeding or cause mortalities, such as *Prorocentrum minimum, Alexandrium monilatum*, *Margalefidinium* (previously *Cochlodium*) *polykrikoides*, *Karlodinium veneficum* (e.g.: Glibert, Alexander, Meritt, North, & Stoecker, 2007; Stoecker, Adolf, Place, Glibert, & Merit, 2008; Li, Glibert, & Gao, 2015). To ensure that the biodeposition experiments compared feeding performance without effects of HAB species, water samples were evaluated microscopically for harmful species immediately prior to experiments.

2.2.1. Chlorophyll a

For chlorophyll *a* (chl *a*) analysis, three replicates of 250–500 mL water were passed through a GF/F glass fiber filter, and filters were stored at −20 °C until analysis. Upon analyses, each filter was placed in a glass tube with 90% acetone and kept refrigerated overnight (20 h) to allow for pigment extraction. Samples were centrifuged at 1000 × *g* for 10 min at 4 °C, and the supernatant was drawn for chlorophyll *a* determination using a model 10-AU Turner Designs Fluorometer pre-calibrated with a chlorophyll *a* standard from Turner Designs Inc. (Sunnyvale, California, USA) or Sigma-Aldrich (Saint Louis, Missouri, USA).

2.2.2. Particle differentiation and quantification by flow-cytometer

Water samples were pre-screened through a 180-μm nylon sieve to remove large particles. Samples then were fixed with 4% (v/v) formaldehyde. Sample were analysed by a C6+ flow cytometer (BD Biosciences, formerly Accuri) equipped with a 488-nm laser flow-cytometer followed the procedures of Li, Veilleux, & Wikfors (2009) with slight modification was used. The flow-cytometer was adjusted to obtain quantitative data on fluorescence at 670 nm (FL3) related to chlorophyll *a*, and forward scatter (FSC), which is related to particle size for each particle that passes the laser interrogation point. Phytoplankton or "detritus" (non-phytoplankton) particles were discriminated based upon the amount of red fluorescence detected as FL3 with a 670 LP filter. Particles were also grouped into two size categories, 2–20 μm, and >20 μm, based upon FSC. Particles with known sizes (DUKE STANDARDS™ microspheres, Thermo Scientific, Waltham, MA, USA) were used to determine particle size ranges with FSC.

2.3. Biodeposition measurements

All Eastern oysters used for the biodepositon study were provided by VIMS and suspended in mesh bags at each site for two weeks before the measurements were conducted. Diploid, triploid, and tetraploid oysters were sampled haphazardly from their grow-out bags before the start of each experiment. Oysters used in the experiments at both sites were from the same populations and ranged in size from 40 to 73 mm. The Pacific oysters used in Korea were spawned in a research hatchery and seeded at 5 mm in a tidal aquaculture farm in the nearby city of Taean for growout and then transferred to Incheon and acclimated. Oyster sizes varied from 59 to 100 mm in shell length (Table 1).

Feeding rates of oysters on natural seston were measured *in situ*. The portable flow-through device that allows these measurements is described elsewhere (Galimany et al., 2011, 2017). Briefly, a submersible pump continuously pumps seawater into an aerated, PVC reservoir tank that feeds into 20 individual chambers. From the reservoir tank, seawater flows through each chamber at a rate of 12 L/h, which allows for homogenous distribution of particles within chambers (for details see Galimany et al., 2011).

Oysters were cleaned of all encrusting organisms prior to placement in a chamber to ensure that filtration was by oysters only. Oysters were positioned in chambers with the hinge facing the water inflow to prevent influence of direct flow upon feeding activity (Eckman, Peterson, & Cahalan, 1989). For each individual measurement with Eastern oysters, 6 diploid oysters, 6 triploid oysters, and 4 tetraploid oysters were added to individual chambers ($n = 16$); additionally, 4 empty oyster shells (controls) were added to separate chambers. Experiments with Pacific oysters in Korea consisted of 8 diploids and 8 triploids ($n = 16$), and again 4 empty-shell controls. Oysters were considered acclimated to the system when they opened valves and began to feed, producing visible pseudofeces deposits and feces (typically 2–3 h). Apart from the number of oysters of each ploidy, set up and management proceeded identically in all biodeposition measurements.

While the oysters were being acclimated to the chambers, oyster gut transit time (GTT) was determined. The GTT was the minimum time for an organic particle to pass through the digestive tract of the oyster after ingestion (Hawkins et al., 1998). Two oysters from each ploidy group were placed in individual containers filled with seawater from the site and with added cultured green algae *Tetraselmis chui* (PLY429) from the NEFSC Milford Laboratory collection. Initially, oysters were closed, thus the time of re-start of filtration as determined by valve gape was noted, and feces were examined until green colored pellets from passage of *T. chui* was detected. The GTT was calculated from the time the oyster first opened valves until the green feces were first produced. This time was used to determine when feces and pseudofeces collection would occur in the biodeposition apparatus.

Once most of the oysters in the chambers were actively feeding, chambers were cleaned of any previously-produced biodeposits, and water-sample collection and biodeposit collection was started: feces and pseudofeces collection corresponded to water sampling time plus gut-transit time. Water samples were collected to determine seston availability. Feces and pseudofeces were collected to determine how the oysters processed the available seston. The GTT is used to offset the collection of biodeposits from the collection of water samples by the amount of time the oysters process ingested seston internally. Water samples (~150 mL) were collected from three locations on the biodeposition system: water entering the reservoir and the two control

Table 1
Technical details of sampling.

Species	Country/Region	Season of experiment	Oyster size (mm ± s.d.)		
			Diploid	Triploid	Tetraploid
Eastern oyster *Crassostrea virginica*	Gloucester Point (GP), Virginia	Spring (May)	53.8 ± 8.8	64.8 ± 5.8	59.5 ± 7.8
		Summer (August)	62.9 ± 3.2	61.4 ± 4.6	65.0 ± 6.4
		Autumn (November)	64.5 ± 6.7	66.3 ± 4.4	55 ± 3.9
	Rappahannock River (RH), Virginia	Spring (May)	58.9 ± 8.7	63.2 ± 5.1	51.0 ± n.a.
		Summer (August)	63.4 ± 7.1	44.6 ± 6.3	64.4 ± 5.0
		Autumn (November)	59.2 ± 7.8	68.8 ± 4.9	65.5 ± 1.4
Pacific oyster *Crassostrea gigas*	Incheon (IN), South Korea	Summer (June)	65.6 ± 4.8	87.4 ± 9.15	n.a.

chambers to allow estimation of particles trapped by the apparatus itself. For each individual oyster, biodeposits were separated and collected with a glass pipette until sufficient biodeposit collection for quantification.

All samples (water seston and mussel biodeposits) were filtered separately on combusted (450 °C for 8 h) pre-weighed, Whatman 25-mm GF/C filters. All samples were rinsed with isotonic ammonium formate, folded in half, stored in aluminum foil, and immediately placed on ice. Filters were frozen until analysis at the laboratory. All filters were processed for total particulate matter (TPM), particulate inorganic matter (PIM), and particulate organic matter (POM). TPM was determined by drying the filters to a constant weight for 5 days at 60 °C. PIM was determined by combusting the filters at 450 °C for 4 h. POM was calculated as the difference between TPM and PIM. The organic content of the seston was calculated as a ratio of the organic and total particulate matter (f = POM/TPM). The PIM and POM from the water and biodeposits were used to calculate feeding variables of oysters (Galimany et al., 2013, 2017; Iglesias et al., 1998). Not all oysters opened during the feeding measurements, thus oysters that did not actively filter were excluded from analysis.

All oysters were collected in individually-labeled bags and placed on ice for subsequent processing. Within 48 h, oysters were measured for shell length (mm) with a caliper and shucked. From each oyster, a small section (3 mm × 3 mm) of gill tissue was extracted, placed in a 1.5-mL Eppendorf tube filled with filtered seawater, and shipped on ice to the Aquaculture Genetics and Breeding Technology Center (ABC) Flow Cytometry Laboratory at the Virginia Institute of Marine Science for flow cytometry analysis to confirm ploidy. Dissection shears were washed with acetone between samples. The remaining soft tissue from each oyster was placed in pre-weighed, aluminum weighing dish and dried to constant weight at 60 °C for determination of tissue dry weight to standardize all feeding variables to 1 g of dried oyster flesh, following the equation:

$$Y_s = Y_e\ (1/W_e)^{bw}$$

here Y_s is the standardized physiological rate, Y_e is the experimentally-determined rate, and W_e is the measured dry body mass. We adopted a *b* value of 0.73 for both oyster species, as determined for *C. virginica* by Riisgård (1988) and for *C. gigas* by Gerdes (1983).

2.4. Filtration and feeding variables

Clearance rate, filtration rate, rejection proportion, organic ingestion rate, absorption rate, absorption efficiency, and selection efficiency were calculated according to Table 2.

Table 2
Calculation of physiological feeding variables.

Parameter	Units	Calculation
Clearance rate (CR)	L/h	(mg inorganic matter from both feces and pseudofeces per unit of time (mg/h))/(mg inorganic matter (PIM; mg/L) in bay water)
Filtration rate (FR)	mg/h	CR · TPM (mg/L) in the bay water
Rejection Rate (RR)	%	[(total rejection rate mg/h)/(total filtration rate (mg/h)] × 100
Organic ingestion rate (OIR)	mg/h	(CR · POM (mg/L) in the bay water) - (rejection rate of organic matter (mg/h))
Absorption rate (AR)	mg/h	OIR – (egestion rate of organic matter)
Absorption efficiency (AE)	fraction	AR/OIR
Selection efficiency (SE)	fraction	1 - [(organic fraction with pseudofeces)/(organic fraction within total particles available in the water)]

2.5. Catabolic metabolism measurement

Procedurally, we followed the protocol described by Lambert et al. (2003). Measurements were carried out with *C. virginica* to explore possible differences in catabolic metabolism of oysters with different ploidy. Flow cytometric analyses measured the rate of oxidation of the non-fluorescent substrate DCFH-DA (InVitrogen product number D6883; Sigma-Aldrich, St. Louis, MO, USA) to a green-fluorescent product dichlorofluorescein (DCF) by oyster hemocytes *in vitro*. The method is based upon the oxidation of intracellular DCFH that is quantitatively related to the oxidative metabolism of hemocytes and measurable on the FL1 detector of a flow cytometer (see Lambert et al., 2003 for details). These analyses took place on August 21st and 22nd, 2018, at the Virginia Institute of Marine Science in Gloucester Point, Virginia, where diploid (n = 17), triploid (n = 16), and tetraploid (n = 14) Eastern oysters, *C. virginica,* had been grown in similar seston and temperature conditions. During analysis, oysters were kept at an ambient water temperature between 29.5 °C and 31.5 °C. Hemolymph was removed from the adductor muscle of each oyster using a needle of 23G x 1" and 1-mL syringe. Immediately after bleeding, 100 μL of hemolymph from each oyster was placed in a 2-mL Eppendorf tube with 200 μL of filtered seawater and 4 μL of 1 mM DCFH-DA solution in DMSO. Tubes were mixed and analysed in a BD Accuri C6+ Flow Cytometer with a threshold of 80,000 on FSC-H. Reading of DCF fluorescence was performed every minute for the first 10 min with a sample acquisition of 10-μL and flow rate of 66 μL/min. A regression plot for each oyster yielded the corresponding slope, which is proportional to the catabolic rate of DCFH-DA by hemocytes. Granular and agranular hemocytes were plotted separately. Again, ploidy analysis of all oysters was confirmed by flow cytometric analyses at ABC. Shell length of each oyster was recorded.

2.6. Statistical analysis

Statistics were calculated separately for each studied species, except for GTT. Data were checked for normality using the distribution fitting model a Shapiro-Wilk test. Individual One-Way ANOVAs were performed to determine significance of season, location, and ploidy upon filtration and feeding variables. For non-normally-distributed data, comparisons of medians were performed using the Kruskal-Wallis test at the 95% confidence interval. Simple regressions (linear model) were used to analyze possible relationships between mean values of FR and size, CR and S and T for each ploidy and time. All analyses were performed using the software Statgraphics Centurion XVII Version 17.1.12 (Statpoint Technologies). Results are shown by species. When results between two studied species of oysters were compared, it is clearly indicated.

For the oysters used in the catabolic metabolism measurement, both shell length and raw slope data were compared using ANOVA followed by Tukey's pairwise tests. Raw slope data from the regression of the mean fluorescence values was square root transformed to meet the assumptions of normality. The software used for these analysis was PAST (Paleontological Statistics Software Package for Education and Data Analysis) Version 3.21 (Paleontological Association, 2001).

3. Results

3.1. Environmental conditions and seawater quality

3.1.1. Virginia, USA

Temperature and salinity varied seasonally but not between sampling sites (Table 3). During the study, temperature ranged from 10.3 °C to 27.6 °C. Although both stations were located on rivers, salinity at Gloucester Point (GP) was higher than Rappahannock (RH). The highest water temperature was reached during the sampling in August, which corresponded to the lowest dissolved oxygen (DO) concentration typical

Table 3
Environmental variables (temperature, salinity, and dissolved oxygen) for all biodeposition measurements. N/A stands for data not available.

Date	Temperature (°C)	Salinity	Dissolved Oxygen (mg/L)	GTT (min)
Gloucester Point (GP)				
Spring (May)	21.7	17.2	N/A	55
Summer (August)	27.2	20.0	2.93	54
Autumn (November)	13.3	19.5	8.14	130
Rappahannock (RH)				
Spring (May)	18.0	14.0	N/A	107
Summer (August)	27.6	15.0	3.2	39
Autumn (November)	10.3	16.4	10.85	108
Incheon (IN)				
Summer (June)	21.0	31.4	10.5	63

of hypoxic conditions in enclosed bays (<3 mg/L; Mizuta, Kasai, Ishii, Yamaguchi, & Nakata, 2014). DO increased to saturated levels in October (Table 3). Water temperature was the coldest in November when DO was saturated. Chl *a* values varied from 1.92 to 12.8 μg/L, doubling in concentration during the summer season in both studied sites in Virginia, when compared to spring and autumn samplings (Fig. 1). Organic content of the seston (f) varied between sites. Only at the RH site was there a decrease in TPM, but an increase in organic content from spring to autumn; whereas, at GP seston was stable throughout the seasons sampled (Fig. 2, Table 4). The particle sizes found in the seston, including for phytoplankton cells, were mostly between 2 and 20 μm in size, which is in the nanoplankton range. Total numbers of microalgal cells were consistent in all sampling seasons, with a slight increase at GP in August matching the higher chlorophyll *a* concentration. The ratio between total phytoplankton and non-phytoplanktonic particle concentration was similar in all samplings, except in August when the ratio increased with more phytoplankton cells and fewer non-phytoplankton particles (Fig. 3). Unfortunately, November data are not available because of sample loss.

3.1.2. Incheon, South Korea

Water temperature during summer was 21.0 °C, similar to spring in Virginia. The concentration of dissolved oxygen was high (DO = 10.5 mg/L), and salinity at the site was 31.4 (Table 3). TPM and organic content were in the same range as the RP American site.

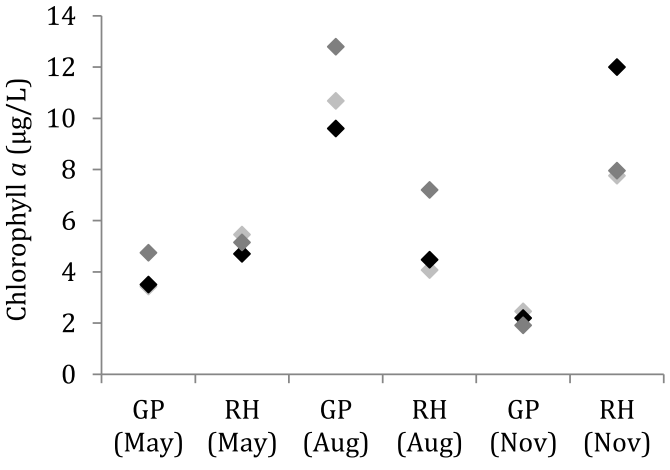

Fig. 1. Seasonal changes in organic content of seston at Gloucester and Rappahannock sites in Virginia, USA.

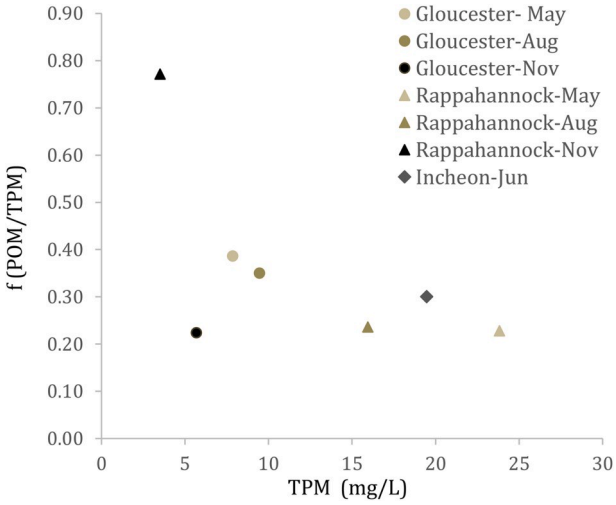

Fig. 2. Chlorophyll *a* values in different seasons at USA stations. Different colors distinguish the 3 replicates.

Table 4
Particulate matter characteristics of the water (mean ± s.d.) in different seasons at different stations.

Date	TPM (mg/L)	PIM (mg/L)	POM (mg/L)	f (POM/TPM)
Gloucester Point (GP)				
Spring (May)	7.85 ± 2.09	4.82 ± 1.59	3.03 ± 0.64	0.39
Summer (August)	9.46 ± 1.03	6.31 ± 0.87	3.31 ± 0.80	0.35
Autumn (November)	5.68 ± 1.63	4.41 ± 1.32	1.27 ± 0.38	0.22
Rappahannock (RH)				
Spring (May)	23.83 ± 4.33	18.40 ± 3.23	5.43 ± 1.38	0.23
Summer (August)	15.94 ± 1.90	12.18 ± 1.63	3.76 ± 0.41	0.24
Autumn (November)	3.50 ± 0.34	1.68 ± 0.25	2.70 ± 0.40	0.77
Incheon (IN)				
Summer (June)	22.02 ± 18.71	16.37 ± 15.86	5.65 ± 6.57	0.30

3.2. Oyster performance

Mean gut transit times (Table 3) of both species were negatively related to seasonal temperature for both species of oyster, as oysters responded with increasingly higher metabolic rates when temperature was higher ($y = -0.1527x + 32.5$; $R^2 = 0.67$; $n = 7$).

3.2.1. Crassostrea virginica

There was no difference attributable to ploidy for any oyster feeding physiological variables (Kruskal-Wallis, Df = 2, h > 0.05). Feeding responses however did vary temporally (all feeding physiological attributes except SE) and spatially (all feeding physiological attributes except AE; Table 5).

Considering the oyster hemocyte metabolic measurements, there was no difference in mean catabolic rate of agranular hemocytes from the different ploidy groups. The mean slope of fluorescence intensity in diploid granular cells, thus oxidative metabolism of those cells, was statistically larger (mean = 24,591) than the mean slope of triploids (mean = 16,935) and tetraploids (mean = 15,890; Fig. 4). Oyster shells were significantly longer in diploids (mean = 59.7 mm) than triploids (mean = 53.2 mm) and tetraploids (mean = 51.3 mm).

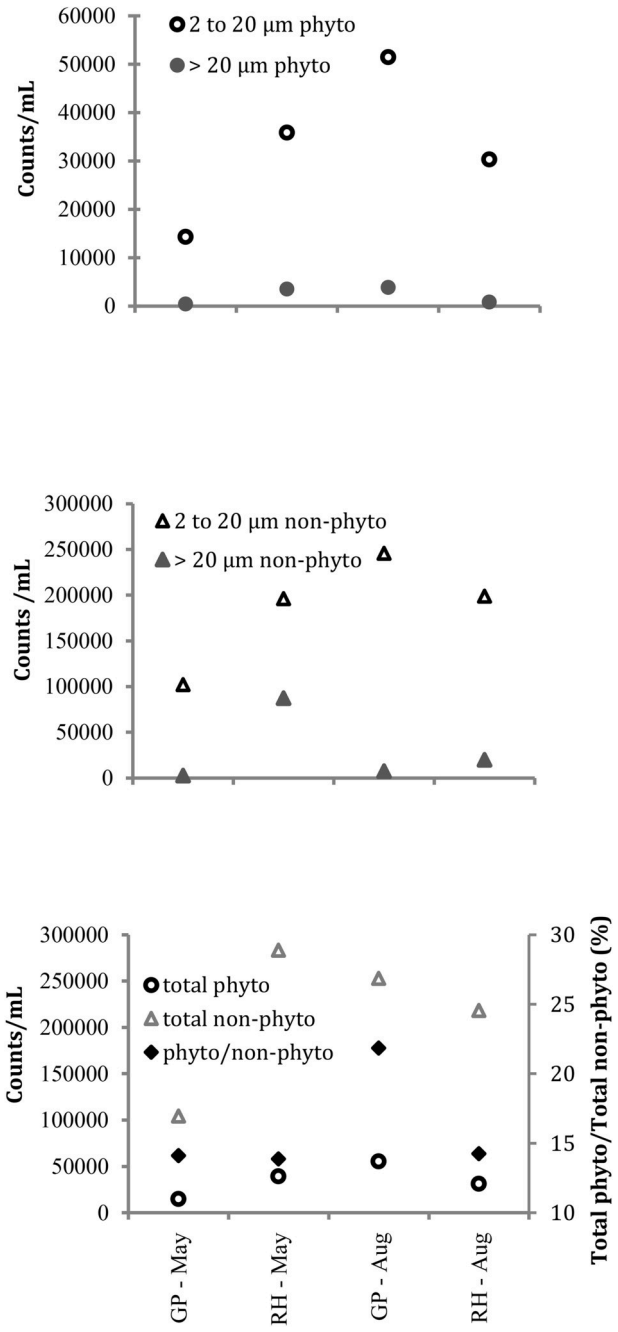

Fig. 3. Phytoplankton and non-phytoplankton seston components at Gloucester Point and Rappahannock River sites, Virginia, USA, in spring and summer. 'Phyto' means phytoplankton and 'non-phyto' means non-phytoplankton.

3.2.2. *Crassostrea gigas*

Differently than Eastern oysters, AE was statistically different between diploid and triploid Pacific oysters (ANOVA, Df = 1, $F = 14.07$, $p < 0.01$), being statistically higher in triploids (Table 5). There was, however, no difference attributable to ploidy for all other oyster feeding physiological variables (Kruskal-Wallis, Df = 1, h > 0.05).

4. Discussion

This study was conducted to determine if filtration and feeding rates in oysters with different ploidy levels were the same. The result is an unambiguous "yes" for Eastern oysters and a preliminary "maybe not" for Pacific oysters. Physiological feeding rates in shellfish vary with species and environmental conditions; most often responses are related to both factors. In the present study, we confirmed expected environmental influences at the studied stations.

Previous investigations showed that clearance rate, as the volume of water cleared of particles per unit of time, in oysters is likely to vary continuously in response to seston composition (Bayne, 2017; Cranford, Ward, & Shumway, 2011). Shellfish are known to remove particles efficiently if both organic and inorganic particles in suspension are larger than 3–5 µm to up to 20 µm (Jorgensen, 1966; Bayne & Newell, 1983). In our Eastern oyster sites, seston quality varied with season, exposing oysters to a range of environmental scenarios. Oysters showed different feeding activities at different sites and seasons, but these responses did not differ between ploidy groups. Some authors found that clearance rates were negatively related to food concentration in both diploids and triploids, but the relationship was especially strong for adult triploid oysters (Kesarcodi-Watson et al., 2001a). For *C. virginica*, our results in August, when chl *a* was higher, did not indicate the same trend. Additionally, triploids and diploids also performed similarly at the Rappahannock site in November where more than 75% of the seston was made up of organic content by weight (Fig. 2; Table 4).

Salinity changes also can affect clearance rate, and for *C. virginica*, it is generally accepted that clearance rates are higher and optimal when salinities are between 15 and 25 (Casas et al., 2018; Galtsoff, 1964). Salinity was within this range at all sampling times in Virginia, but at the lower end of the ideal range at the Rappahannock site in spring and summer (S = 14 and 15, respectively). At the latter relatively low salinity site, oysters had the highest clearance rates (CR = 3.7–6.8 L/h^{-}, Table 5) and filtration rates (FR = 61.5–113.2 mg/L^{-}, Table 5) in the present study. Nevertheless, overall there was no statistical relationship between clearance rate and salinity ($R^2 = 6.75.10^{-3}$; $F = 0.12$, $p = 0.730$); whereas, CR increased significantly with higher temperatures ($R^2 = 0.62$; $F = 29.55$, $p < 0.01$).

Similarly, organic ingestion rate (OIR), the proportion of filtered material rejected as pseudofeces (RR), and selection efficiency (SE) were not different between ploidy groups, indicating that oysters of different ploidy do not select particles differently. This corroborates previous studies in *S. commercialis* wherein pseudofeces production in diploids and triploids was not significantly different across a wide range of food concentrations (Kesarcodi-Watson et al., 2001a). SE is thought to vary considerably with turbidity as selection is not as efficient at high seston loads (Gosling, 2015, p. 524), but this was the only feeding variable that did not vary temporally in the present study. This is possibly because of limitation in selection process, as both rejecting the few inorganic particles when organic content increases and selecting more organics when 'f' decreases is difficult. Additionally, previous studies report great variability in SE responses only with different diet sources and when TPM was greater than 20 mg/L^{-} (Ward & Shumway, 2004), which happened only at Rappahannock and Incheon sites in the present study.

In the present study, AE, defined as the efficiency with which ingested ration is absorbed, was significantly different between ploidy groups only for Pacific oysters in Incheon, where the organic content in the TPM was relatively low (f = 0.30; Fig. 2; Table 4) but still similar to GP where no differences were observed between ploidy levels in Eastern oysters. This comparison suggests that *C. gigas* diploids are able to better use the organic content in TPM at those environmental conditions of seston than *C. virginica*. Optimal environmental conditions for the performance of Pacific oysters, given temperature (T = 21 °C) and oxygen levels (DO > 10 mg/L), may have also facilitated higher AE (Allen & Burnett, 2008; Bourlés et al., 2009). In other studies, absorption efficiency in Sydney rock oysters was inversely related to size in triploids, but not in diploids (Kesarcodi-Watson, Klumpp, & Lucas, 2001b). Because a single experiment was performed with *C. gigas*, in which AE was found to differ between ploidy groups, results should be interpreted with caution and more experiments should be performed.

Filtration rate provides an estimate the amounts of energy and materials captured per unit time (Casas et al., 2018) and are a function of

Table 5
Mean values (mean ± s.d.) of physiological responses of oysters *Crassostrea virginica (*measurements in the United States*) and Crassostrea gigas (*measurements in South Korea*)* standardized by 1 g weight.

Location	Ploidy	Season	CR (L/h)	FR (mg/h)	RR (%)	OIR (mg/h)	AR (mg/h)	AE	SE
Gloucester	2N	Spring	2.71 ± 0.53	20.95 ± 4.18	40.76 ± 7.63	5.99 ± 1.40	4.03 ± 1.40	0.66 ± 0.10	0.53 ± 0.12
Point (GP)	3N	Spring	2.92 ± 0.62	22.57 ± 4.37	30.62 ± 13.78	6.74 ± 2.05	4.23 ± 1.65	0.61 ± 0.10	0.48 ± 0.15
	4N	Spring	1.47 ± n.a.	11.61 ± n.a.	20.38 ± n.a.	3.82 ± n.a.	2.50 ± n.a.	0.65 ± n.a.	0.94 ± n.a.
	2N	Summer	4.69 ± 0.43	45.75 ± 4.29	34.24 ± 2.66	14.58 ± 1.10	11.21 ± 0.89	0.77 ± 0.01	0.49 ± 0.02
	3N	Summer	5.63 ± 0.98	54.95 ± 9.67	47.16 ± 11.68	15.76 ± 3.61	12.38 ± 2.81	0.79 ± 0.02	0.44 ± 0.06
	4N	Summer	5.46 ± 0.99	53.26 ± 10.00	32.65 ± 10.74	17.05 ± 3.55	13.19 ± 2.09	0.78 ± 0.05	0.49 ± 0.04
	2N	Autumn	2.74 ± 2.11	13.51 ± 9.64	31.40 ± 19.63	2.44 ± 1.47	1.50 ± 1.14	0.56 ± 0.16	0.31 ± 0.21
	3N	Autumn	3.60 ± 0.85	18.97 ± 5.48	46.73 ± 31.76	2.33 ± 2.48	1.96 ± 0.73	0.72 ± 2.33	0.21 ± 0.23
	4N	Autumn	0.77 ± 0.72	4.24 ± 4.29	56.28 ± 25.49	0.52 ± 0.43	0.23 ± 0.21	0.44 ± 0.17	0.27 ± 0.22
Rappahannock (RH)	2N	Spring	2.08 ± 0.75	52.52 ± 18.80	67.59 ± 5.20	6.18 ± 3.42	4.69 ± 2.04	0.69 ± 0.65	0.27 ± 0.13
	3N	Spring	1.76 ± 0.55	44.31 ± 13.91	66.74 ± 9.97	4.81 ± 2.23	3.73 ± 2.09	0.74 ± 0.12	0.26 ± 0.14
	4N	Spring	1.26 ± 0.56	31.75 ± 14.09	64.16 ± 9.10	2.77 ± 0.22	2.03 ± 0.20	0.73 ± 0.01	0.19 ± 0.08
	2N	Summer	3.71 ± 0.94	61.54 ± 14.19	69.94 ± 6.92	6.75 ± 2.01	4.09 ± 1.56	0.60 ± 0.11	0.22 ± 0.03
	3N	Summer	6.80 ± 1.32	113.22 ± 34.47	74.22 ± 6.82	12.28 ± 1.00	8.23 ± 0.29	0.67 ± 0.03	0.22 ± 0.00
	4N	Summer	4.35 ± 1.87	71.82 ± 24.65	73.28 ± 6.23	8.39 ± 4.63	5.79 ± 3.37	0.68 ± 0.05	0.22 ± 0.02
	2N	Autumn	0.48 ± 0.21	1.82 ± 0.86	36.14 ± 19.03	1.15 ± 0.56	0.84 ± 0.47	0.71 ± 0.07	0.52 ± 0.09
	3N	Autumn	0.83 ± 0.70	3.11 ± 2.52	16.06 ± 17.95	2.22 ± 1.97	1.66 ± 1.55	0.74 ± 0.07	0.19 ± 0.43
	4N	Autumn	0.08 ± n.a.	0.33 ± n.a.	54.05 ± n.a.	0.15 ± n.a	0.11 ± n.a.	0.53 ± n.a.	0.41 ± n.a.
Incheon (IN)	2N	Summer	1.64 ± 0.61	23.40 ± 8.72	33.27 ± 9.38	6.26 ± 2.38	5.66 ± 2.20	0.90 ± 0.02*	0.80 ± 0.04
	3N	Summer	1.41 ± 0.81	25.91 ± 14.98	41.34 ± 14.42	9.94 ± 5.98	9.36 ± 5.65	0.94 ± 0.02*	0.78 ± 0.08

* Significant ($p < 0.05$) differences between ploidy.

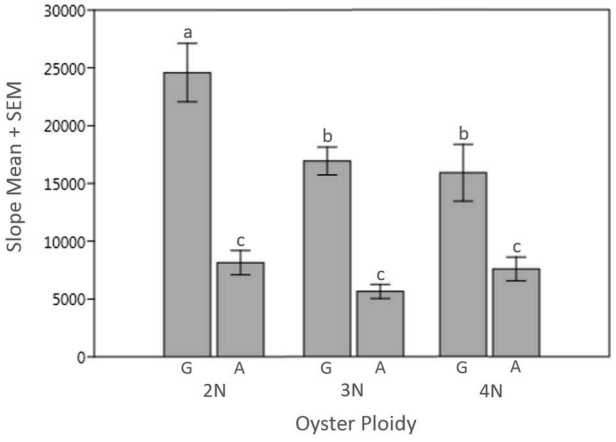

Fig. 4. Comparison between values of the slope of DCFH-DA oxidation by oyster hemocytes *in vitro* in diploids (2N), triploids (3N) and tetraploids (4N). 'G' denotes granular cells; 'A' equals agranular cells. Lowercase letters indicate statistically-significant contrasts at the 95% significance level. Bars indicate the standard error of the mean (SEM).

different factors. Weight-specific filtration rate decreases with oyster size, i.e., the rate per unit weight is higher in smaller oysters, as a small oyster filters proportionally more water than a larger oyster (Shumway, 1996). Coincidently, filtration rates were higher for small triploid oysters used at RH during the summer sampling, although the relationship between the two variables was low ($R^2 = 0.149$; $F = 2.79$, $p = 0.114$). Increased physiological activities observed in August might also be influenced by the hypoxic condition of the water in that season. In Chesapeake Bay, hypoxic conditions have been recorded for both Rappahannock and York rivers in 95% and 75% of surveys reported, for deep waters and bottom layers, respectively, in summer when water temperature exceeded 20 °C (Kuo, Park, & Moustafa, 1991). In this work, however, the DO levels during August summer samplings were hypoxic in coastal surface areas. This might have affected response of oysters that tried to obtain more oxygen by increasing the amount of water passing the gills, thereby increasing filtration rate. Triploid response was not significantly different from other oysters, which agrees with Shpigel and collaborators (1992) who found no significant differences in rates of oxygen consumption between diploid and triploid Pacific oysters at both low (8–15 °C) and high temperatures (30 °C), although triploid consumption rates tended to be higher, and consumption for both oyster groups increased at 30 °C. Nevertheless, some studies have reported high mortality of triploid oysters concomitant with periods of low dissolved oxygen and elevated seawater temperatures, although authors did not explain why triploids would be more susceptible (Cheney et al., 2000).

Studies of toxin accumulation in different ploidy oysters have shown that triploids have higher rates of digestive enzyme activity (amylase activity) and increased hemocyte density and functional variables in the hemolymph, such as reactive oxygen species production, when compared to diploids, which was interpreted as evidence of higher metabolic activity in triploids (Haberkorn et al., 2010). These findings are consistent with an expectation that triploids would be able to maximize digestion, absorption and food assimilation (higher amylase activity), and have improved feeding rates, absorption efficiency, and growth efficiency because of higher heterozygosity in triploids (Magoulas et al., 2000). Surprisingly, our results do not confirm this expectation, as feeding of both diploids and triploids seems to occur similarly at comparable environments, except for a single result with *C. gigas*.

As no statistically-significant differences in filtration and feeding between diploid, triploid, and tetraploid oysters were found in the biodeposition measurements, we measured metabolism as oxidation rate of the non-fluorescent substrate DCFH-DA to a fluorescent product, DCF, by oyster hemocytes *in vitro*, using flow cytometry to complement the biodeposition measurements. Catabolic rates of agranular hemocytes were the same for all ploidy groups and lower than those of granular hemocytes, as granular oxidation rates include oxidative burst during immune function (Wikfors & Alix, 2014). Mean granular hemocyte oxidation rate of diploid oysters was statistically higher than means for triploid and tetraploid oysters, which were statistically indistinguishable. These results indicate that either granular hemocytes from diploid oysters have higher basal metabolic rates than those of triploids or tetraploids, or that the diploid granular hemocytes were more active in immune response at the time of sampling. Nevertheless, as agranular hemocytes are not phagocytic (de Freitas Rebelo et al., 2013; Wikfors & Alix, 2014), and agranular cell catabolic rates were similar between ploidies, the metabolic rates, estimated by oxidation rate of DCFH-DA, seem to be similar between ploidies although immune function may be different.

In summary, an initial hypothesis that triploids would feed

differently than diploids and tetraploids is not confirmed in the present work. According to Guo and Allen (1994), nutrient requirements of polyploids may be higher than for diploids. McCarthy and collaborators (2016) cited this assertion, in addition to supposedly higher filtration rates, to theorize an advantage of triploids that would be able to maximize food resources in food-replete environments. Based upon our findings that oysters with different ploidy seem to feed similarly, the high nutrient requirements of triploids could be considered a negative trait undermining the previously expected high performance of that group of oysters. We have, however, no evidence that this is true for the Eastern oyster polyploids. Additionally, our catabolism results did not confirm the higher metabolism hypothesis for triploids (Haberkorn et al., 2010). It could be the case that triploid advantage, if any, was not apparent under the characteristics of our sites, as in 'translated' to detectable feeding performance. This line of thought is similar to what Wadsworth, Wilson, and Walton (2019) described for growth (whole wet weight) advantage in mated triploids, which only become apparent – namely 'translate to growth' – with increased study length.

A second explanation for lack of difference in performance between ploidies in the present work could be in genetic diversity. Hawkins et al. (2000), working with induced triploids, found that improved physiological performance, such as filtration rate, absorption efficiency from ingested organics, and net energy balance in triploids, was associated with allelic diversity rather than quantitative effects of ploidy status – a fact that could be true also for biological triploids. If so, as oysters used in this presented study originated from breeding programs in single hatcheries where broodstock have been selected over the years, it might be that diversity in triploids has not been retained in the lines tested. Although triploids are still arguably more heterozygous (Stanley, Hidu, & Allen, 1984) that 'smoothed' diversity might not have been enough to have an effect upon the physiological performance attributes measured in our study. Thus, genetically manipulated oysters and natural diploids, the latter also from a local, closed population, would have thus performed similarly.

Importantly, as our study was limited to biological polyploid oysters, the performance comparison between diploids and chemically-induced triploids, which are deemed quite different from the former (Dégremont et al., 2015; Hawkins et al., 2000), could produce different results (Wadsworth et al., 2019).

5. Conclusions

Our results indicate the following: 1. Eastern oyster feeding behavior did not vary with ploidy, although filtration and feeding varied with changes in the environment as expected. 2. Basal metabolism also did not vary with ploidy; therefore, superior growth in triploids compared to diploids under most conditions appears to be fundamentally a consequence of lower energy allocation to gametogenesis in triploids than in spawning diploids. 3. Attributing mortalities in triploids under certain conditions to higher metabolic demand and nutrient requirements, as proposed by McCarthy et al. (2016) is not supported by our findings. 4. Physiological consequences of polyploidy in oysters remains a research topic of both scientific and practical importance. Future studies, following up on the present one, to understand causes of differences in performance of oysters of different ploidy are important to lead to better informed decisions about the benefits of stocking oyster farms with diploid or triploid oysters and to enable optimization of site selection and aquaculture practices.

CRediT authorship contribution statement

Darien D. Mizuta: Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Gary H. Wikfors:** Conceptualization, Formal analysis, Methodology, Validation, Investigation, Writing - review & editing, Project administration, Supervision, Funding acquisition. **Shannon L. Meseck:** Investigation, Validation. **Yaqin Li:** Investigation, Resources, Data curation. **Mark S. Dixon:** Investigation, Resources. **Hyun Jeong Lim:** Investigation, Resources, Data curation. **In Joon Hwang:** Investigation, Resources. **Magalí Bazzano:** Investigation, Data curation, Visualization. **Steven Pitchford:** Investigation, Data curation, Visualization.

Acknowledgements

This work was funded by NOAA Internal Competitive Aquaculture Grant, by the South Korean National Institute of Fisheries Science (R2018012), a JPA project between the U.S. and South Korea, and by a fellowship award from the United States National Academy of Sciences. The authors would like to thank Dr. Standish K. Allen Jr. (ABC Director, VIMS) for his valuable comments in the early versions of this manuscript, Lucie-Liane Duchesne (UniLaSalle, France) for technical assistance during undergraduate internship at NOAA and Melissa Krisack (NEFSC- NOAA) for support in the laboratory.

References

Allen, S. K. (1983). Flow cyrometry. Assaying experimental polyploid fish ans shellfish. *Aquaculture, 33*, 317–328.

Allen, S. M., & Burnett, L. E. (2008). The effects of intertidal air exposure on the respiratory physiology and the killing activity of hemocytes in the Pacific oyster, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology, 357*, 165–171.

Allen, S. K., Downing, S. L., & Chew, K. K. (1989). Hatchery for producing triploid oyster, Washington Sea grant publ. *WSG, 89–3*, 27pp.

Barber, B. J., & Mann, R. (1991). Sterile triploid *Crassostrea virginica* (Gmelin, 1791) grow faster than diploids but are equally susceptible to *Perkinsus marinus*. *Journal of Shellfish Research, 19*, 445–450.

Bass, D. A., Parce, J. W., Dechatelet, L. R., Szejda, P., Seeds, M. C., & Thomas, M. (1983). Flow cytometric studies of oxidative product formation by neutrophils: A graded response to membrane stimulation. *The Journal of Immunology, 130*(4), 1910–1917.

Bayne, B. (2017). *Biology of oysters* (1st ed., Vol. 41). London: Academic press.

Bayne, B. L., & Newell, R. C. (1983). Physiological energetics of marine molluscs. In A. S. M. Saleuddin, & K. M. Wilbur (Eds.), *The Mollusca, physiology, Part 1* (Vol. 4, pp. 407–515). New York, NY: Academic Press.

Bender, M. E. (1986). *The York river: A brief review of its physical, chemical and biological characteristics*. Virginia: Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary, Gloucester Point.

Bourlés, Y., Alunno-Bruscia, M., Pouvreau, S., Tollu, G., Leaguay, D., Arnaud, C., et al. (2009). Modelling growth and reproduction of the pacific oyster *Crassostrea gigas*: Advances in the oyster- DEB model through application to a coastal pond. *Journal of Sea Research, 62*, 62–71.

Casas, S. M., Lavaud, R., La Peyre, M. K., Comeau, L. A., Filgueira, R., & La Peyre, J. F. (2018). Quantifying salinity and season effect on eastern oyster clearance and oxygen consumption rates. *Marine Biology, 165*(90), 1–13.

Cheney, D. P., MacDonald, B. F., & Elston, R. A. (2000). Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *Journal of Shellfish Research, 19*, 353–359.

Cranford, P. J., Ward, J. E., & Shumway, S. E. (2011). Bivalve filter feeding: Variability and limits of the aquaculture biofilter. In S. E. Shumway (Ed.), *Shellfish aquaculture and the environment* (1st ed., pp. 81–124). Chichester: Wiley.

Dégremont, L., Garcia, C., & Allen, S. K., Jr. (2015). Genetic improvement for disease resistance in oysters: A review. *Journal of Invertebrate Pathology, 131*, 226–241.

Dégremont, L., Garcia, C., Frank-Lawale, A., & Allen, S. K., Jr. (2012). Triploid oysters in the Chesapeake bay: Comparison of diploid and triploid *Crassostrea virginica*. *Journal of Shellfish Research, 31*(1), 21–31.

Eckman, J. E., Peterson, C. H., & Cahalan, J. A. (1989). Effects of flow speed, turbulence, and orientation on growth of juvenile bay scallops *Argopecten irradians concentricus* (Say). *Journal of Experimental Marine Biology and Ecology, 132*, 123–140.

Fao. (2018). *Global aquaculture production 1950-2016. Fishery statistical collections*. Available at: http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en. Last access: 20 November 2018 at 2:30 pm.

de Freitas Rebelo, M., de Souza Figueiredo, E., Mariante, R. M., Nobrega, A., de Barros, C. M., & Allodi, S. (2013). New insights from the oyster *Crassostrea rhizophorae* on bivalve circulating hemocytes. *PloS One, 8*, Article e57384.

Galimany, E., Ramón, M., & Ibarrola, I. (2011). Feeding behavior of the mussel *Mytilus galloprovincialis* (L.) in a mediterranean estuary: A field study. *Aquaculture, 314*, 236–243.

Galimany, E., Rose, J. M., Dixon, M. S., & Wikfors, G. H. (2013). Quantifying feeding behavior of ribbed mussels (*Geukensia demissa*) in two urban sites (Long Island Sound, USA) with different seston characteristics. *Estuaries and Coasts, 36*, 1265–1273.

Galimany, E., Wikfors, G. H., Dixon, M. S., Newell, C. R., Meseck, S. L., Henning, D., et al. (2017). Cultivation of the ribbed mussel (*Geukensia demissa*) for nutrient bioextraction in an urban estuary. *Environmental Science and Technology, 51*, 13311–13318.

Galtsoff, P. S. (1964). The American oyster: US fish and wildlife service. *Fishery Bulletin, 64*, 1–480.
Gerdes, D. (1983). The pacific oyster *Crassostrea gigas*: Part I. Feeding behaviour of larvae and adults. *Aquaculture, 31*(2–4), 195–219.
Glibert, P. M., Alexander, J., Meritt, D. W., North, E. W., & Stoecker, D. K. (2007). Harmful algae pose additional challenges for the oyster restoration: Impacts of the harmful algae *Karlodinium veneficum* and *Prosocentrum minimum* one early life stages of the oysters *Crassostrea virginica* and *Crassostrea ariakensis*. *Journal of Shellfish Research, 26*(4), 919–925.
Gosling, E. (2015). *Marine bivalve molluscs*. West Sussex, UK: John Wiley & Sons, Ltd.
Guévélou, E., Carnegie, R. B., Small, J. M., Hudson, K., Reece, K. R., Rybovich, M., et al. (2019). Tracking triploid mortalities of eastern oysters *Crassostrea virginica* in the Virginia portion of the Chesapeake Bay. *Journal of Shellfish Research, 38*, 1–13.
Guo, X., & Allen, S. K., Jr. (1994). Sex determination and polyploid gigantism in dwarf surfclam (*Mulinia lateralis* say). *Genetics, 138*, 1199–1206.
Guo, X., & Allen, S. K., Jr. (1996). All triploid Pacific oysters (*Crassotrea gigas* Thumberg) produced by mating tetraploids and diplois. *Aquaculture, 142*, 149–1161.
Haberkorn, H., Lambert, C., Le Goic, N., Gueguen, M., Moal, J., Palacios, E., et al. (2010). Effects of *Alexiandrium minutum* exposure upon physiological and hematological variables of diploid and triploid oysters. *Crassostrea gigas. Aquat. Toxicol., 97*(2), 96–108.
Hawkins, A., Bayne, B., Bougrier, S., Héral, M., Iglesias, J., Navarro, E., et al. (1998). Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. *Journal of Experimental Marine Biology and Ecology, 219*, 87–103.
Hawkins, A. J. S., Magoulas, A., Heral, M., Bougriey, S., Naciri-Graven, Y., Day, A. J., et al. (2000). Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. *Genetical Research, 76*, 273–284.
Hyer, P. V. (1977). *Water quality model of York river, Virginia. Special scientitic report No. 146*. Virginia: Virginia Institute of Marine. Science, Gloucester Point.
Ibarra, A. M., Ascencion-Michel, R., Ramirez, J. L., Manzano-Sarabia, M., & Rodriguez-Jaramilo, C. (2017). Performance of diploid and triploid *Crassostrea gigas* (Thunberg, 1793) grown in tropical versus temperate natural environmental conditions. *Journal of Shellfish Research, 36*(1), 119–139.
Iglesias, J., Urrutia, M., Navarro, E., & Ibarrola, I. (1998). Measuring feeding and absorption in suspension-feeding bivalves: An appraisal of the biodeposition method. *Journal of Experimental Marine Biology and Ecology, 219*, 71–86.
Jorgensen, C. B. (1966). *Biology of suspension feeding*. Oxford: Pergamon Press.
Kalyanaraman, B., Darley-Usmar, V., Davies, K. J., Dennery, P. A., Forman, H. J., Grisham, M. B., et al. (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: Challenges and limitations. *Free Radical Biology and Medicine, 52*(1), 1–6.
Kesarcodi-Watson, A., Klumpp, D. W., & Lucas, J. S. (2001a). Comparative feeding and physiological energetics in diploid and triploid Sydney rock oysters (*Saccostrea commercialis*) II. Influences of food concentration and tissue energy distribution. *Aquaculture, 203*, 195–216.
Kesarcodi-Watson, A., Klumpp, D. W., & Lucas, J. S. (2001b). Comparative feeding and physiological energetic of diploid and triploid Sydney rock oysters, Saccostrea commercialis I. Effects of oyster size. *Aquaculture, 203*(1–2), 177–193.
Kuo, A. Y., & Neilson, B. J. (1987). Hypoxia and salinity in Virginia estuaries. *Estuaries, 10*(4), 277–283.
Kuo, A. Y., Park, K., & Moustafa, M. Z. (1991). Spatial and temporal variabilities of hypoxia in the Rappahannock River, Virginia. *Estuaries, 14*(2), 113–121.
Lambert, C., Soudant, P., Choquet, G., & Paillard, C. (2003). Measurement of Crassostrea gigas hemocyte oxidative metabolism by flow cytometry and the inhibiting capacity of pathogenic vibrios. *Fish & Shellfish Immunology, 15*, 225–240.
Li, J., Glibert, P. M., & Gao, Y. (2015). Temporal and spatial changes in Chesapeake Bay water quality and relationships to *Prorocentrum minimum, Karlodinium veneficum*, and CyanoHAB events, 1991-2008. *Harmful Algae, 42*, 1–14.
Li, Y., Veilleux, D. J., & Wikfors, G. H. (2009). Particle removal by Northern bay scallops Argopecten irradians irradians in a semi-natural setting: Application of a flow-cytometric technique. *Aquaculture*, 237–245.
Magoulas, A., Kotoulas, G., Gerard, A., Naciri-Graven, Y., Dermitzakis, E., & Hawkins, A. J. S. (2000). Comparison of genetic variability and parentage in different ploidy classes of the Japanese oyster *Crassostrea gigas*. *Genetical Research, 76*(3), 261–272.
Matthiessen, G. C., & Davis, J. P. (1992). Observations on growth rate and resistance to MSX (*Haplosporidium nelsoni*) among diploid and triploid eastern oysters (*Crassostrea virginica* (Gmelin, 1791)) in New England. *Journal of Shellfish Research, 11*(449–45496), 237–245.
McCarthy, A., Crawford, C., Eriksen, R., & Ross, D. J. (2016). Dietary preferences, growth, and condition of triploid and diploid pacific oysters, *Crassostrea gigas* (thunberg), in little swanport estuary, tasmania, Australia. *Aquaculture Research, 47*, 3352–3358.
Mizuta, D. D., Kasai, A., Ishii, K. I., Yamaguchi, H., & Nakata, H. (2014). Effects of artificial upwelling on the environment and reared oyster *Crassostrea gigas* in Omura Bay, Japan. *Bulletin of the Japanese Society of Fisheries Oceanography, 78*(1), 13–27.
Murray, T. J., & Hudson, K. (2012). Virginia shellfish aquaculture situation and outlook report: Results of 2011 Virginia shellfish aquaculture crop reporting survey. Virginia Sea grant, VSG-12-07, VIMS marine resource report 2012-04. *Gloucester*, 20. VA.
Nell, J. A. (2002). Farming triploid oysters. *Aquaculture, 120*, 69–88.
Park, K., Kuo, A. Y., & Neilson, B. J. (1996). A numerical model study of hypoxia in the tidal Rappahannock River of Chesapeake bay. *Estuarine, Coastal and Shelf Science, 42*, 563–581.
Riisgård, H. U. (1988). Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. *Marine Ecology Progress Series*, 217–223.
Shapiro, H. M. (2005). *Practical flow cytometry*. John Wiley & Sons.
Shpigel, M., Barber, B. J., & Mann, R. (1992). Effects of elevated temperature on growth, gametogenesis, physiology and biochemical composition in diploid and triploid Pacific oysters, *Crassotrea gigas*, Thunberg. *Journal of Experimental Marine Biology and Ecology, 161*, 15–25.
Shumway, S. E. (1996). Natural environmental factors. Pp. 467-513. In V. S. Kennedy, R. I. E. Newell, & A. F. Eble (Eds.), *The eastern oyster: Crassostrea virginica*. College Park, Maryland: Maryland Sea Grant College Program.
Sin, Y., Wetzel, R. L., & Anderson, I. C. (1999). Spatial and temporal characteristics of nutrient and phytoplankton dynamics in the York river estuary, Virginia: Analysis of long-term data. *Estuaries, 22*(2), 260–275.
Stanley, J. G., Hidu, H., & Allen, S. K., Jr. (1984). Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. *Aquaculture, 37*, 147–155.
Stanley, J. G., Allen, S. K., Jr., & Hidu, H. (1981). Polyploidy induced in American oyster, *Crassostrea virginica*, with cytochalasin B. *Aquaculture, 23*, 1–10.
Stoecker, D. K., Adolf, J. E., Place, A. R., Glibert, P. M., & Merit, D. W. (2008). Effects of the dinoflagellates *Karlodinium veneficum* and *Prorocentrum minimum* on early life history stages of the eastern oyster (*Crassostrea virginica*). *Marine Biology, 154*(1), 81–90.
Wadsworth, P. C. (2018). Comparing triploid and diploid growth and mortality in farmed oysters, *Crassostrea virginica*. In *The northern Gulf of Mexico*. Auburn, Alabama: Master of Science thesis for the Graduate Faculty of Auburn University.
Wadsworth, P., Wilson, A. E., & Walton, W. C. (2019). A meta-analysis of growth rate in diploid and triploid oysters. *Aquaculture, 499*, 9–16.
Walton, W. C., Rikard, F. S., Chaplin, G. I., Davis, J. E., Arias, C. R., & Supan, J. E. (2013). Effects of ploidy and gear on the performance of cultured oysters *Crassostrea virginica*: Survival, growth, shape, condition index and *Vibrio* abundances. *Aquaculture, 414–415*, 260–266.
Ward, J. E., & Shumway, S. E. (2004). Separating the grain from the chaff: Particle selection in suspension and deposit-feeding bivalves. *Journal of Experimental Marine Biology and Ecology, 300*, 83–130.
Wikfors, G. H., & Alix, J. H. (2014). Granular hemocytes are phagocytic, but agranular hemocytes are not, in the Eastern Oyster *Crassostrea virginica*. *Invertebrate Immunity, 1*, 15–21.