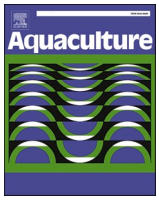
Aquaculture 595 (2025) 741613

Contents lists available at ScienceDirect

Aquaculture

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



Triploid eastern oysters (*Crassostrea virginica*) display high susceptibility to microbial infections but only during early ontogenetic stages

Christopher J. Brianik [a], Emmanuelle Pales Espinosa [a], Ming Liu [b], Pete Topping [c], Gregg Rivara [d], Ximing Guo [e], Dina Proestou [f], Bassem Allam [a,*]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11790, USA
[b] Morgan State University, Patuxent Environmental and Aquatic Research Laboratory, 10545 Mackall Road, Saint Leonard, MD 20685, USA
[c] Peconic Baykeepers, 167 Red Creek Rd, Hampton Bays, NY 11946, USA
[d] Cornell Cooperative Extension, Suffolk County Marine Environmental Learning Center, 3690 Cedar Beach Rd, Southold, NY 11971, USA
[e] Rutgers University, Department of Marine and Coastal Sciences, 6959 Miller Avenue, Port Norris, NJ 08349, USA
[f] USDA ARS National Cold Water Marine Aquaculture Center, 120 Flagg Road, Kingston, RI 02881, USA

ARTICLE INFO

Keywords:
Aquaculture
Hatchery
Shellfish
Triploid
Vibrio
Disease

ABSTRACT

Over the last two decades, oyster aquaculture has largely shifted from the use of diploids to the near-exclusive use of triploids in many regions, with some hatcheries now producing >90 % triploid oyster spat. The increased demand for triploids is primarily driven by substantial growth advantages and more consistent meat quality associated with sterile triploid animals, with most data suggesting triploids and diploids display comparable adult survivorship. Although adult performance appears similar, anecdotal reports from farmers have suggested that triploids may exhibit greater frailty during earlier age classes, particularly in response to bacterial infections. Due to the extent of triploid production now taking place, the possibility of early ontogenetic frailty in triploids could pose a significant risk to oyster aquaculture production. To evaluate these claims, two cohorts of half-sibling diploid and triploid eastern oyster lines were generated in 2020 and 2021. A subset of larvae and juveniles from these lines were then exposed to a cocktail of bacterial (*Vibrio*) pathogens and monitored for viability. The remaining oysters were then allowed to grow for another 2 months before being deployed in either Peconic Bay, New York (2020), or Patuxent River, Maryland (2021), where their survivorship was followed for another year. Results showed that triploids were at significantly greater risk of mortality during the larvae and juvenile stages, though differential mortality decreased with age. These trends were consistent across the two spawning events, and the extent of early ontogenetic triploid frailty was observed to vary between the lines tested. This work provides valuable data for hatchery managers and farmers alike and suggests areas where specific attention and further work are required.

1. Introduction

Aquaculture is a rapidly growing industry that has nearly doubled in size over the last 2 decades with the value of eastern oyster (*Crassostrea virginica*) alone having more than tripled from 2005 to 2018 (USDA, 2006; USDA, 2019; FAO, 2022). In recent years, oyster aquaculture has largely shifted from the use of diploid (2n) to triploid (3n) oysters, with triploids now accounting for more than 90 % of oyster aquaculture production in France and Virginia, over a third of North America's West Coast production (reviewed in Brianik and Allam, 2023) and ~70 % of Pacific oysters grown in China (Jiang and Mu, 2021). The near exclusive use of triploids in many regions is primarily due to elevated growth rates (~30 %), and more consistent meat quality associated with the sterile triploids as well as comparable adult survivorship thus increasing the yield for farmers (Dégremont et al., 2012; Walton et al., 2013; Wadsworth et al., 2019a; Brianik and Allam, 2023). Triploidy can be induced in a variety of ways, with the most popular method being the cross-fertilization of tetraploid sperm (4n) with diploid eggs which has largely replaced traditional chemical induction methods that relied on the use of toxic chemicals and that were less effective (Guo et al., 1996; Wang et al., 1999). Critical to the rapid growth of aquaculture and the production of triploid animals are shellfish hatcheries, which spawn and

* Corresponding author.
E-mail address: Bassem.Allam@stonybrook.edu (B. Allam).

https://doi.org/10.1016/j.aquaculture.2024.741613
Received 20 March 2024; Received in revised form 12 August 2024; Accepted 10 September 2024
Available online 12 September 2024

raise oysters from fertilization to seed size (typically 2-25 mm) with nearly all farmers reliant upon hatchery-derived seed (also referred to as spat). For hatcheries and growers, the switch from diploid to triploid production has been relatively seamless as triploids can be grown following the same methods developed for diploids (excluding spawning requiring tetraploids or chemical treatment) allowing for the near-exclusive production of triploids without hatchery protocol modification or reexamination.

In hatchery settings, larval oysters are grown under dense conditions allowing even small hatcheries to produce millions of seed annually for farmers. The high output from these shellfish hatcheries paired with the specific location requirements needed to operate (i.e., access to high-quality seawater, protected space for nursery operations), has resulted in relatively few hatcheries responsible for supporting an ever-growing industry with ~60 shellfish hatcheries supplying clams, scallops, mussels and oysters to over 1300 farmers operating along the East coast of North America and the Gulf of Mexico (Zemeckis, 2020; ECSGA, 2024). Such consolidation can have its advantages but can also create significant vulnerabilities in production as bacterial outbreaks have caused mass hatchery crashes (Brown, 1981; Richards et al., 2015) and are capable of reducing production by 59 % (Elston et al., 2008) leading sometimes to seed shortages for farmers. Biosecurity measures can eliminate some of the risks, however, a pathogen-free environment is virtually impossible to ensure in hatcheries and outbreaks can still result from microbes brought in with the spawning broodstock (Sainz-Hernández and Maeda-Martínez, 2005; Elston et al., 2008). These outbreaks are typically associated with *Vibrio* spp. with multiple species known to be highly pathogenic to oyster larvae at relatively low concentrations (Richards et al., 2015). Moreover, problems with *Vibrio* are expected to increase as sea temperatures continue to rise (Elston et al., 2008), suggesting that these issues may become increasingly problematic for hatcheries in general. As a result, there is an increasing need to identify oyster traits or aquaculture techniques that may help reduce the incidence of disease as well as lines that may be more resistant to outbreaks.

Despite triploids having widespread use in oyster aquaculture, there remains surprisingly little information about how this technology impacts disease dynamics, particularly during early life stages. For adult oysters, parasite susceptibility, overall immune performance, and survivorship are often reported to be similar between triploids and diploids (Duchemin et al., 2007; Dégremont et al., 2012; Walton et al., 2013; Wadsworth et al., 2019a). However, different life stages often present varying vulnerabilities that may not reflect other ontogenetic stages. For instance, triploid turbot (*Scophthalmus maximus*) have been reported to be more susceptible to *Vibrio* pathogens while at juvenile stages (Isidan et al., 2021) even though adult survival and immune competence are equivalent (Budiño et al., 2006; Cal et al., 2006). For oysters, the bulk of mortality typically occurs during and between the larvae-spat stages when oysters are at heightened risk of bacterial infections. Anecdotal reports from farmers in the northeast have suggested that the triploid spat they have purchased are often “frail”, displaying poor survival compared to diploids after being deployed on their farms (Long Island oyster farmer, personal communication). These reports are at least partially influenced by the different lines (genetic lineage) that are often sourced for diploids and triploids in this region, though data from Azéma et al. (2016) suggest that triploid spat may be at higher risk to bacterial pathogens than diploids. However, conflicting observations have been made on slightly older oysters with no difference in overall survival based on ploidy (De Decker et al., 2011). Due to these conflicting data and aquaculture’s increasing reliance on triploids, there is a need to understand if ploidy and certain genetic pairings increase the risk of bacterial outbreaks.

In this study, half-sibling triploid and diploid eastern oyster (*Crassostrea virginica*) lines were produced to compare the influence of ploidy and maternal source (i.e., genetic background) on oyster survivorship across different ontogenetic stages. The data were collected using two different spawns to further enhance the robustness of the study. For the larvae and juvenile stages, experiments were conducted by experimentally exposing oysters to bacterial pathogens from the *Vibrio* genus, while older age classes were deployed in the field and monitored for survival. Results from early-age classes were then related to field outcomes to assess for early indicators of performance. The findings support that specific attention needs to be given to early life stages of triploid oysters.

2. Methods

2.1. Spawning and larval rearing

Diploid and triploid oysters were produced at the Suffolk County Marine Environmental Learning Center (SCMELC), Southold, NY in late April of 2020 (spawn 2020) and 2021 (spawn 2021). The breeding strategy is detailed in Table 1. For both spawning events, eggs were collected from local or regional diploid females via strip spawning. Eggs from each female (12 dams per line, excluding Rhode Island during spawn 2021 which only had 8 females) were held separately and evenly split before being fertilized using pooled sperm from either diploid (20 sires per spawn) or tetraploid (12 sires per spawn) oysters from a selected disease resistant line (NEH line) developed at Rutgers University, NJ. This process resulted in three diploid and three triploid half-sibling lines per spawn for a total of 12 total lines.

After fertilization, oysters were raised following standard hatchery protocols (Helm et al., 2004). In brief, larvae were initially held in 400 L conicals at 24 °C with full water changes performed three times a week. After 2–3 weeks, oysters started to set when they were ~200 μm in size, and pediveligers were then transferred to downwellers to favor metamorphosis. Once large enough (typically 1–2 mm), oyster juveniles were then transferred to an upwelling system where they remained until they were deployed in the field (see below). No size-based culling was ever performed to maintain maximal genetic diversity, though non-selective thinning was performed as needed to maintain comparable density across the different crosses. Oyster ploidy was confirmed using flow cytometry analysis of DNA content following standard methods outlined in Guo et al. (1996).

2.2. Vibrio challenge

2.2.1. Larvae

One week post-fertilization, oyster larvae from each line were collected and seeded into 6-well microplates at ~150 larvae per well in 6 mL of filter-sterilized seawater collected from the source hatchery (25 PSU) and maintained at room temperature (20 °C). Bacterial species, *Vibrio tubiashii*, *V. coralliilyticus*, *V. splendidus*, and *Vibrio* (*Listonella) anguillarum,* were grown for 48 h on marine agar and then resuspended in sterile artificial seawater (SASW, 25 PSU). Bacterial suspensions were adjusted spectrophotometrically (optical density at 600 nm) then mixed together to create a *Vibrio* cocktail that was used to dose half the wells (3 wells per line) containing oyster larvae to attain a final concentration of about 2.5×10^3 colony-forming units (CFUs) mL^{-1} per strain. These bacterial concentrations represent the approximate LD50 for bivalve larvae (Richards et al., 2015; Schwaner et al., 2020). The remaining three wells received an equivalent amount of SASW to serve as controls (six wells per line, three exposed and three control). Oyster viability was monitored visually for two days using a dissection microscope and dead larvae were removed as they were counted. The larvae were not fed during this experiment and were considered dead based on a lack of ciliary movement, unresponsiveness, or occurrence of empty shells.

2.2.2. Juveniles

Juvenile experiments were similar to larvae experiments with slight modifications. Juvenile oysters were collected six weeks post-fertilization and seeded into 6-well microplates (1 oyster line per

Table 1
The breeding layout and cohort name codes used for both spawning events.

Spawn 2020					Spawn 2021				
♀	♂	Line produced	Triploid %	Diploid%	♀	♂	Line produced	Triploid %	Diploid%
NEH (2n)	NEH (2n)	HND (2n)	0	100	NEH (2n)	NEH (2n)	ND (2n)	4.2	87.5
	NEH (4n)	HNT (3n)	100	0		NEH (4n)	NT (3n)	100	0
Connecticut (2n)	NEH (2n)	HCD (2n)	0	100	Central Long Island (2n)	NEH (2n)	ID (2n)	0	100
	NEH (4n)	HCT (3n)	100	0		NEH (4n)	IT (3n)	33.3*	54.2*
Eastern Long Island (2n)	NEH (2n)	HED (2n)	0	100	Rhode Island (2n)	NEH (2n)	RD (2n)	4.2	95.8
	NEH (4n)	HET (3n)	100	0		NEH (4n)	RT (3n)	100	0

* Results generated using the IT line should be carefully interpreted since it showed a high percentage of diploid oysters as a likely result of husbandry errors.

plate), though only 35 individuals were seeded into each well due to their increased size at this stage. Before the experiment, all oysters were assessed for signs of viability using a dissection microscope (responsiveness to touch, active filtering). Half the wells (three per plate/line) were then dosed with the same *Vibrio* strains used for larvae, though concentrations were increased to ~1.2×10^4 CFU mL^{-1} per strain, while the remaining three received an equivalent amount of SASW to serve as controls. Oysters were held at room temperature (20 °C) and monitored daily for 9 (spawn 2020) or 10 (spawn 2021) days.

2.3. Adult field deployments

Oysters not selected (same lines, different individuals) to be used in the larval, or juvenile challenges were maintained following standard hatchery procedures, though no sized-based culling was administered at any point. Oysters were maintained on upwellers separated by oyster line until the average size of each line was between 8 and 10 mm in length, after which they were deployed in Peconic Bay Long Island (N 40.91446, W -72.54391) or in the Patuxent River, Maryland (N 38.39413, W -76.50343) for spawn 2020 and 2021, respectively. Deployments occurred in late July for both spawns. Both of these locations represent general areas where aquaculture activity is already present but are locations where relatively high oyster mortalities are known to occur. Oysters were deployed on the bottom in triplicate ADPI bags (high-density polyethylene mesh bags commonly used in oyster aquaculture), with the spawn 2020 initially stocked at 500 oysters per bag, which was increased to 1000 oysters per bag for initial stocking for spawn 2021. Routine maintenance (i.e., bag cleaning) was performed as needed, and oyster survival was assessed seasonally until reaching 1.5 years of age which represents the local average time to market size.

2.4. Statistical analysis

All statistical analyses were conducted using R version 4.1.0. Normality and homoscedasticity assumptions were confirmed using Shapiro-Wilk and Bartlett's tests, respectively. All percentage data was arcsine square root transformed. Students *t*-test, one-way and two-way analysis of variance (ANOVA) were used to determine if differences were significant for parametric data. When data failed to meet parametric assumptions a Kruskal-Wallis test was used instead. Juvenile survival data was compared using a log-rank test. Post-hoc comparisons were performed using Tukey Kramer post hoc test or Dunn's test. All results were deemed significant at $\alpha = 0.05$.

3. Results

3.1. Ploidy status

Ploidy levels in the produced cohorts are summarized in Table 1. Cross-fertilization of diploid eggs with NEH tetraploid sperm resulted in 100 % triploid induction for lines HNT, HCT and HET during spawn 2020. Lines NT and RT produced during the second spawning event (spawn 2021) also had 100 % triploid induction, however, line IT was only 33 % triploid, and diploid lines RD and ND had minor (4.2 %) triploid contamination. Line IT and ID were removed from any ploidy-based comparisons due to the low levels of triploids present in IT, however, data from these lines were still used for line-based comparisons (i.e., maternal source effect) where results from diploids and triploids oysters from the same genetic background were pooled.

3.2. Vibrio pathogen challenges

3.2.1. Larvae

Oyster larvae from both spawns were exposed to *Vibrio* pathogens at one week post-fertilization to compare survival trends between ploidy, lines, and spawns. Mortality was noticeable in larvae challenged with *Vibrio* and both experiments (spawns) were terminated two days post-pathogen exposure. For both 2020 and 2021 spawns, challenged diploids had significantly higher survival than triploids (one-way ANOVA, $p < 0.0001$, $n = 18$ for 2020, $n = 12$ for 2021), with an overall average survival of 27.3 % (±29.8) and 77.7 % (±16.9) for triploids and diploids, respectively (Figs. 1 and 2). Control (unchallenged) survivorship was high for all groups excluding NT from spawn 2021. When comparing spawns, the average survival of oysters from spawn 2020 was significantly (Kruskal-Wallis test, $p = 0.017$, $n = 36$) greater than those from 2021 (about 20 % higher overall survivorship). When assessing maternal origin on survival outcomes (assessing triploids and diploids from the same maternal source together) there were no significant differences in survivorship (one-way ANOVA, $p = 0.202$, n = 36). However, when the maternal source is assessed by accounting for ploidy, the eastern Long Island (HED, or HET) lines tend to perform best. Overall, HED displayed

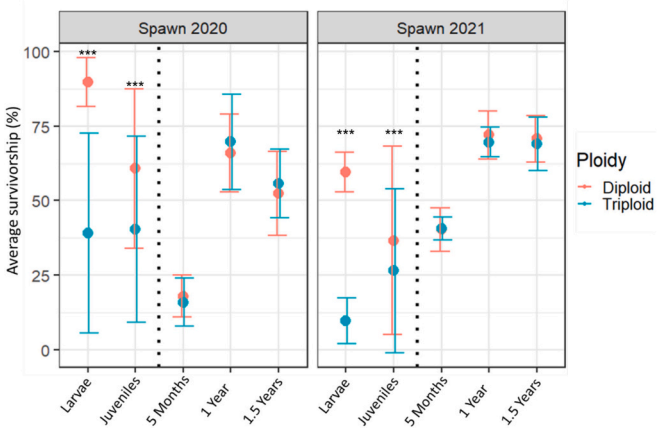


Fig. 1. Average percent survival (± standard deviation) of diploid (red) and triploid (blue) oysters from both spawns at each of the sampling points. The vertical dashed black line separates laboratory experiments (larvae two days post exposure and juveniles nine days post exposure) from field deployment (time points references on the x-axis correspond to the age of oysters). *** denote significance ($p < 0.001$; *t*-test for larvae and log-rank test for juveniles, see Figs. 2 and 3 for detailed larvae and juvenile data). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

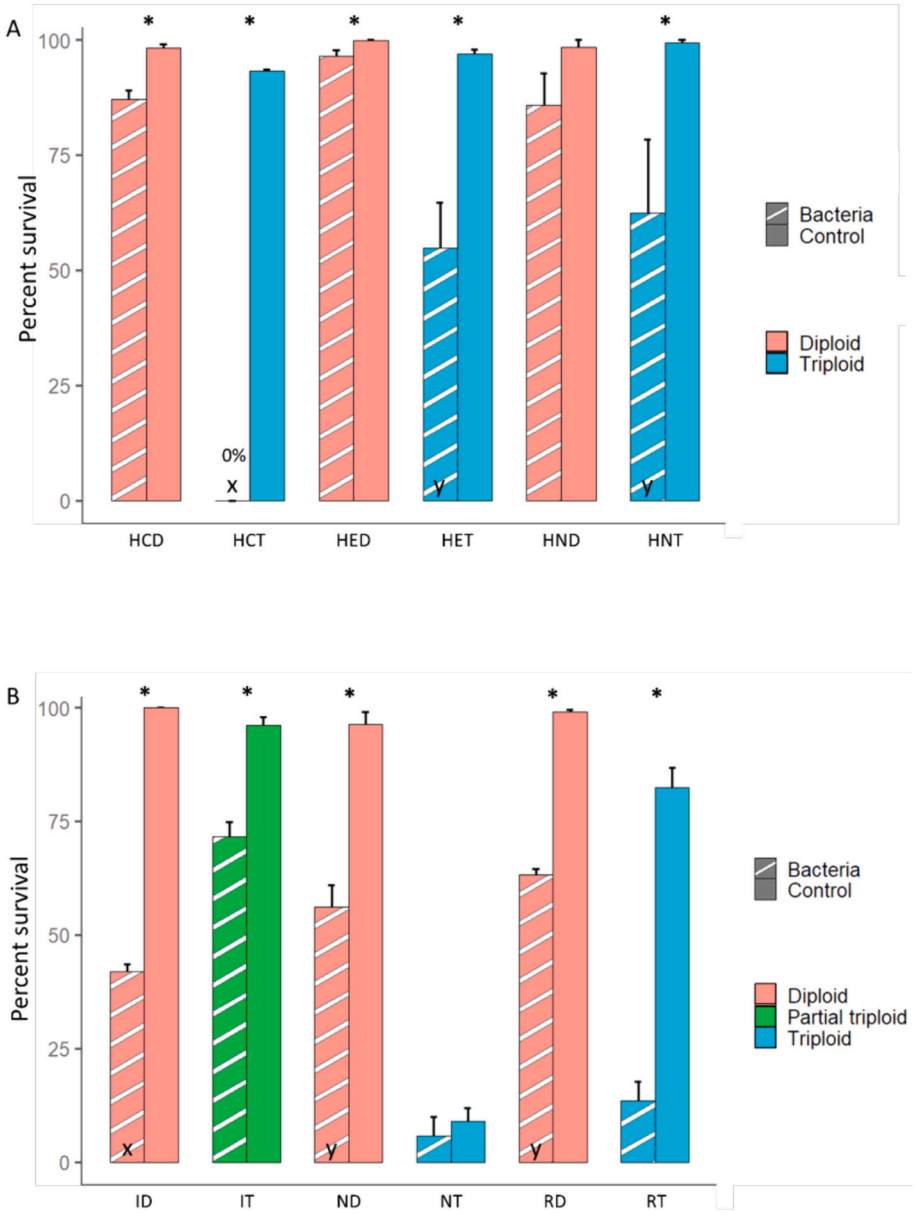


Fig. 2. Average percent survival (± standard error) of diploid and triploid larvae two days after exposure to a *Vibrio* cocktail. A) oysters produced in 2020. B) Oysters produced in 2021. Oysters from the central Long Island cross (ID, IT) were excluded from ploidy comparisons due to mixed ploidy (see footnote in Table 1), though they were still used when assessing line differences. * denote significance ($p < 0.05$; t-test) between exposed and control oyster larvae from each line. Different letters (x and y) represent significant differences between oyster lines based on maternal source when controlling for ploidy ($p < 0.05$, 1 way ANOVA or Kruskal-Wallis tests).

the highest survivorship among challenged larvae across all lines investigated while HCT displayed the lowest survival.

3.2.2. *Juveniles*

At the juvenile stage (six weeks post-fertilization), oysters from each spawn were also exposed to *Vibrio* pathogens and monitored for mortality. At this age class, mortality was slower, and the experiment lasted longer, and as such a Kaplan-Meier log-rank test was used to compare ploidy differences. In this case, triploid oysters exposed to *Vibrio* pathogens displayed significantly greater mortality than their diploid counterparts (Log-rank test, $p < 0.001$) for both spawns, while control survivorship in all lines was high (>90 %) and statistically equivalent (Fig. 3). Although ploidy-based differences were relatively consistent between spawns, mortality dynamics were significantly different between spawns 2020 and 2021 (Log-rank test, $p < 0.0001$). All mortality in spawn 2020 occurred before day 5, with triploids reaching their LT50 on day 4, whereas mortality in spawn 2021 primarily occurred after day 6 with the LT50 of triploids reached on day 8 (Fig. 3). By day 9 the average survival rates of exposed oysters from both spawns were 34.8 % ± 29 (mean ± standard deviation) and 51.1 % ± 30 for triploids and diploids, respectively. When comparing cumulative mortality on day 9, there were no significant differences based on the oyster maternal source (diploids and triploids assessed separately; one-way ANOVA, $p = 0.84$, $n = 18$ for either diploids or triploids,), or between either spawn (Kruskal-Wallis, $p = 0.10$, $n = 36$), though oyster from spawn 2020 tended to have higher survivorship than spawn 2021.

3.3. *Field grow out*

3.3.1. *Two months post-field deployment (5 months post-fertilization)*

Three months after fertilization, oysters were deployed in either the Peconic Bay, Long Island, for spawn 2020 or in the Patuxent River,

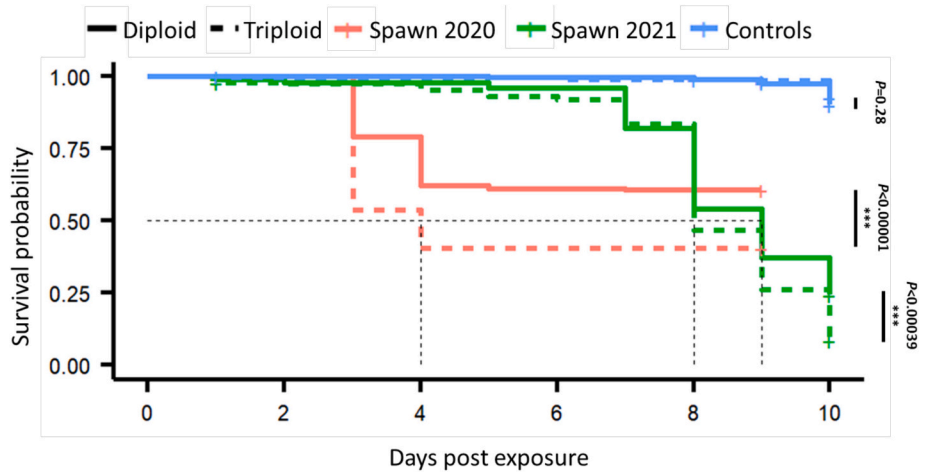


Fig. 3. Kaplan-Meier survival plot of diploid and triploid juvenile oysters exposed to a *Vibrio* cocktail. The thin black dashed lines represent the LD50 for each one of the lines that experiences >50 % mortality. Results of control (unchallenged) oysters from 2020 and 2021 were similar so they were plotted together for simplicity.

Maryland for spawn 2021, to assess survival trends between diploid and triploid half-siblings, and the consistency of these results across spawns. The first survival assessment was conducted two months post-deployment (5 months post-fertilization) with substantial mortality observed among all groups at both locations (Fig. 1). At this time point, oysters from spawn 2020 had an average mortality rate of ~83 % ±7.4 which was significantly higher (Student's *t*-test, $p < 0.0001$, $n = 36$) than the mortality of oysters from spawn 2021 which averaged ~66 % ±10, though these data were collected from different locations so this comparison should be interpreted with caution. Gross observation of oysters (both live and dead) from 2020 revealed classical signs of juvenile oyster disease (JOD; caused by *Aliiroseovarius crassostreae*; i.e., cupped shells, asymmetrical growth of the shells, brown deposit on the inner face of the shell; Fig. 4) while oysters from spawn 2021 did not. When comparing survival rates of triploid and diploid oysters at each location, both diploid and triploid oysters had equivalent survival (two-way ANOVA, $p = 0.62$, $n = 30$) for both spawns (Fig. 1). When comparing the maternal source's influence on survival (each spawn was separately assessed due to the distinct growing conditions, making across-spawn comparisons inappropriate) significant differences were observed. For spawn 2020, eastern Long Island lines (HED, HET) displayed significantly higher survival than Connecticut (HCT, HCD) or NEH (HNT, HND) lines (Kruskal-Wallis, $p < 0.003$, $n = 18$), while for spawn 2021 the central Long Island lines (ID, IT) had significantly lower survival than the lines originating from NEH (NT, ND), or Rhode Island (RT, RD; Kruskal-Wallis, $p < 0.003$, n = 18; Fig. 5A).

Fig. 4. Five-month old oysters deployed in the Peconic Bay displayed typical gross signs of juvenile oyster disease (JOD), namely uneven valve margins (top) and conchiolin deposits on the inner face of the shell (bottom). The ruler shown is in cm.

3.3.2. *Nine months post field deployment (1-year post-fertilization)*

The second assessment of oyster survival was conducted in the spring (i.e., ~nine months after deployment) when oysters were one year old and after spending their first winter in the field. For both spawns, the overall mortality rates decreased compared to the initial post-deployment mortality, and mortality levels were no longer significantly different between spawns (Fig. 1). The average interval mortality between 2 and 9 months post-deployment was ~34 % ± 12 (mean ± standard deviation) for both spawns (Fig. 1), though the cumulative mortality of spawn 2020 was still higher at 89 % ± 6 while spawn 2021 was at 76 % ± 6 (Fig. 5B). Diploid and triploid oysters from both spawns were again found to have non-significant differences in survival when comparing either the interval or cumulative mortality rates (accounting for spawn; two-way ANOVA, $p > 0.05$, $n = 30$; Figs. 1 and 5B). Maternal source was again found to have a significant (one-way ANOVA, $p < 0.05$, $n = 18$ for either diploids or triploids) influence on the cumulative mortality at this time point, and this was driven by original mortality post-deployment since differences between lines for interval mortality (mortality occurring between 2 and 9 months) were nonsignificant. Overall, eastern Long Island lines performed better than the NEH lines but were no longer significantly better than the Connecticut lines for the 2020 spawn, and the central Long Island lines again performed significantly worse than Rhode Island or NEH lines during 2021.

3.3.3. *Fifteen months post-field deployment (18 months post-fertilization)*

The final field survival assessment was conducted after the oysters had been in the field for ~15 months which corresponds to the average growing time needed to achieve market size in the regions used for deployment. Overall cumulative survival rates for both spawns were low, with an average mortality rate of 94 % ± 2.5 (average ± standard deviation) and 86 % ± 3 for spawns 2020 and 2021, respectively (Fig. 5C). Interval mortality rates for this period had similar trends to the prior sampling point and were significantly (Kruskal-Wallis, $p < 0.001$, $n = 36$) higher for spawn 2020 (46 % ± 12) than for spawn 2021 (31 % ± 9, Fig. 1). Both diploid and triploid oysters displayed near identical

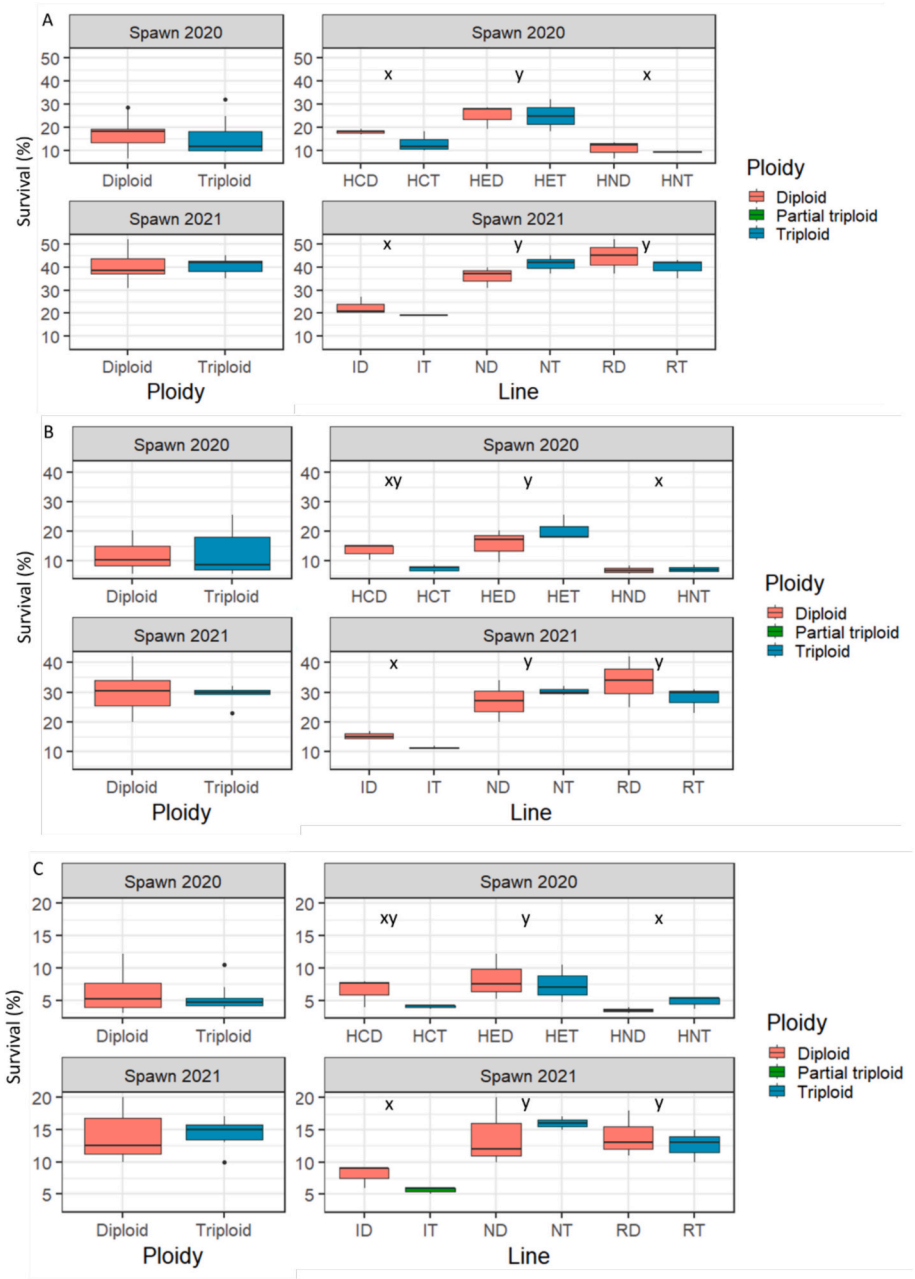


Fig. 5. Box and whisker plots showing cumulative survival of oysters from both spawns aggregated by ploidy (left panels) or displayed as individual cohorts (right panels). A) Survival of 5-month old oysters (deployed in the field for 2 months). B) Survival of 1-year old oysters (9-month deployment including winter). C) Final assessment of cumulative survival in 18-month old oysters. Within each plot, significant differences ($p < 0.05$) in survival between oysters derived from different maternal sources are indicated by different letters (x and y).

interval and cumulative mortality rates when controlling for maternal source and spawn, finding no significant ploidy-based differences for any comparison (2-way ANOVA, $p > 0.75$, $n = 30$). Maternal source of the oyster lines was again found to have a significant influence on the cumulative mortality (one-way ANOVA, $p < 0.05$, $n = 18$ for either diploids or triploids). Specifically, HED/HET had greater survivorship than HND/HNT for spawn 2020 while ID/IT had significantly lower survivorship than all other lines for spawn 2021 (Fig. 5C). Interval mortality did not significantly differ based on maternal source (Fig. S1C).

3.4. Survival correlations

Interval mortality rates at each time point were assessed and correlated to understand if early mortality trends of oysters exposed to vibrio pathogens would be reflective of later survival outcomes. Considering all experimental oyster lines, there was a strong significant positive correlation between larvae survival and juvenile survival when exposed to *Vibrio* pathogens (Pearson's correlation, $R^2 = 0.71$, $p = 0.0055$, $n = 12$; Fig. 6). In contrast, there were no significant correlations between larvae (two days post exposure) or juvenile (nine days post exposure) survival and field survivorship whether considering interval or cumulative mortality levels (Fig. S2).

4. Discussion

Triploids have become a staple of oyster aquaculture, with their predominance likely to continue into the foreseeable future, and

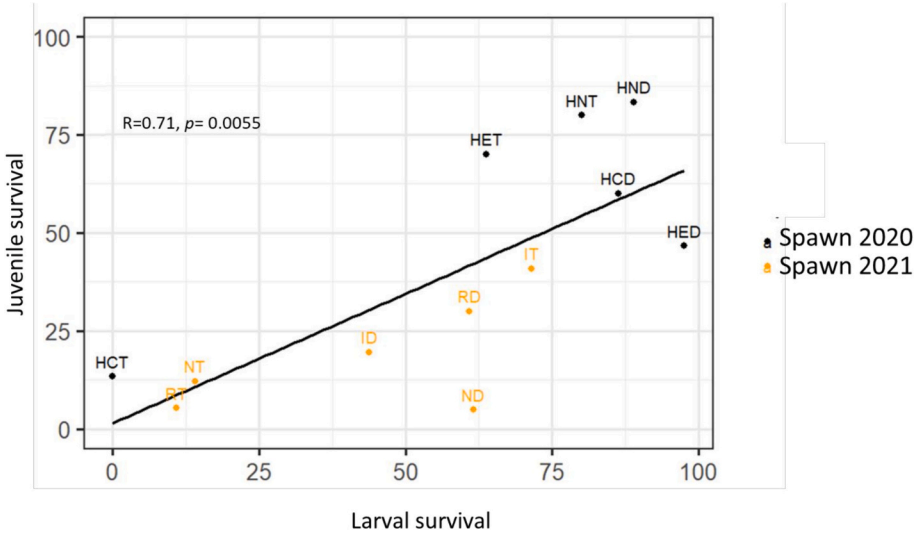


Fig. 6. Correlation analysis showing a positive relationship between larval and juvenile survival for oysters exposed to a *Vibrio* cocktail at two and nine days post exposure, respectively. Refer to Table 1 for code names used for oyster lines.

hatcheries likely to allocate more resources towards their production. To date, most studies have demonstrated that triploids have comparable survivorship, disease resistance, and stress tolerance (though some recent reports suggest dual stressors may be an exception; George et al., 2023) relative to their diploid counterparts, however, nearly all previous data has focused on adult performance (Duchemin et al., 2007; Wadsworth et al., 2019a; reviewed in Brianik and Allam, 2023). Consequently, assessments of the usually more vulnerable early life stages have received little attention, despite anecdotal reports from farmers indicating possible issues shortly after deployment (Long Island oyster farmer, personal communication). As such, this study sought to assess the resilience of triploids relative to diploids as they aged, using a combination of *Vibrio* exposure experiments and field deployments.

Oysters are often regarded as some of the most resilient animals due to their ability to thrive in volatile estuarine environments, however, this assertion often overlooks the high mortality rates experienced by the larval and juvenile stages. Bacterial infections are substantial contributors to mortality during these stages (Garnier et al., 2007; Elston et al., 2008; Richards et al., 2015), with host factors such as growth rates and pedigree known to impact survival outcomes (Davis and Barber, 1999). Here we observed that ploidy is also a factor that can influence susceptibility to bacterial infections with triploids being significantly more vulnerable to *Vibrio* pathogens during early developmental stages. The differential mortality between diploids and triploids seems to be directly related to the age of the animals, as diploid survival advantage decreased from ~3× to ~1.5× greater than triploid survival between the larval (one week post-fertilization) and juvenile (six weeks post-fertilization) stages. These results support the observations previously made by Azéma et al. (2016) who reported superior survivorship in diploid *C. gigas* spat (4–8 months) exposed to *V. aestuarianus,* but similar survivorship of diploids and triploids past the spat stage. The early ontogenetic triploid frailty in that study lasted notably longer than what we observed in our study (no ploidy-based differences at five months post-fertilization), which may result from the different bacterial species used in both studies or possible use of cytochalasin B for triploid induction by Azéma et al. (2016), as this method is known to negatively impact early-term growth and survival compared to the tetraploid cross-breeding method we used here (Wang et al., 1999; Wadsworth et al., 2019a). Alternatively, *C. gigas* may have slightly differing ploidy dynamics compared to *C. virginica*, as other triploid features such as the extent of sterility can vary between triploids of these species (Matt and Allen Jr, 2021; reviewed in Brianik and Allam, 2023) so comparisons between different species should be made with caution. Regardless, similar results have been reported in several fish species where young-of-the-year triploids displayed enhanced vulnerabilities to bacterial pathogens (Jhingan et al., 2003; Weber et al., 2013; Isidan et al., 2021), and generally present higher rates of deformities (Fraser et al., 2012) as compared to diploids. The reason for this ploidy disparity and triploid frailty during early ontogenetic stages is unclear. Fortunately, changes in husbandry methodologies (specifically nutrient supplementation and lower temperatures for triploids) have been shown to regulate immune responses (Ignatz et al., 2020) and reduce the incidence of deformities for triploid fish (Fraser et al., 2012; Taylor et al., 2015). This suggests that a reassessment of hatchery methods may help to bolster triploid performance which could hopefully reduce or shorten the extent of triploid frailty during the larvae and juvenile stages.

Once deployed in the field, ploidy had negligible influence on the survival outcomes of oyster lines whether assessing cumulative or interval mortality. These results are consistent with several previous studies that reported similar survivorship between triploid and diploid *C. virginica* (Dégremont et al., 2012; Walton et al., 2013; Wadsworth et al., 2019a). Deviations from this generality do exist, though these examples are typically the result of dual stress events (Wadsworth et al., 2019b; Bodenstein et al., 2021), for which triploids appear to be more susceptible (reviewed in Brianik and Allam, 2023; George et al., 2023). It should be noted that the field locations in this study were chosen for known occurrences of high mortality rates, though this did not lead to differential mortality between diploids and triploids for either spawn. Interestingly, the presence of JOD also did not cause any ploidy-based differences during the first field assessment in 2020, further supporting that triploid "frailty" decreases over time and may primarily be a concern for hatchery managers. One possible explanation is that weaker individuals in the triploid cultures disproportionately died during the hatchery/nursery period, resulting in hardier oysters being used for deployment than those used in the *Vibrio* challenges, although similar mortality trends were noted between diploid and triploid half-siblings during these early stages (excluding when experimentally exposed to the *Vibrio* cocktail) suggesting that such scenario is unlikely. Regardless, the findings from this study conflict with local anecdotal observations from farmers reporting issues with triploid seed performance, though, these differences may be attributed to the source and size of the triploid seed used. Oyster seed can be sourced at significantly smaller sizes (e.g., ~2 mm) as compared to the size we deployed here (8–10 mm) with younger (smaller) seed often times much cheaper for a farmer to purchase (Helm et al., 2004; Hensey, 2020). As such, some farmers prefer to buy smaller (i.e., cheaper) seed, but may be inadvertently increasing the

risk of early ontogenetic triploid frailty leading to their anecdotal observations. Additionally, while sources of diploid oyster seed in NY are available, triploid oysters grown in NY are virtually always imported from remote northern states (no local source of triploids and importation of southern shellfish stocks to NY is prohibited), making these triploids less adapted to the local environment and possibly diminishing their productivity as oysters are known to display high degrees of endemicity (Proestou et al., 2016).

Genetic background is a critical component of successful aquaculture efforts, with several oyster lines now available in most regions to cater to specific growing conditions (e.g., low salinity, fast growth, disease resistance). The ability to selectively breed oysters has had great success in developing these unique lines, with the benefits of selective breeding often maintained in triploid oysters (Hand et al., 2004). However, differing parental genomes may exhibit varying levels of tolerance to triploidization, leading to line-specific ploidy effects. For example, in salmon, certain combinations of parental genomes are less tolerant of triploidization resulting in reduced immune function of triploids from certain lines (Johnson et al., 2004). In our study, the Connecticut oysters (HCT, HCD) may represent one such example in oysters, as the larvae and juveniles from these two lines had the largest ploidy disparity of all the lines tested. Moreover, the Connecticut triploid (HCT) line tended to underperform compared to its diploid (HCD) counterpart during the field component of this experiment too, whereas other triploid-diploid pairs had more even responses throughout. The identification of other lines that may produce suboptimal triploids should be further investigated, as detection of lines that display increased early ontogenetic triploid frailty may aid in risk management. Follow up experiments working with larvae from a broad array of oyster lines may help to identify possibly weak lines, as larvae and juvenile results had a strong correlation, allowing hatcheries to avoid lines that may increase the chance of bacterial outbreaks. Final cumulative mortality in this study was relatively reflective of the mortality observed within the first 2 months post-deployment, with maternal source not found to influence interval mortality at 9 or 15 months post-deployment. These results suggest that if a farmer is capable of reducing the mortality of oysters early on, all the lines used for this study offer comparable survivorships, and that triploids present no greater risk than diploids.

5. Conclusions

Triploid oysters display enhanced vulnerability to vibriosis during their early developmental stages, though this increased frailty decreases as they age with adult survivorship not impacted by ploidy. It should be noted that only *Vibrio* pathogens were used in this study and further investigations may evaluate whether heightened sensitivity of triploid larvae and juveniles can be detected against other microbial pathogens. Regardless, early ontogenetic frailty of triploids observed in our study appeared to be influenced by the maternal background, with general results consistent between disparate spawns. Taken together, hatchery managers should have heightened awareness while culturing triploids, and ensure that biosecurity measures are as high as practically possible. Increased attention should also be given to how differing oyster lines respond to triploidization, to avoid particularly frail crosses. Following a microbial contamination event, hatcheries may benefit from producing diploids for a period of time, as complete removal of contaminants can be challenging, and diploids could be less likely to foster a bacterial resurgence. For farmers, this study supports that triploids are beneficial, as they do not pose an elevated risk of mortality events. While our field data revealed no concerns regarding triploid survivorship, farmers may still find benefit from avoiding small triploid seed to mitigate the potential for early ontogenetic triploid frailty. Future works should be conducted to reevaluate and possibly optimize the standard husbandry practices for triploids, as this area of research has received little to no attention and minor changes may be able to promote triploid performance. Predictions indicate that *Vibrio* induced hatchery mortality events may become more prevalent as temperatures continue to rise. As such the predominance of triploids in aquaculture may be unknowingly putting many hatcheries at greater risk necessitating mitigation strategies and the refinement of this technology to avoid serious economic troubles for the oyster industry.

CRediT authorship contribution statement

Christopher J. Brianik: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Emmanuelle Pales Espinosa:** Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Ming Liu:** Funding acquisition, Data curation, Conceptualization. **Pete Topping:** Data curation. **Gregg Rivara:** Funding acquisition, Data curation, Conceptualization. **Ximing Guo:** Investigation, Funding acquisition, Data curation, Conceptualization. **Dina Proestou:** Funding acquisition, Data curation, Conceptualization. **Bassem Allam:** Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgments

This research was supported by grants from the New York Sea Grant Institute (project R/XG-33), the Northeastern Regional Aquaculture Center (2018-38500-28885), and the Atlantic States Marine Fisheries Commission (award number NA18NMF4720321). We thank Dr. Gary Richards (USDA) for sharing one of the bacterial strains used in the challenge experiments. The authors would also like to acknowledge Dr. Caroline Schwaner for her assistance during some of the larvae and juvenile experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741613.

References

Azéma, P., Travers, M.A., Benabdelmouna, A., Dégremont, L., 2016. Single or dual experimental infections with Vibrio aestuarianus and OsHV-1 in diploid and triploid Crassostrea gigas at the spat, juvenile and adult stages. J. Invertebr. Pathol. 139, 92–101.

Bodenstein, S., Walton, W.C., Steury, T.D., 2021. Effect of farming practices on growth and mortality rates in triploid and diploid eastern oysters Crassostrea virginica. Aquac. Environ. Interact. 13, 33–40.

Brianik, C., Allam, B., 2023. The need for more information on the resistance to biological and environmental stressors in triploid oysters. Aquaculture 577, 739913.

Brown, C., 1981. A study of two shellfish-pathogenic Vibrio strains isolated from a Long Island hatchery during a recent outbreak of disease [New York]. J. Shellfish Res. 1 (1), 83–87.

Budiño, B., Cal, R.M., Piazzon, M.C., Lamas, J., 2006. The activity of several components of the innate immune system in diploid and triploid turbot. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 145 (1), 108–113.

Cal, R.M., Vidal, S., Gómez, C., Álvarez-Blázquez, B., Martínez, P., Piferrer, F., 2006. Growth and gonadal development in diploid and triploid turbot (Scophthalmus maximus). Aquaculture 251 (1), 99–108.

Davis, C.V., Barber, B.J., 1999. Growth and survival of selected lines of eastern oysters, Crassostrea virginica (Gmelin 1791) affected by juvenile oyster disease. Aquaculture 178 (3–4), 253–271.

De Decker, S., Normand, J., Saulnier, D., Pernet, F., Castagnet, S., Boudry, P., 2011. Responses of diploid and triploid Pacific oysters Crassostrea gigas to Vibrio infection in relation to their reproductive status. J. Invertebr. Pathol. 106 (2), 179–191.

Dégremont, L., Garcia, C., Frank-Lawale, A., Allen, S.K., 2012. Triploid oysters in the Chesapeake Bay: comparison of diploid and triploid Crassostrea virginica. J. Shellfish Res. 31 (1), 21–31.
Duchemin, M.B., Fournier, M., Auffret, M., 2007. Seasonal variations of immune parameters in diploid and triploid Pacific oysters, Crassostrea gigas (Thunberg). Aquaculture 264 (1–4), 73–81.
East coast Shellfish Growers Association, 2024. Representing the Needs of Aquaculture and the Environment. Available at https://ecsga.org/.
Elston, R.A., Hasegawa, H., Humphrey, K.L., Polyak, I.K., Häse, C.C., 2008. Re-emergence of Vibrio tubiashii in bivalve shellfish aquaculture: severity, environmental drivers, geographic extent and management. Dis. Aquat. Org. 82 (2), 119–134.
FAO, 2022. The State of World Fisheries and Aquaculture 2022. FAO, Rome, Toward blue transformation. https://doi.org/10.4060/cc0461en.
Fraser, T.W., Fjelldal, P.G., Hansen, T., Mayer, I., 2012. Welfare considerations of triploid fish. Rev. Fish. Sci. 20 (4), 192–211.
Garnier, M., Labreuche, Y., Garcia, C., Robert, M., Nicolas, J.L., 2007. Evidence for the involvement of pathogenic bacteria in summer mortalities of the Pacific oyster Crassostrea gigas. Microb. Ecol. 53, 187–196.
George, M.N., Cattau, O., Middleton, M.A., Lawson, D., Vadopalas, B., Gavery, M., Roberts, S.B., 2023. Triploid Pacific oysters exhibit stress response dysregulation and elevated mortality following heatwaves. Glob. Chang. Biol. 29 (24), 6969–6987.
Guo, X., DeBrosse, G.A., Allen Jr., S.K., 1996. All-triploid Pacific oysters (Crassostrea gigas Thunberg) produced by mating tetraploids and diploids. Aquaculture 142 (3–4), 149–161.
Hand, R.E., Nell, J.A., Thompson, P.A., 2004. Studies on triploid oysters in Australia: XIII. Performance of diploid and triploid Sydney rock oyster, Saccostrea glomerata (Gould, 1850), progeny from a third generation breeding line. Aquaculture 233 (1–4), 93–107.
Helm, M.M., Bourne, N., Lovatelli, A., 2004. Hatchery Culture of Bivalves: A Practical Manual.
Hensey, S.M., 2020. Improving the (off)-Bottom Line: Assessing the Costs and Benefits of Different Culture Techniques on an Alabama Commercial Oyster Farm (Doctoral dissertation,. Auburn University.
Ignatz, E.H., Braden, L.M., Benfey, T.J., Caballero-Solares, A., Hori, T.S., Runighan, C.D., Fast, M.D., Westcott, J.D., Rise, M.L., 2020. Impact of rearing temperature on the innate antiviral immune response of growth hormone transgenic female triploid Atlantic salmon (Salmo salar). Fish Shellfish Immunol. 97, 656–668.
Isidan, H., Ozturk, R.C., Aydin, I., 2021. Effect of ploidy status of juvenile turbot, Scophthalmus maximus, on innate immune response and disease susceptibility against Aeromonas salmonicida, Vibrio anguillarum and viral hemorrhagic septicemia virus. Bull. Eur. Assoc. Fish Pathol. 41 (4), 146–159.
Jhingan, E., Devlin, R.H., Iwama, G.K., 2003. Disease resistance, stress response and effects of triploidy in growth hormone transgenic coho salmon. J. Fish Biol. 63 (3), 806–823.
Jiang, R., Mu, Y., 2021. Analysis of agglomeration characteristics of Chinese oyster breeding industry. Chin. Fish. Econ 39, 55–61.
Johnson, R.M., Shrimpton, J.M., Heath, J.W., Heath, D.D., 2004. Family, induction methodology and interaction effects on the performance of diploid and triploid Chinook salmon (Oncorhynchus tshawytscha). Aquaculture 234 (1–4), 123–142.
Matt, J.L., Allen Jr., S.K., 2021. A classification system for gonad development in triploid Crassostrea virginica. Aquaculture 532, 735994.
Proestou, D.A., Vinyard, B.T., Corbett, R.J., Piesz, J., Allen Jr., S.K., Small, J.M., Li, C., Liu, M., DeBrosse, G., Guo, X., Rawson, P., 2016. Performance of selectively-bred lines of eastern oyster, Crassostrea virginica, across eastern US estuaries. Aquaculture 464, 17–27.
Richards, G.P., Watson, M.A., Needleman, D.S., Church, K.M., Häse, C.C., 2015. Mortalities of eastern and Pacific oyster larvae caused by the pathogens Vibrio coralliilyticus and Vibrio tubiashii. Appl. Environ. Microbiol. 81 (1), 292–297.
Sainz-Hernández, J.C., Maeda-Martínez, A.N., 2005. Sources of Vibrio bacteria in mollusc hatcheries and control methods: a case study. Aquac. Res. 36 (16), 1611–1618.
Schwaner, C., Barbosa, M., Connors, P., Park, T.J., de Silva, D., Griffith, A., Gobler, C.J., Espinosa, E.P., Allam, B., 2020. Experimental acidification increases susceptibility of Mercenaria mercenaria to infection by Vibrio species. Mar. Environ. Res. 154, 104872.
Taylor, J.F., Waagbø, R., Diez-Padrisa, M., Campbell, P., Walton, J., Hunter, D., Matthew, C., Migaud, H., 2015. Adult triploid a tlantic salmon (S almo Salar) have higher dietary histidine requirements to prevent cataract development in seawater. Aquac. Nutr. 21 (1), 18–32.
United States Department of Agriculture, 2006. 2005 Census of Aquaculture.
United States Department of Agriculture, 2019. 2018 Census of Aquaculture.
Wadsworth, P., Wilson, A.E., Walton, W.C., 2019a. A meta-analysis of growth rate in diploid and triploid oysters. Aquaculture 499, 9–16.
Wadsworth, P., Casas, S., La Peyre, J., Walton, W., 2019b. Elevated mortalities of triploid eastern oysters cultured off-bottom in northern Gulf of Mexico. Aquaculture 505, 363–373.
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, 260–266.
Wang, Z., Guo, X., Allen Jr., S.K., Wang, R., 1999. Aneuploid Pacific oyster (Crassostrea gigas Thunberg) as incidentals from triploid production. Aquaculture 173 (1–4), 347–357.
Weber, G.M., Wiens, G.D., Welch, T.J., Hostuttler, M.A., Leeds, T.D., 2013. Comparison of disease resistance between diploid, induced-triploid, and intercross-triploid rainbow trout including trout selected for resistance to Flavobacterium psychrophilum. Aquaculture 410, 66–71.
Zemeckis, D., 2020. East Coast Shellfish Hatchery and Nursery Directory, Rutgers University Cooperative Extension. New Jersey Agricultural Experiment Station, p. 6.