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Costs of induced defenses dissipate by maturity for diploid and triploid oysters

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ABSTRACT

Oyster reef restoration efforts and on-bottom aquaculture are frequently plagued with high predation rates. Oysters are phenotypically plastic, and rearing juvenile oysters, *Crassostrea virginica*, with predator cues causes them to grow stronger shells that increases survivorship in the field. However, induced defenses (e.g., shell hardening in oysters) are often associated with cost-benefit trade-offs, and the extent the increased shell strength persists into adulthood and alters the growth of somatic and reproductive tissues remains unknown. We raised diploid oysters (used in reef restoration) and triploid oysters (used in aquaculture) with and without predator cues for one month before placing individuals on an oyster farm to grow to market size. Oyster shell characteristics, soft tissue mass, and reproductive investment were measured periodically over one year of culture and compared across treatments. Both diploid and triploid oysters had significantly stronger and smaller shells than controls at the end of their nursery period. However, while diploid shells became 15 % stronger and 17 % smaller than controls, triploid shells became 28 % stronger and 23 % smaller. Additionally, triploid oysters exposed to predator cues returned to the size of controls faster and maintained their shell strength differences longer than diploids. Differences in soft tissue mass between treatments mirrored the patterns exhibited in shell size and weight with greater initial physiological costs and faster recovery for triploid individuals. There was no significant difference in somatic or reproductive tissue mass between induced and control oysters of the same ploidy after seven months in the field. Triploid oysters were 15–110 % larger than diploids depending on the characteristic measured at maturity. Additionally, there was a significant interaction between treatment and ploidy because induced triploids had marginally greater growth than their control counterparts while induced diploids had marginally less growth than controls. These findings demonstrate that physiological costs of oysters reacting to predators in early life stages are minimal by the time individuals reach maturity. Early exposure to predator cues is a promising tool for improving oyster survivorship in restoration and aquaculture operations, especially in regions with high predation pressure.

1. Introduction

Oysters are one of the most degraded marine habitats, with ~85 % of native reefs lost globally (Beck et al., 2011; McAfee and Connell, 2021). Harvests of wild oysters have declined substantially accompanied by the loss of their ecosystem services, where in the United States current levels are <2 % of their peak in the 1890s (Mackenzie, 2007; zu Ermgassen et al., 2013; Wijsman et al., 2019). Consequently, oyster aquaculture was the greatest source of bivalves globally in 2020 (FAO, 2023) in an effort to both supplement the loss of the wild fishery and to facilitate the restoration of oyster reefs and their benefits.

Oyster aquaculture often involves spawning larvae in a hatchery, rearing them for several weeks, and then moving cohorts into the field where they are often grown as “single seed” individuals within suspended cages. For restoration in areas that are recruitment limited, remote setting (i.e., spat-on-shell) is also used for oyster stock enhancement and restoration by allowing oyster larvae to settle onto shells or hard substrates before placing them in the field. High predation rates remain one of the greatest hurdles to both oyster aquaculture and restoration. Even in protected operations, such as off-bottom culturing,

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farms can lose 28 % of their biomass from predators (Richard et al., 2020) whereas unprotected operations can lose >90 % of planted juveniles within weeks (Mackenzie, 1970). While farmers have developed a number of methods to reduce mortality from predators, ranging from growing bivalves in protective containers (Gosling, 2008) to mass removal of predators (Calderwood et al., 2016), the most effective traditional techniques for reducing predation are too labor intensive for large-scale commercial growers or restoration efforts.

Early exposure of juvenile oysters to predator chemical cues in the hatchery appears to be an inexpensive technique for inducing oysters to grow stronger shells and reducing predator mortality in the field (Belgrad et al., 2021, 2023). Oysters are known to strengthen their shells in response to chemical exudates from crustacean (e.g. crabs; Newell et al., 2007) and gastropod (e.g. oyster drills; Lord and Whitlatch, 2012, Ponce et al., 2020) predators as well as the metabolites homarine and trigonelline (Roney et al., 2023). By rearing juveniles with caged predators or chemical cues, hatcheries can readily induce millions of individuals simultaneously and improve out-planting success, where survivorship can be increased by >50 % a year after planting on restored reefs (Russell et al., 2023; Lin et al., 2024). However, induced defenses are frequently associated with energetic costs that can result in reduced growth and fecundity (Harvell, 1990; Cronin, 2001; Relyea, 2002). Preliminary observations indicate that induced oysters also allocate less resources to growth during the induction period (Combs et al., 2019). Yet, oysters are a highly phenotypically plastic species whose growth depends on local environmental conditions (Rybovich et al., 2016; Lebreton et al., 2021), and it remains unknown the extent such initial energetic costs of early shell changes in response to predation risk persist into later life stages and alter the soft-tissue growth or fecundity of individuals. Long-term studies on the effects of induction are necessary to better evaluate the costs and benefits of this new hatchery technique.

While reef restoration operations use natural diploid oysters, many farm operations culture triploid oysters due to the different advantages the two ploidies provide. Triploid oysters appeal to farmers because of their enhanced taste profile and substantially faster growth rate that can shorten culture time over diploids (Maguire et al., 1994; Nell, 2002; Walton et al., 2013). This increased triploid somatic tissue and shell growth is generally considered to be a consequence of polyploid gigantism as well as their gonadal dysgenesis that causes partial sterility and is thought to cause individuals to shunt energy away from reproduction towards growth (Guo and Allen Jr, 1994; Nell, 2002; Chen et al., 2024). Conversely, reef restoration efforts utilize diploid stocks to ensure that oyster fecundity remains high and reefs become self-sustaining. Due to these inherent differences in energy allocation between ploidies, predator induction has a high potential to affect diploid and triploid oysters differently, but this has yet to be examined.

We quantified the extent predator induction affects oyster growth and reproduction as well as tested whether ploidy shapes the strength of the induction response in a long-term culture experiment. Here, we exposed diploid and triploid oysters to either a predator cue treatment (caged blue crabs) or control of no cue in the nursery before raising the oysters to maturity on an oyster farm and periodically checking their size, soft tissue mass, and reproductive investment. We hypothesized that differences in oyster characteristics across induction treatment would diminish over time as the oysters mature while triploids were expected to respond to predator cues more strongly than diploids due to triploids' propensity to not allocate energy reserves towards reproduction.

2. Methods

2.1. Oyster culturing for induction

Oysters (*Crassostrea virginica*) were spawned at the Auburn University Shellfish Laboratory (AUSL; Dauphin Island, AL, USA) in mid-May 2021. Diploids and triploids were spawned as half-siblings from the same maternal broodstock. Diploids were spawned via thermal shock methods using eggs and sperm from the broodstock line LAFT, with roots from Texas, Louisiana, Alabama, and Florida lines. For triploids, eggs from the LAFT line were strip spawned and fertilized with sperm from a tetraploid line (MAPCK) acquired through thermal shock methods. Ploidy was verified within 2 weeks post fertilization using standard techniques through flow cytometry and a CyFlow Ploidy Analyser (Chaiton and Allen, 1985). The resulting “eyed” larvae were set on microcultch to produce single-set spat.

Approximately 8000 diploid and 8000 triploid oysters were taken from this spawn and reared in upweller systems for one month. Oysters were held in four flow-through tanks (2.4 m × 0.9 m, water depth = 0.4 m) with two 57 cm diameter cylindrical upwellers in every tank. One upweller in each tank would contain all diploids while the second upweller would contain triploids (2000 diploid and 2000 triploid seed per tank). Filtered seawater (200 μm) was pumped from the Gulf of Mexico near Dauphin Island ensuring oysters received natural phytoplankton for food while filtering out predatory zooplankton. Oysters were initially suspended in the upwellers on 300 μm mesh, and after si × days the seawater filter was removed and oysters were suspended on a 500 μm mesh followed by 1 mm mesh to accommodate their growth. These holding containers mimicked normal nursery procedures for single seed (Matthiessen, 2001) which enables large-scale growth of oysters suitable for industrial applications, but does not offer the ability for high replicate tank numbers. However, previous work in these systems revealed that oysters exposed to predator cue treatments in big industrial scale tanks exhibit the same morphology patterns observed in small scale, highly replicated experiments (Robinson et al., 2014; Scherer et al., 2016, 2017; Combs et al., 2019; Belgrad et al., 2021).

Half of the oysters were reared in water with predator exudates by holding four live adult blue crabs, *Callinectes sapidus*, in two of the flow-through tanks (8 crabs total) while the remaining two tanks did not have crabs and served as a control (hereafter known as induced and control oysters respectively). Crabs were held in two partitioned baskets to prevent crabs from consuming the experimental oysters or each other while control tanks had empty crab cages. Each crab was fed one adult oyster daily (∼5.0 cm in length) to maximize predation risk cues, causing experimental oysters to receive exudates from both crabs and injured oysters as they were being consumed. Oyster upwellers were rotated daily to prevent tank placement effects. Crabs were replaced at least every other week to ensure predators remained healthy and to replace crabs that died.

2.2. Shell morphology measurements

After four weeks in the hatchery, subsets of oysters were removed to assess shell morphology and determine if exposure to crabs had caused shell changes including hardness and size. Fifty oysters were removed from each upweller ($n = 100$ individuals/treatment) and their shell size and shell crushing force measured (Robinson et al., 2014; Scherer et al., 2016). Shell length was measured from the umbo to the outer growth edge to the nearest 0.01 mm using digital calipers. We quantified the force needed to break each oyster shell using a penetrometer (Kistler force sensor 9203 and Kistler charge amplifier 5995). The probe of the force sensor was placed equidistant from the shell edges and perpendicular to the shell surface. Gentle, consistent pressure was applied until the shell cracked, and the maximum force needed to break the shell (N) was recorded. This technique is a standard proxy of shell hardness (Robinson et al., 2014). We divided shell crushing force by shell length to produce a size-standardized metric of shell strength (i.e. standardized crushing force, N/mm) because larger individuals naturally have a stronger shell as a byproduct of their size. Soft tissue was not quantified at this time due to the small oyster size that make measurements logistically challenging and error prone.

2.3. *Farm grow-out operations*

All remaining oysters were brought to AUSL's oyster farm in Grand Bay, AL (30° 22′ 29.45" N, 88° 18′ 53.11" W) to grow to maturity and assess the long-term costs of induction. The farm is near shore with an average low tide water depth of 0.75–1.25 m and modest tidal variation (~0.5 m). The substrate consists of firm mud and salinity typically ranges between 15 and 25. Experimental oysters were grown on the farm using standard off-bottom procedures (Walton et al., 2013). Oysters were placed in adjustable long-line baskets (BST, Ltd.) with oysters from different tanks and ploidies being kept in separate baskets (initially 2 baskets/treatment, ~2000 oysters/basket, 8 baskets total). Oysters were thinned periodically and split into additional baskets after two months (2 baskets/treatment, ~500 oysters/basket, 16 baskets total) and 6 months (3 baskets/treatment, 150 oysters/basket, 24 baskets total) into the grow out operation to maintain individuals at stocking densities typical for commercial oyster culture operations within the Gulf of Mexico. Baskets were clipped to an adjustable long-line that was raised above the high tide water line weekly for ~24 h air exposure to control fouling and reduce predator abundance.

2.4. *Oyster growth and condition assessment*

Subsamples of oysters were collected at 16, 124, 209, and 322 days in the field to assess changes in shell strength and tissue production (40, 40, 50, and 50 individuals sampled per treatment respectively with equal numbers of individuals from each nursery tank of origin). Sampling ended early June 2022 during peak spawning season (Ingle, 1951). All oysters had their shell morphology assessed in the same manner as described above in section 2.2. To determine if induction altered the growth of soft tissues, oysters collected at 16, 124, 209, and 322 days in the field were dissected and their tissue separated from the shell. Additionally, oysters collected on days 209 and 322 in the field had their reproductive tissue isolated via forceps and scalpel to assess relative reproductive effort. The reproductive tissue, remaining soft tissue, and shell were dried separately to constant weight at 70 °C. The dry mass of the reproductive tissue was divided by the total dry mass of the oyster soft tissue to produce a size independent metric of investment towards reproduction (i.e., gonadosomatic index, GSI; Kyomo, 1988) while mass of the reproductive tissue was added to the remaining dry soft tissue mass to quantify the overall tissue production of oysters.

2.5. *Statistical analyses*

To determine if oysters induced and assess the initial physiological costs of induction across ploidy, we ran generalized linear mixed models (GLMMs; R package: lme4) on oyster characteristics the day oysters left the nursery and were planted in the field (day 0) and after 16 days in the field. Induction treatment (blue crab cue, no-predator control) and ploidy (diploid, triploid) were set as fixed effects while tank (day 0 models) or holding basket (day 16 models) was treated as a random effect to control for nonindependence among individuals (Bolker et al., 2009). Separate models were performed for the dependent variables shell length and shell strength at day 0 as well as shell length, shell standardized crushing force, shell mass, and total soft tissue dry mass at day 16 (6 models). Every model used a Gamma distribution. All interactions were initially included in every model and nonsignificant interactions were removed stepwise, from the most comple × interaction terms to the simplest, following the protocol of Crawley, 2013 to help resolve the significance of main effects. Tukey's multiple comparison tests were used to determine pairwise differences in oyster characteristics (R package: DTK). All statistical analyses were conducted using R v4.1.2. (R Development Core Team, 2020).

To identify how induction may alter the growth patterns of diploid and triploid oysters over time, we ran generalized linear models (GLMs) with Gamma distributions that encompassed data across all the sample periods. Here, time in the field was added as a third explanatory variable along with induction and ploidy treatments. Separate models were run for the dependent variables shell length, shell standardized crushing force, shell mass, and total soft tissue dry mass. All interactions were initially included in every model and nonsignificant interactions were sequentially removed as described above (Crawley, 2013). In these models, a significant interaction between time and another treatment denoted that the treatment altered the growth patterns of oysters.

To assess the long-term consequences of induction on oyster morphology and physiology, we ran separate GLMMs on oyster shell length, shell standardized crushing force, shell mass, somatic tissue dry mass, reproductive tissue dry mass, and GSI at 322 days in the field during peak spawning season. We also ran separate GLMMs on oyster somatic tissue dry mass, reproductive tissue dry mass, and GSI at 209 days in the field prior to the spawning season to evaluate how oyster gonads were developing across experimental treatments (Ingle, 1951). These nine models used induction treatment and ploidy as fixed effects while holding basket was treated as a mixed effect. All models had a Gamma distribution, except for the analyses of reproductive tissue mass and GSI which utilized the more generalized Tweedie distribution (R package: glmmTMB). Tukey's multiple comparison tests were used to determine pairwise differences in oyster characteristics.

3. Results

3.1. *Initial induction and physiological costs*

Oysters responded strongly to blue crab predators in the nursery. Predator cue exposure significantly increased oyster shell strength as induced (i.e., exposed to blue crabs) diploid oysters were, on average, 15 % stronger than controls while induced triploids were 28 % stronger than controls at the end of the nursery period (coefficient = 0.04, t = 3.43, p = 0.0006; Fig. 1A). However, ploidy did not have a significant effect on shell strength initially (coefficient < 0.01, t = 0.22, p = 0.8297)

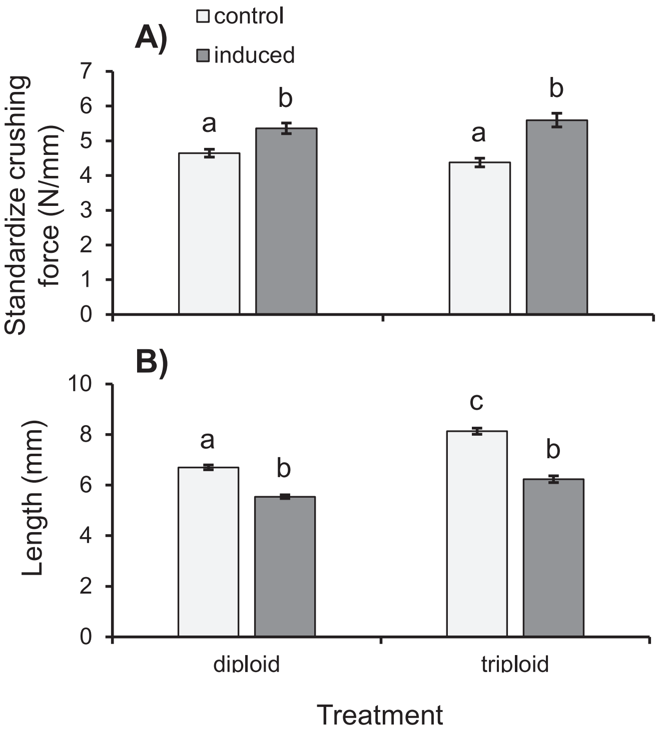

Fig. 1. Mean ± SE diploid and triploid oyster shell A) standardized crushing force (N/mm) and B) length (mm) after either being exposed to predator cues (induced) in the nursery for one month or reared without cues as a control (n = 100). Letters denote statistical differences.

and there was not a significant interaction between induction and ploidy treatments (estimate = 0.02, $t = 1.06$, $p = 0.2912$). Cue exposure also significantly reduced the shell size of oysters where induced individuals were 21 % smaller on average than controls (coefficient = −0.03, $t = -4.73$, $p < 0.0001$; Fig. 1B). Additionally, ploidy significantly influenced shell size as control triploid oysters were significantly larger than control diploids (coefficient = 0.02, $t = 2.99$, $p = 0.0028$). There was not a significant interaction between induction and ploidy treatments on shell size (coefficient < −0.01, $t = -0.68$, $p = 0.4975$).

Induced oysters continued to have 26 % stronger shells on average than controls after 16 days in the field (coefficient = 0.07, $t = 1.63$, $p = 0.1030$; Fig. 2A). However, this was not statistically different since holding basket had a large effect on shell strength where the average shell strength of the weakest induced oyster baskets would approximately equal the shell strength of the strongest control baskets. Induction also had notable costs on oyster physiology as oysters with prior exposure to predator cues had, on average, 22 % smaller shells (coefficient = −0.16, $t = -3.63$, $p = 0.0003$; Fig. 2B), 45 % lighter shells (coefficient = −3.40, $t = -4.49$, $p < 0.0001$; Fig. 2C), and 54 % less soft tissue (coefficient = −81.96, $t = -3.24$, $p = 0.0012$; Fig. 2D) than controls. Ploidy also had a substantial effect on oysters as triploid oysters had greater shell lengths (coefficient = 0.10, $t = 2.28$, $p = 0.0225$), shell weights (coefficient = 1.85, $t = 2.30$, $p = 0.0213$), and soft tissue amounts (coefficient = 45.87, $t = 1.79$, $p = 0.0728$) than diploids, but marginally weaker shell strengths (coefficient = 0.03, $t = 0.57$, $p = 0.5660$). These differences across ploidy were most apparent among control oysters, and consequently the physiological cost of triploids strengthening their shell (difference between induced and control oyster shell length, shell mass, and soft tissue mass) was on average 36 % greater than the cost for diploids. There were no significant interactions between induction and ploidy treatment at day 16 in the field ($p > 0.05$)

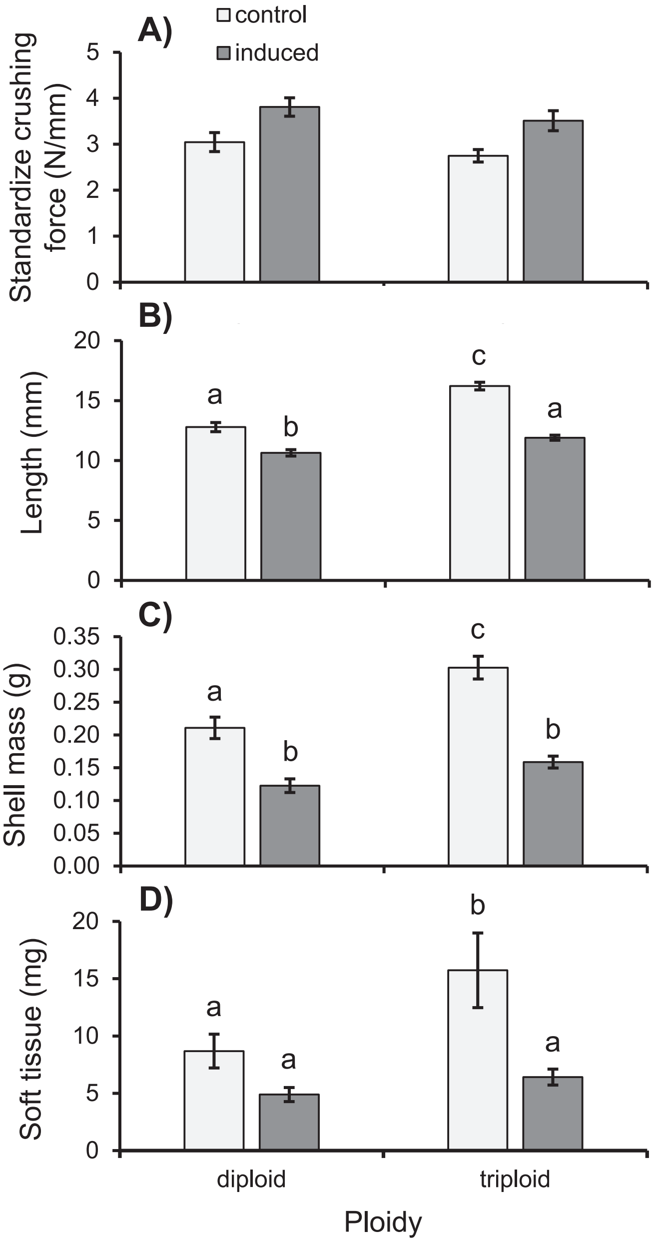

Fig. 2. Mean ± SE A) shell standardized crushing force (N/mm), B) shell length (mm), C) shell mass (g), and D) soft tissue mass (mg) of diploid and triploid induced and control oysters after 16 days in the field ($n = 40$). Letters denote statistical differences.

3.2. *The effect of induction and ploidy on growth patterns*

Both induction and ploidy shaped how oysters grew over time. The influence of these treatments was most comple × with shell hardness which had a significant three-way interaction between induction, ploidy, and time (Table 1). While both induced and control diploid oysters grew stronger shells at similar rates, triploid oyster shell strength grew less rapidly and differed by induction treatment (Fig. 3A). Shell length growth rate was also significantly influenced by induction and ploidy (Table 1). Triploid oysters grew rapidly in size, particularly those that were induced with predator cues, while diploid oysters grew slower (Fig. 3B). Ploidy, but not induction treatment, had a significant effect on the growth rate of shell mass and soft tissue mass (Table 1). Like with shell length, triploid oysters grew substantially faster than diploids (Fig. 3C,D). Indeed, the effect ploidy had on growth patterns was most

Table 1
Statistical results from generalized linear mixed models examining the effect induction (nursery exposure to predator cue or no cue), ploidy (diploid or triploid), and time in the field (0–322 days) have on oyster shell strength (N/mm), shell length (mm), shell mass (g), and soft tissue mass (g). Significant interactions between time and a treatment denote that the treatment altered the growth curve of oysters.

	Shell Strength			Shell Length		
Source of Variation	Coef	t	*p*	Coef	t	*p*
Induction	0.0191	2.20	**0.0283**	0.0066	2.05	**0.0406**
Ploidy	0.0079	0.92	0.3597	0.0103	3.19	**0.0015**
Time	4.1×10^{-4}	18.62	**<0.0001**	1.9×10^{-4}	19.65	**<0.0001**
Induction x Ploidy	0.0360	2.70	**0.0070**	7.1×10^{-4}	0.39	0.6984
Induction x Time	6.1×10^{-5}	1.89	0.0597	2.1×10^{-5}	2.03	**0.0425**
Ploidy × Time	6.2×10^{-5}	1.80	0.0728	2.6×10^{-5}	2.50	**0.0128**
Induction × Ploidy × Time	1.3×10^{-4}	2.60	**0.0094**	4.0×10^{-6}	0.19	0.8464
	Shell Mass			**Soft Tissue Mass**		
Source of Variation	Coef	t	*p*	Coef	t	*p*
Induction	0.0148	1.01	0.3113	0.0911	0.86	0.3921
Ploidy	0.0428	2.89	**0.0039**	1.2584	4.08	**<0.0001**
Induction × Ploidy	6.6×10^{-4}	15.22	**<0.0001**	0.0104	12.69	**<0.0001**
Induction × Time	0.0046	1.00	0.3199	0.1403	1.16	0.2470
Ploidy × Time	3.9×10^{-5}	0.85	0.3951	0.0008	0.82	0.4141
Induction × Ploidy × Time	1.1×10^{-4}	2.36	**0.0188**	0.0027	2.72	**0.0067**

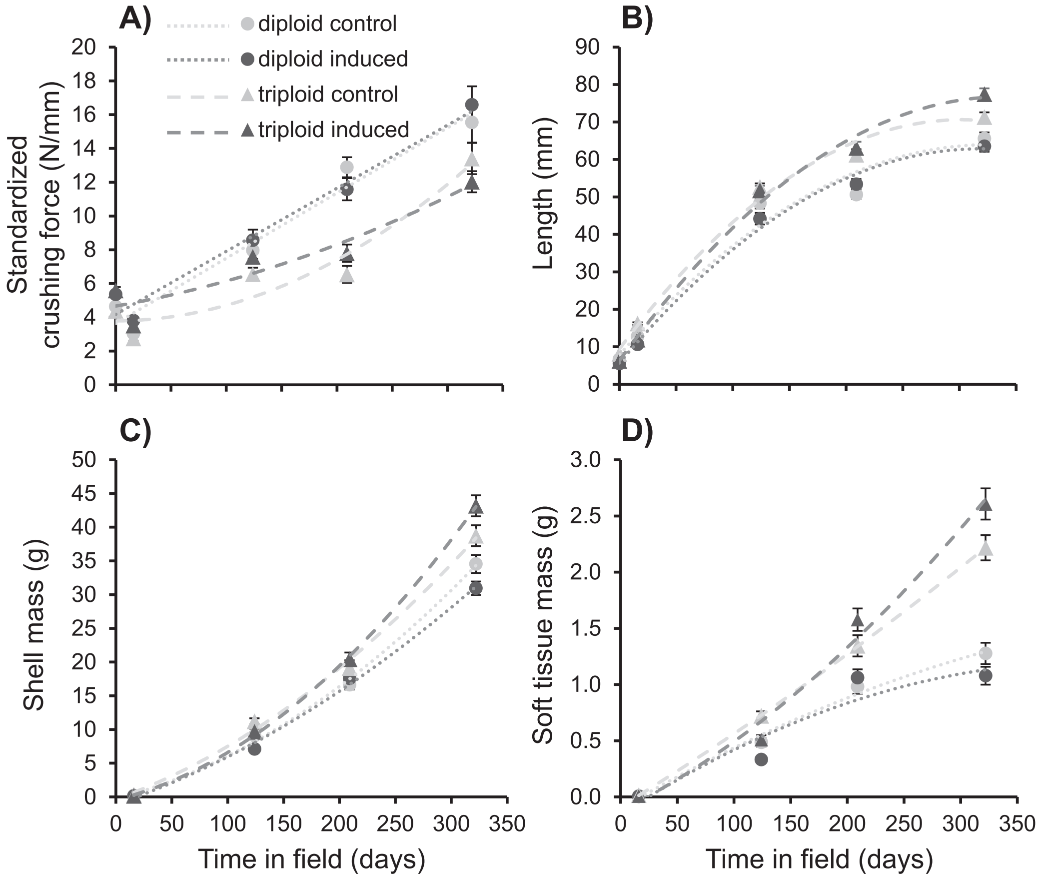

Fig. 3. Mean ± SE A) shell standardized crushing force (N/mm), B) shell length (mm), C) shell mass (g), and D) total soft tissue mass (g) of diploid and triploid induced and control oysters over 322 days in the field (n = 40–50).

pronounced with soft tissue mass where triploid growth was almost exponential while diploid growth was logarithmic (Fig. 3D).

3.3. Long-term consequences of induction and ploidy

After 322 days in the field, induction in the nursery no longer had a significant effect on shell strength, and induced oysters of the same ploidy were no longer significantly smaller than control oysters in any of the measured parameters (Table 2, Fig. 4). Instead, ploidy had a substantial effect on oyster characteristics where triploid oyster shell size-standardized strength was 21 % weaker than diploids while triploid shell length, shell mass, somatic tissue mass, and gonad mass were 15 %, 25 %, 110 %, and 50 % greater than diploids respectively. Triploid oysters' remarkable investment towards somatic tissue also caused triploids to have a 24 % lower GSI than diploid oysters (Fig. 4F). Interestingly, induction had a significant interaction with ploidy for shell length, shell mass, and somatic tissue mass as induced diploids would be marginally smaller than their control counterparts while induced triploids would be marginally larger than their controls (Table 2, Fig. 4B,C,D). However, triploid shell length was the only parameter where there was a significant difference between induction treatments of the same ploidy.

Prior to the spawning period (209 days in the field), oysters exhibited the same amount of reproductive tissue mass regardless of treatment (Supplemental Table 1, Supplemental Fig. 1). However, their overall reproductive investment was, on average, 77 % lower for triploids than diploids because triploids produced significantly more somatic tissue than diploids. Induction did not have a significant effect on soft tissues at this time point (Supplemental Table 1).

Table 2
Statistical results from generalized linear mixed models examining the effects of induction (nursery exposure to predator cue or no cue) and ploidy (diploid or triploid) on oyster shell strength (N/mm), shell length (mm), shell mass (g), somatic tissue mass (g), gonad tissue mass (g), and gonadosomatic inde × after 322 days in the field.

	Shell Strength			Somatic Tissue		
Source of Variation	Coef	t	*p*	Coef	t	*p*
Induction	0.0040	0.68	0.4986	0.1547	1.80	0.0725
Ploidy	0.0229	3.285	**0.0010**	0.6055	8.54	**<0.0001**
Induction × Ploidy	0.0126	1.30	0.1926	0.2206	2.31	**0.0207**
	Shell Length			Gonad Tissue		
Source of Variation	Coef	t	*p*	Coef	z	*p*
Induction	0.0005	1.07	0.2827	0.2431	0.69	0.4932
Ploidy	0.0028	6.62	**<0.0001**	0.7944	2.26	**0.0236**
Induction × Ploidy	0.0016	2.62	**0.0088**	0.6099	1.23	0.2189
	Shell Mass			Gonadosomatic Index		
Source of Variation	Coef	t	*p*	Coef	z	*p*
Induction	0.0034	2.05	**0.0406**	0.1370	1.25	0.2107
Ploidy	0.0091	6.08	**<0.0001**	0.2761	2.52	**0.0118**
Induction × Ploidy	0.0060	2.86	**0.0042**	0.0139	0.06	0.9490

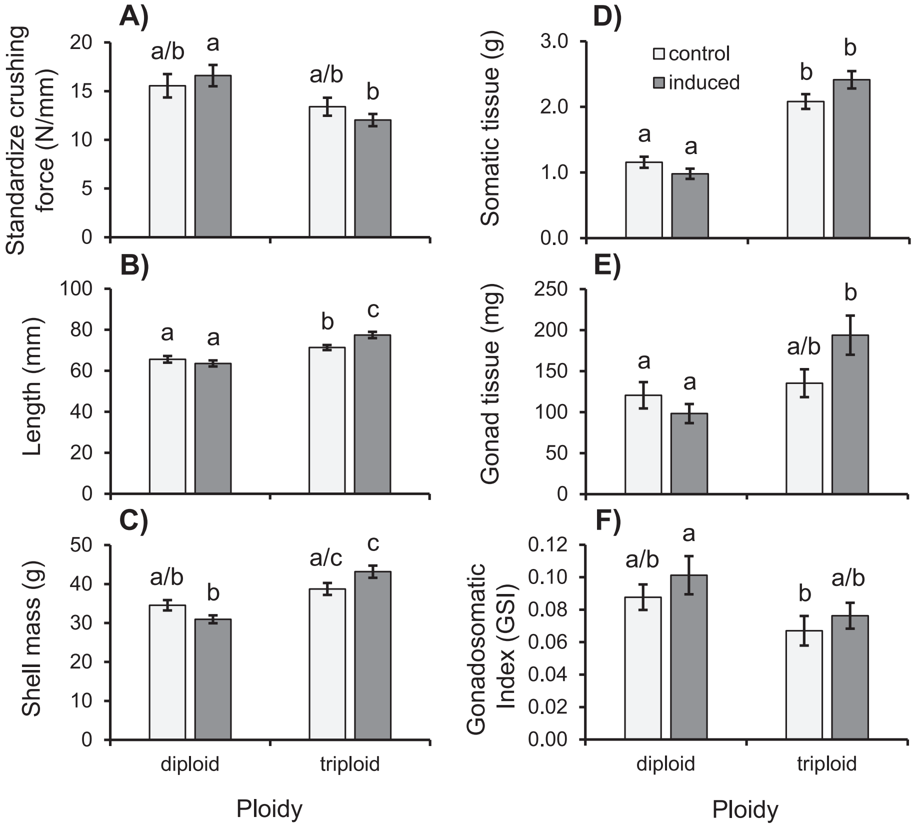

Fig. 4. Mean ± SE A) shell standardized crushing force (N/mm), B) shell length (mm), C) shell mass (g), D) somatic tissue mass (g), E) gonad tissue mass (mg), and F) gonadosomatic inde × (GSI) of diploid and triploid induced and control oysters after 322 days in the field ($n = 50$). Letters denote statistical differences.

4. Discussion

Although oyster induction had large physiological costs initially, by the end of the experiment these costs were essentially nonexistent. Instead, oyster ploidy dominated the growth patterns of individuals. Interestingly, triploid oysters responded more dramatically to predator cues than diploids, possibly because triploids maintain greater energetic reserves due to inherent differences in reproductive effort. These results quantify the growth rate for different oyster culture methods and highlight the value of a new technique to improve oyster aquaculture.

Induced defenses frequently come with energetic costs (Harvell, 1990; Cronin, 2001; Relyea, 2002), and our oysters likewise exhibited induction costs in the form of reduced shell size and soft tissue mass (Fig. 2). However, differences in oyster characteristics across induction treatment gradually decreased over time after oysters were no longer exposed to predator cues (Figs. 3 and 4). Both the benefits (stronger shells) and costs (smaller sizes) of induction were negligible after seven months in field. This is likely because oysters are extremely phenotypically plastic and growth is strongly dependent on the local environment where temperature, salinity, and food availability govern metabolic performance (Matthiessen, 2001; Rybovich et al., 2016; Galimany et al., 2017; Lebreton et al., 2021). The differences between induced and control oysters, while relatively large at their small size and young age out of the nursery, were extremely small compared to size at adulthood. Thus, induction treatment differences were likely progressively overshadowed as local environmental conditions increasingly governed oyster growth on the farm. These results indicate that early exposure to predator cues in the nursery is ideally suited for helping juveniles survive through early life stages when they are most vulnerable to predators (Belgrad et al., 2021, 2023), but is unlikely to confer survival benefits at later life stages.

Triploid oysters grew substantially faster than diploids, similar to previous studies (Nell, 2002; Walton et al., 2013). While the faster triploid growth matched the growth curves of diploids for shell length and shell mass (Fig. 3B,C), triploid growth patterns for soft tissue diverged, following a nearly geometric growth curve whereas diploid tissue growth was more logarithmic (Fig. 3D). Such faster triploid soft tissue growth highlights the advantage of farmers using triploid oysters as this can enable crops to reach market size quicker and allow more crop turnover.

Triploid oysters also exhibited markedly stronger induction responses and faster recovery of induction costs than diploids. This likely stems from the overall faster growth of triploids, and these findings support the theory that the fast growth rate of triploids may be fueled in part by having larger energy reserves due to gonadal dysgenesis (Guo and Allen Jr, 1994; Nell, 2002; Chen et al., 2024). Remarkably, triploid oysters produced more total reproductive tissue than diploids (Fig. 4E), which was mostly a function of triploids being larger overall, but only control triploids had statistically less investment towards reproduction than induced diploids when differences in individual size were considered (Fig. 4F). This suggests that triploids may be investing more towards reproduction than conventionally thought as we also encountered several instances of triploids spawning (Supplemental Video 1). However, our analysis only measured bulk gonad tissue and did not consider differences in gamete quality. Histological analyses of Pacific oyster, *Crassostrea gigas*, gonads found lower abundances of mature spermatozoa and higher numbers of apoptotic sperm cells in triploid oysters than in diploids (Chen et al., 2024). Thus, there may be differences in the quality of gametes the two ploidies produce due to their genetic differences, and more refined measurements of reproductive output would be necessary to quantify total fecundity differences.

This study is among the first to examine the long-term consequences of induced defenses in oysters to adulthood. Surprisingly, induced oysters invested slightly more towards reproduction than control oysters, although this investment was not significantly different for oysters of the same ploidy (Fig. 4F). Reproductive responses to predator cues are common and highly species dependent. These may range from early to delayed reproduction as well as larger to smaller overall brood or egg size depending on the species and life history strategy (Kats and Dill, 1998). One potential explanation for why induced oysters had greater GSI values than controls is because they were producing more gametes to increase the probability that more offspring escape predation in what was perceived to be a risky environment. As oysters are an r-selected species that rely on timing mass spawns together for fertilization and overwhelming predators (Ingle, 1951; Pianka, 1970; Bishop and Peterson, 2006), increasing the number of gametes produced in a high predation pressure environment may confer greater odds that genes are passed to the next generation rather than focusing on gamete quality and performance of offspring that have low individual survival probability. However, more in-depth studies are necessary to evaluate changes in the quantity and quality of gametes produced to quantify overall fecundity differences.

In conclusion, induced defenses against predators are a widespread phenomenon, and many bivalve species commonly cultured by the industry are known to grow stronger shells in the presence of predators (Leonard et al., 1999; Nakaoka, 2000; Bishop and Peterson, 2006). Given the speed oysters were able to ameliorate induction costs over time and how similar induced oysters were to controls at adulthood, rearing individuals with predator cues in the nursery is likely to yield a net gain in brood performance. The benefits of using predator cues in the nursery are likely to be especially high in on-bottom aquaculture practices and restoration efforts where predation plagues the industry (Matthiessen, 2001; Mann and Powell, 2007; Gosling, 2008; Wijsman et al., 2019), as even small increases in survival can change the economics of bivalve aquaculture. The ability of aquaculture practitioners to manipulate bivalve shell properties for commercial gain is only likely to grow as more research explores identifying the predator molecules that stimulate these shell changes and develops species specific cue exposure regimes.

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CRediT authorship contribution statement

Benjamin A. Belgrad: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Carter Lin:** Visualization, Methodology, Investigation, Data curation. **Christa Russell:** Visualization, Methodology, Investigation, Data curation. **Randi Cannon:** Visualization, Methodology, Investigation, Data curation. **Jessica Lunt:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Data curation. **Delbert L. Smee:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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