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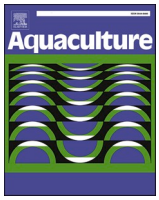

Review

The need for more information on the resistance to biological and environmental stressors in triploid oysters

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ABSTRACT

Triploid bivalves, specifically oysters, have become an increasingly popular aquaculture product over the last 3 decades due to their superior growth and meat quality. Despite a significant growth advantage, there remain surprisingly few consistent physiological or metabolic differences between diploid and triploid bivalves. This review summarizes the technical approaches used for triploid production before focusing on how triploidy affects key physiological performances, including growth and survivorship, tolerance to environmental stressors, resistance to disease, and immune performances. Survival trends appear to be similar between diploid and triploid oysters, though some immunological features appear to be enhanced in the larger cells present in triploid animals. Broad disease trends also appear to be similar between diploid and triploid oysters, although this topic remains relatively under-investigated and infrequently considers the genetic background of evaluated stocks, hence large knowledge gaps concerning how ploidy may influence host-parasite dynamics remain. Triploid oysters also seem to be equally capable of tolerating common environmental stressors, but coinciding stress appears to impact them more severely than diploids. The vast majority of work concerning triploid bivalves derive from field-based studies and as such considerable knowledge gaps exist outside of growth and survival prompting the need for more controlled approaches. Specifically, the impact of larger triploid cells that contain 50% more DNA on basic molecular and cellular functioning remains largely unexplored and may represent a fertile ground for fundamental and applied research.

1. Introduction

Aquaculture is the fastest growing food industry in the world with its contribution to global food production exceeding 100 million tons in 2016 alone (FAO, 2018). Advances in technologies have greatly contributed to this dramatic growth with the use of triploid animals being one of the most popular techniques currently used. Triploid animals have an extra set of chromosomes (3n) compared to their diploid (2n) counterparts, which makes most triploid animals functionally sterile. Triploidy was initially achieved by forcing the retainment of polar bodies during meiosis via physical or chemical stress (discussed below; Fig. 1) with triploidization now widely adopted for numerous species including several oyster species, salmonids, shrimp, and turbot (Nell, 2002; Xiang et al., 2006; Budiño et al., 2006; Fraser et al., 2012). Among oyster farmers, triploid animals are particularly popular and estimated to account for ~90% of oyster spat produced in France (Dégremont et al., 2010), a third of north American west coast aquaculture production (Guo, 2009), and approximately 90% of oysters sold in Virginia in 2017 (Hudson, 2018). Demand for triploid oysters is primarily due to the generally increased growth rates as triploid oysters grow ~30% faster than their diploid counterparts (Fig. 2), as well as the consistently high meat quality observed in triploids resulting from their sterility (Barber and Mann, 1991; Matthiessen and Davis, 1992; Nell, 2002; Wadsworth et al., 2019a). Not surprisingly, the improved growth rate associated with triploid oysters is highly advantageous as it can result in greater meat yields (Dégremont et al., 2012; Wadsworth et al., 2019a) and can allow stocks to reach market sizes several months earlier, limiting risks such as adverse weather events or disease related losses (Hand et al., 2004; Nell and Perkins, 2005). The advanced growth rate observed in triploid oysters is believed to be a result of energy reallocation from reproduction to somatic growth (Allen Jr and Downing, 1986), with possible contributions from increased genetic heterozygosity (Mallia et al., 2006; Hawkins et al., 1994) and triploid cell gigantism (Guo and Allen, 1994a; Guo et al., 1996). Although increased genetic heterozygosity, sterility, and cell gigantism are maintained in other triploid species, triploid growth rates are primarily non

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advantageous for most bivalves other than oysters (Guo and Allen, 1994a; Maldonado-Amparo et al., 2004; El-Wazzan and Scarpa, 2009; Table 1) and are highly variable between studies on fish (Maxime, 2008).

Although the growth advantage of triploids is mostly associated with oysters, the employment of triploid technologies has increased drastically over the last few decades as demand for sterile hatchery animals has become increasingly popular. For example, maturation in Atlantic salmon (*Salmo salar*) reduces flesh quality and growth leading to an estimated 4–9% loss in gross profits (Fraser et al., 2012) increasing the appeal of sterile triploid salmon. Similarly, spawning in oysters is associated with reduced glycogen content and meat quality (Nell, 2002). As a result, sterile triploids provide a more consistent product throughout the year and are more valuable during the reproductive periods of diploid animals.

In addition to providing a more consistent food product, the use of sterile triploids is also regarded as the most efficient and cheapest mechanism to prevent genetic spillover of hatchery stocks into wild populations. Hatchery lines are frequently selected for fast growth or specific economic features that do not necessarily translate to environmental longevity, making it critical that aquaculture processes do not reduce or negatively alter the genetic characteristics of wild stocks (Naylor et al., 2005; Maxime, 2008; Benfey, 2016). The ability of triploids to be deployed but not reproduce in the environment has also allowed the use of non-native species for bioremediation and other purposes without the undesired effect of permanent establishment (Zajicek et al., 2011). It must be noted though, that triploids are not completely sterile with the maximum reproductive potential of mated triploid and diploid *Crassostrea gigas* estimated to be 0.1075%, therefore complete containment cannot be ensured (Guo and Allen, 1994b). Triploid technologies have helped to expand aquaculture over the last few decades; however, aquaculture is still a highly volatile industry influenced by unpredictable abiotic and biotic factors.

One of the largest factors limiting bivalve aquaculture growth is disease related losses, as millions of dollars are lost globally to diseases such as Ostreid herpesvirus 1 in *C. gigas* (Pacific oyster), *Haplosporidium nelsoni* and *Perkinsus marinus* in *C. virginica* (eastern oyster), or *Mucochytrium quahogii* (formerly known as QPX) in *Mercenaria mercenaria* (hard clam or northern quahog) (Ford and Haskin, 1987; Segarra et al., 2010; Geraci-Yee et al., 2021). Such pathogens frequently lead to mass mortality events resulting from the exploitation of naive hosts, or through exhaustion during natural stress events (e.g., reproduction, low oxygen, high temperatures). Resulting from their reduced gonad development (minor gonad production is common but typically ceases before production of mature gametes; See Section 3.2), it has been proposed that triploids may offer a more stable resource as the absence of reproductive stress may free up energy reserves that could enhance their tolerance to other stressors (Meyers et al., 1991; Nell, 2002). However, the ability to withstand pathogens is ultimately derived from an animal's physiology and how its immune system can respond to insults such as consistent exposure to pathogens, a common occurrence for all marine animals. As triploid technologies become increasingly popular, understanding the health and immunological aspects of triploids is critical to understanding the durability and reliability of implementing this technology. This review aims to address how triploid technologies have impacted bivalve aquaculture. First, a short summary of technical

Fig. 2. Comparison of half-sibling diploid (left) and triploid (right) *Crassostrea virginica* after 1.5 years of growth in the same location, displaying the commonly observed triploid growth advantage.

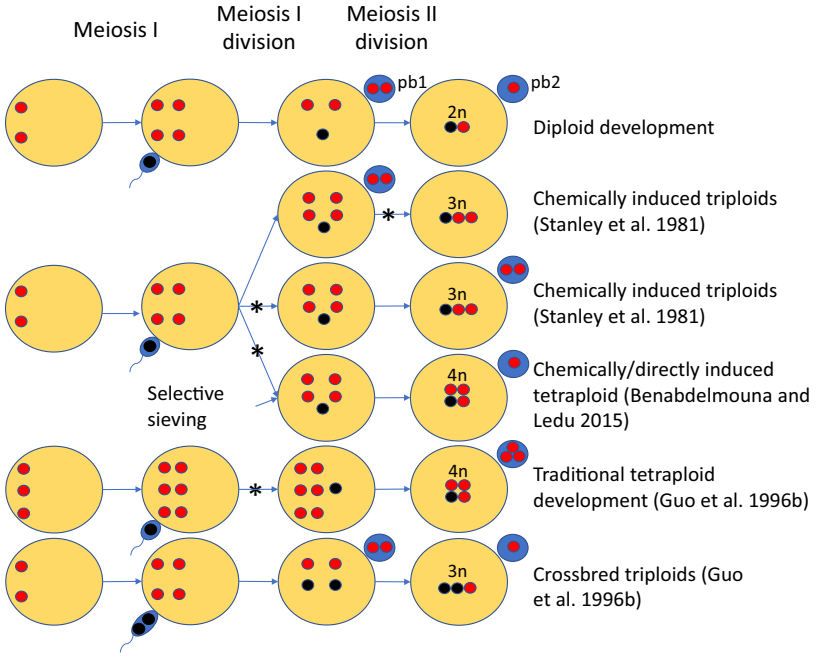

Fig. 1. A diagram showing the different routes in which diploid, triploid, and tetraploid oysters can be produced in hatchery settings. Asterisk (*) indicate the use of an inducer (primarily although not exclusively the actin polymerization inhibitor cytochalasin B) to inhibit polar body (pb) formation.

Table 1
Representative summary of studies (from most recent to oldest) contrasting performance of diploid and triploid mollusks. When multiple triploid induction methods were tested in a study only the best performing method is presented. Triploid percent rate sometimes changes over time and as such triploid % reports data from the last time point provided. Mortality data also represent cumulative levels reported on the latest time point provided. Ages shown in the "Stage" column are relative to post fertilization date unless stated otherwise. The table excludes papers that do not include direct diploid-triploid comparisons, studies that investigate hybrid triploids between different species, or those missing both survival or growth data. Growth rate and mortality display the performance of triploids as compared to their diploid counterparts. Cross: triploid produced by crossing diploid and tetraploid animals. NA: not available. DMAP: 4-Dimethylaminopyridine. CB: Cytochalasin B. The equal sign (=) indicates similar performance between triploids and diploids.

Study	Location	Field vs Lab study	Species	Stage	Triploid induction	Triploid%	Triploid growth rate	Triploid weight	Triploid mortality
Bodenstein et al., 2023	Louisiana, USA	Field	*C. virginica*	4 months–16 months	Cross	NA	= (height: low salinity site): Higher (height: moderate salinity site)	NA	Higher
Li et al., 2022	Shandong, China	Lab (heat stress)	*C. gigas*	18 months	Cross	NA	NA	NA	Higher
Yang, 2022	Florida, USA	Field	*C. virginica*	<1 year >1 year	CB CB	NA	Higher (height: 2/6 spawns/locations): = (height: 4/6 spawns/locations) Higher	Higher (wet: 3/6 spawns/locations): = (wet: 3/6 spawns/locations) Higher (whole)	NA NA
Osterheld et al., 2021	Québec, Canada	Lab	*Mytilus edulis*	Larvae (<15 days)	DMAP	90%	Higher	NA	=
Haure et al., 2021	Charente-Maritime, France	Lab	*C. gigas*	1 year old	Cross	NA	NA	Higher (whole)	NA
Bodenstein et al., 2021	Louisiana, USA	Field	*C. virginica*	1 year	Cross	NA	Higher	NA	Higher
Matt et al., 2020	Virginia, USA	Field	*C. virginica*	22 mm–82 mm	Cross	>90%	Higher (height)	Higher (wet)	= (except 1 site in June in which triploid mortality was observed)
De La Rosa et al., 2020	Magdalena, Colombia	Lab Field	*Argopecten nucleus* *A. nucleus*	<2.5 months >2.5 months	Cold-shock Cold-shock	59% 2%	= (length) = (length)	NA = (wet)	= Higher
Melo et al., 2020	Santa Catarina Island, Brazil	Lab	*C. gigas*	3–11 months	DMAP	25–75%	= (height)	= (whole)	Higher
Houssin et al., 2019	Normandy, France	Field	*C. gigas*	6 months-1 year	Cross	31–100%	NA	NA	Higher
Guévélou et al., 2019	Virginia, USA	Field	*C. virginica*	3–9 months	Cross	>98%	NA	NA	=
Ma et al., 2019	Shandong Province, China	Lab	*Patinopecten yessoensis*	0–90 days	Hyperosmotic shock (60 ppt)	46%	Higher (length)	NA	Higher
Qin et al., 2019	Guangdong, China	Lab Field	*Crassostrea hongkongensis* *C. hongkongensis*	Larvae (3–15 days) 90–600 days	CB CB	90–100% 90–100%	Higher (height) Higher (height)	NA Higher (whole)	= Lower
Wu et al., 2019	Guangxi Province, China	Lab Field	*Crassostrea sikamea* *C. sikamea*	Larvae (3–15 days) 180–450 days	CB CB	77% 58%	Lower (height) Higher (height)	NA Higher (wet)	Higher =
Wadsworth et al., 2019b	Alabama, USA	Field	*C. virginica*	6–18 months	Cross	NA	Higher (height: 3/4 sites)	Higher (dry: 3/4 sites)	Higher
Barreto-Hernández et al., 2018	Santa Marta, Colombia	Lab	*A. nucleus*	Larvae (2–15 days)	DMAP	39%	=	NA	Higher
Ibarra et al., 2017	Baja, Mexico	Field	*C. gigas*	4 months-1 year	Cross	94%	Higher (height)	Higher (wet)	=
Zhang et al., 2017	Guangdong Province, China	Lab Field	*C. hongkongensis* *C. hongkongensis*	1–15 days 90–360 days	CB CB	100% 100%	Higher Higher (height)	NA Higher (wet)	= (after day 15) Lower
Azéma et al., 2016	Charente-Maritime, France	Lab (OSHV-1 exposure)	*C. gigas*	4, 8, 16, 25 months	CB	NA	NA	NA	Higher in spat 2 stage: = other 3 stages
Callam et al., 2016	Virginia, USA	Field	*C. virginica*	6–18 months	Cross	100%	Higher (height)	Higher (wet)	NA
Dégremont et al., 2016	Charente-Maritime, France	Lab and Field (OsHV-1 exposure)	*C. gigas*	0.4–2.4 g	CB	86–95%	= (yield)	= (yield)	=
McCarthy et al., 2016	Tasmania, Australia	Field	*C. gigas*	~60-100 mm	NA	NA	Higher	NA	NA

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Table 1 (*continued*)

Study	Location	Field vs Lab study	Species	Stage	Triploid induction	Triploid%	Triploid growth rate	Triploid weight	Triploid mortality
Stone et al., 2013	South Carolina, USA	Field	*C. virginica*	3–11 months	Cross	NA	Higher (height)	Higher (ash free dry at 5/6 sites)	NA
Walton et al., 2013	Alabama, USA	Field	*C. virginica*	1–2 years	Cross	NA	Higher (height, length and width)	Higher (wet and dry)	=
Jouaux et al., 2013	Normandy, France	Lab (temperature stress)	*C. gigas*	24 month old	Cross	NA	NA	NA	Lower (fed), = (not fed)
Dégremont et al., 2012	Virginia, USA	Field	*C. virginica*	7–30 months	Cross	NA	Higher (height)	Higher (whole)	=
Pernet et al., 2012	Thau lagoon, France	Field	*C. gigas*	8–30 months	NA	NA	NA	NA	Lower
Meng et al., 2012	Liaoning, China	Field	*Patinopecten yessoensis*	0–24 months	Hypotonic shock	7%	Lower (height, length and width)	Lower (wet)	= (after 8 months)
De Decker et al., 2011	Charente-Maritime, France	Lab (vibrio injections)	*C. gigas*	>1 year	Cross & CB	>95%	NA	NA	Higher
Dégremont et al., 2010	Northwest and West of France	Lab (heat shock stress)	*C. gigas*	Juvenile (<1 year)	Cross	NA	NA	NA	Lower in 1/3 of trials: = in 2/3 of trials
Pernet et al., 2010	Thau lagoon, France	Field	*C. gigas*	1–1.5 years	Cross	NA	= (length)	= (wet)	=
Haberkorn et al., 2010	Morbihan, France	Lab (HAB exposure)	*C. gigas*	>20 months	NA	NA	NA	Lower (wet)	NA
El-Wazzan and Scarpa, 2009	Florida, USA	Lab	*Mercenaria mercenaria*	14–18 weeks	CB	18–94%	Lower (length)	Lower (whole)	NA
Liu et al., 2009	New South Wales, Australia	Lab	*Haliotis rubra*	7–37 months	DMAP and CB	DMAP = 100% CB = 51%	= (length)	= (whole)	=
Normand et al., 2009	Charente-Maritime, France	Lab	*C. gigas*	5 months	Cross and CB	>95%	NA	Higher (wet)	NA
Normand et al., 2008	Charente-Maritime, France	Field	*C. gigas*	Adult >3 years	CB	NA	Higher (shell weight)	= (dry)	NA
Schoonbee, 2008	Danger Point, South Africa	Field	*Haliotis midae*	0–2 years	Pressure shock	NA	= (length)	= (whole)	NA
Duchemin et al., 2007	Brittany, France	Field	*C. gigas*	1–2.5 years	NA	>99%	NA	NA	NA
Harding, 2007	Virginia, USA	Field	*C. virginica*	6 months- 2 years	NA	NA	NA	Higher (dry)	NA
Okumura et al., 2007	Iwate, Japan	Lab	*Haliotis discus hannai*	Juveniles (6 days post settlement)	Caffeine	>90%	NA	NA	Higher
Mallia et al., 2006	Tamil Nadu, India	Lab	*Crassostrea madrasensis*	3–15 days 2–12 months	DMAP DMAP	NA NA	Higher Higher (height)	NA Higher (wet & dry)	= NA
Gagnaire et al., 2006	Charente-Maritime, France	Field	*C. gigas*	>1.5 years	Cross	NA	NA	NA	Lower
Nell and Perkins, 2005	New South Wales, Australia	Field	*C. gigas*	Adult (>2 years)	Cross	100%	Higher (height)	Higher (whole)	Lower
Troup et al., 2005	New South Wales, Australia	Field	*Saccostrea glomerata*	21–42 weeks 42–160 weeks	CB CB	NA NA	NA NA	= Higher (wet)	NA =
Liu et al., 2004	New South Wales, Australia	Lab	*H. rubra*	20 weeks post settlement	DMAP and CB	DMAP = 100% CB = 82.5%	= (length)	NA	=
Hand et al., 2004	New South Wales, Australia	Field	*S. glomerata*	7–37 months	CB	89–91%	= (shell weight)	Higher (wet/ whole weight)	=
Brake et al., 2004	Prince Edward Island, Canada	Field	*M. edulis*	3–24 months	DMAP	28%	Higher (length)	Higher (dry)	NA
Maldonado-Amparo et al., 2004	Baja peninsula, California	Field	*Nodipecten subnodosus*	2 months-2 years	CB	60%	Lower (length)	Lower (whole)	=
Garnier-Géré et al., 2002	Charente-Maritime, France	Field	*C. gigas*	1–5 year old	CB or DMAP	NA	Higher (shell weight)	Higher (dry and whole)	=

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Table 1 (*continued*)

Study	Location	Field vs Lab study	Species	Stage	Triploid induction	Triploid%	Triploid growth rate	Triploid weight	Triploid mortality
Smith et al., 2000	New South Wales, Australia	Field	*S. glomerata*	Adult >3 years	CB	88%	Higher (height)	Higher (whole)	=
Calvo et al., 1999	Virginia, USA	Field	*C. virginica*	Adult 1–2 years	CB	85%	NA	NA	NA
Hand et al., 1999	New South Wales, Australia	Field	*S. glomerata*	Adult >2 years	CB	>83%	Higher (height)	Higher (whole)	=
Hand, 1998	New South Wales, Australia	Field	*S. glomerata*	Spat-2.5 years	CB	88%	Higher (height:12/13 sites), = 1 site	Higher (whole)	Lower (6/13 sites): =(6/13 sites): Higher (1/13 sites)
Guo et al., 1996	New jersey, USA	Lab and field	*C. gigas*	Larvae-3 months 3–10 months	Cross Cross	100% 100%	Higher NA	= (whole) Higher (wet and whole)	= NA
Goulletquer et al., 1996	La Tremblade, France	Field	*C. gigas*	>1 year	CB	53–70%	Higher (length)	Higher (whole)	Higher
Shpigel and Spencer, 1996	Wales, & Israel	Lab and Field	*Tapes philippinarum*	20-30 mm	CB	1–20%	NA	= (whole)	NA
Maguire et al., 1995	Tasmania, Australia	Field	*C. gigas*	6 months-3 years	CB	76%	Shell (weight)	= (whole)	=
Nell et al., 1995	New South Wales, Australia	Lab	*Tapes dorsatus*	2–18 weeks post fertilization	CB	56–85%	= (width)	NA	=
Nell et al., 1994	New South Wales, Australia	Field	*S. glomerata*	4 months-2.5 years	CB	85%	Higher (height)	Higher (whole)	=
Shpigel et al., 1992	Virginia, USA	Lab (temperature treatments)	*C. gigas*	>9 g whole weight	CB	75%	Higher	NA	=
Matthiessen and Davis, 1992	Massachusetts, USA	Field	*C. virginica*	Spat (45 days)- Adult (> 2 years)	CB	>99%	Higher (height)	Higher (whole)	=
Meyers et al., 1991	Virginia, USA	Lab	*C. virginica, C. gigas*	Adult	NA	NA	NA	NA	=
Barber and Mann, 1991	Virginia, USA	Field	*C. virginica*	3–16 months	CB	96%	Higher (height)	Higher (whole)	NA
Diter and Dufy, 1990	France	Lab	*Ruditapes philippinarum*	larvae -metamorphosis	CB	76%	= (length)	NA	Higher
Yamamoto et al., 1988	Miyagi, Japan	Lab	*C. gigas*	<25 days	Heat-shock	83%	Higher (length)	NA	= (at 24 h)
Allen Jr and Downing, 1986	Humboldt Bay, California	Field	*C. gigas*	>1 year	CB	96%	NA	Higher (dry and wet)	Lower
Stanley et al., 1981	Maine, USA	Lab Field	*C. virginica* *C. virginica*	0–24 h 8 months	CB CB	59% 74%	NA = (height)	NA NA	Higher =

approaches used to produce triploid organisms is provided, before delving into how triploidy affects key physiological performances such as growth, survivorship, and resilience to common environmental stressors. Finally, available information on immune performance in triploid bivalves is provided, highlighting areas where further research is needed. Overall, this paper focuses on bivalve mollusks with an emphasis on oysters although a brief summary of information generated from other aquaculture species is included to provide context, especially when such studies are limited or nonexistent in bivalves.

2. Induction of triploidy

2.1. Triploidy in fish

Triploidy can be a natural phenomenon and has been documented among several orders of wild fish (Rasch et al., 1970; Maxime, 2008). However, for commercial purposes obtaining a sufficient number of triploids involves manipulation during the fertilization process for both fish and bivalve aquaculture. In short, triploidy in fish is primarily induced via physical methods such as thermal shock or hydrostatic pressure shock which works by inhibiting the second polar body extrusion of recently fertilized eggs. Physical methods of triploid induction were demonstrated in a wide variety of fish species including salmon (Teskeredžić et al., 1993; Fraser et al., 2015), turbot (Piferrer et al., 2000) and grass carp (Zajicek et al., 2011) to name a few (see Tiwary et al., 2004 or Maxime, 2008 for a more extensive review of triploid induction and detection methodology in fish).

2.2. Ploidy manipulation in bivalves

In bivalves, the first manmade triploids were successfully produced in the late 1970s by exposing recently fertilized eggs of *C. virginica* to the chemical cytochalasin B (0.1 to 1 mg/l; Stanley et al., 1981; Table 1, Fig. 1). Cytochalasin B induces triploids by blocking polar body formation during meiosis I or meiosis II (Fig. 1), although blocking meiosis I is more difficult to perform, resulting in lower survivorship, and increases chances of aneuploidy (Wang et al., 1999). Overall treatments with cytochalasin B lead to 88% triploid induction on average (Wadsworth et al., 2019a). Several other methods of triploid induction have been developed throughout the years with different methods working best for certain species (see Table 1 in Yang et al., 2018). Of the two most commercially relevant oyster species, *C. gigas* and *C. virginica*, tetraploid-diploid crossbreeding (also called genetic triploids) methods developed by Guo et al. in the 1990s have emerged as the most effective method, capable of producing 100% triploid offspring (Guo et al., 1996; Calvo et al., 1999; Callam et al., 2016; Fig. 1) and have since become the industry standard for these species. The capacity to consistently produce 100% triploid spawns greatly facilitates the ability of farmers and researchers to directly compare batches of diploid and triploid oysters, removing recurring sampling problems due to varying ploidy levels produced via chemical induction. The crossbreeding method uses the sperm from tetraploid (4n) males to fertilize diploid (2n) eggs, removing the need for harmful and costly chemicals such as cytochalasin B, which is highly toxic (Nell, 2002). In addition to higher triploidy induction success rates, crossbreed triploids have been shown to have superior growth and juvenile survival compared to triploids produced using cytochalasin B to block meiosis II (Wang et al., 1999; Wadsworth et al., 2019a). Tetraploid development for new species and lines is an ongoing process with *Crassostrea angulata,* the Portuguese oyster, being one the most recently produced (Zhang et al., 2022). Unfortunately, tetraploid development has not been successful in all oyster species, making certain species such as the Sydney rock oyster (*Saccostrea glomerata* formerly *S. commercialis*) still require the use of chemical induction (Dove et al., 2020; Table 1).

Although crossbreeding methods have helped expand the use of triploid oysters, one drawback is that genetic triploids rely on established tetraploid lines. As a result, it is difficult for farmers to selectively breed locally adapted triploids as half of the parental genes must be sourced from one of the few tetraploid lines already available. This limitation is unfortunate as oysters are known to have a high degree of local adaptation, possibly limiting the benefits derived from these triploids (Burford et al., 2014; Bible and Sanford, 2016). Moreover, tetraploid lines are difficult to develop as they have primarily relied on breeding mature female triploids (>2 years; made using chemical induction methods) with male diploids and then treating the successfully fertilized eggs with cytochalasin B to block polar body one. This process results in a low survivorship with <1% of fertilized eggs making it to the spat stage (Guo et al. 1994b). Successful direct development of tetraploids from diploid stocks was demonstrated by Benabdelmouna and Ledu (2015) using *C. gigas,* by blocking polar body 1 formation. Chemical treatment during meiosis I can yield triploids, tetraploids and aneuploids, with tetraploids growing significantly slower than their siblings. Benabdelmouna and Ledu (2015) took advantage of the decreased tetraploid growth and were able to cultivate tetraploids by size specific sieving, though this method remains seldom used despite its apparent simplicity. In fact, “traditional” tetraploid development via fertilization of mature triploid oocytes is still the dominant method although this remains a strenuous task as triploids are practically sterile (Guo and Allen, 1994b), and resulting tetraploid larvae display enhanced mortality compared to both triploid and diploid larvae (Li and Li, 2022), justifying why few tetraploid lines are available. It should be noted that the sterility associated with triploid *C. gigas* is more consistent among the chemically induced triploids rather than the crossbred triploids, although this observation is less apparent for *C. virginica* (Matt and Allen Jr, 2021).

3. Environmental impacts on triploids

3.1. Effect of salinity fluctuations

Oysters inhabit volatile estuarine environments (often times intertidal) where they are renowned for their capacity to thrive in a broad variety of environmental conditions. *C. virginica*, for example, has a distribution encompassing nearly the entire east coast of North America, where it is known to withstand considerable temperature and salinity gradients ranging from −2 to 36 °C and 5 to 42 PSU, respectively (Kennedy et al., 1996). Droughts, heavy rainfall, tidal cycles and routine oyster handling procedures make exposure to these extreme conditions common in oyster aquaculture (Wadsworth et al., 2019b; Bodenstein et al., 2021). Due to their large geographic ranges, the frequency, intensity and types of stressors experienced by oysters are not uniform and have resulted in strong genotype-by-environment interactions leading to a great deal of endemicity in oysters (Proestou et al., 2016). As such, locally produced lines are often the best performers, regardless of ploidy, with specialized oyster lines now developed specifically for commonly encountered stressors (e.g., low salinity tolerant LOLA line from the Virginia Institute of Marine Science).

Salinity is commonly regarded as one of the most critical factors controlling the survival, growth, disease burden, and general success of bivalve aquaculture in coastal areas. However, information on the impact of salinity variations on triploid animals is limited with conflicting reports. For instance, three separate field studies were performed along salinity gradients in the Chesapeake Bay (all three studies had low, medium, and high salinity sites ranging from 8.3 to 26.3 PSU) and reported different survival trends in triploid *C. virginica*. The first study found that triploids had similar mortality levels as compared to their diploid counterparts across the salinity gradient (Calvo et al., 1999; Table 1), while the second study reported increased survival among triploids as compared to diploids at all locations albeit the trends were non-significant due to high cohort variance (Dégremont et al., 2012). Results from the third, and most genetically controlled (used exclusively tetraploid derived half-siblings), grow-out experiment contradicted both

previous studies and showed that half-sibling triploids only outperformed diploids in high salinity sections of the Chesapeake Bay, while they had equivalent and lower survival in sites with moderate and low salinity, respectively (Callam et al., 2016). Further, Callam et al. (2016) noted that there was a strong genotype-environment interaction in both diploids and triploids, suggesting the differences found between these studies may be a result of different oyster lines being used. In fish, in vitro work using triploid fish cells suggested triploids may be in fact more tolerant to hypoosmotic challenge due to the beneficial (smaller) surface area-to-volume ratio in triploid cells as compared to diploids (Ballarin et al., 2004). The larger volume of triploid cells could provide a larger pool of amino acids, which aids in osmoregulation (Pierce, 1982) and can act in tandem with the smaller area-to-volume ratio which limits water exchange at the cell surface. This was not the case though, as in vivo studies showed variable effect of triploidy on salinity tolerance, with many investigations reporting similar responses to salinity fluctuations among diploid and triploid fish (reviewed by Fraser et al., 2012). Changes in area-to-volume ratios likely influence processes other than osmotic regulation in cells since increased intracellular distances may lead to compromised signal transduction in larger cells, and impair membrane transport processes (Maxime, 2008), however these aspects of triploid biology remain largely unaddressed.

3.2. *Effect of temperature fluctuations in the context of partial fertility in triploids*

Gametogenesis is well documented to be severely reduced in triploid bivalves however it is not fully inhibited, with some evidence suggesting factors such as prolonged high temperatures may promote gonad development in triploids (although still reduced compared to diploids) (Shpigel et al., 1992; Guo et al. 1994b; Samain and McCombie, 2008; Ibarra et al., 2017; Melo et al., 2020). In a field study comparing how triploid growth advantage would be modulated under temperate and tropical environments, Ibarra et al. (2017) observed that the number of oocytes in triploids *C. gigas* was 60% and 18% compared to diploids in the tropical and temperate locations, respectively. Similar results were observed under laboratory conditions as chemically induced *C. gigas* triploids maintained at 30 °C for 35 days were shown to have advanced reproductive stages (spawning and reabsorption), while oysters held at 8–15 °C did not (Shpigel et al., 1992). Moreover, while producing the first tetraploid oyster lines, Guo and Allen, 1994b observed triploid fecundity to be an order of magnitude higher in their quarantined systems than in the field, which they attributed to prolonged increased temperatures in their system (although these animals still had fewer gametes as compared to diploids). However, temperature alone does not fully explain variations in triploid gonad development reported between different studies. For instance, Matt and Allen Jr (2021) observed that 19% of triploid *C. virginica* sampled from the Chesapeake Bay had substantial gonad development, while Wadsworth et al. (2019b) found that 20% of triploids from their location in the Gulf of Mexico had mature gonads, even though sites in the Gulf are markedly warmer throughout the year. Unfortunately, data concerning the impacts of temperature on triploid physiology are largely observational from field grow-out studies with numerous variables (genetic background, trophic resources, salinity, tidal air exposure, etc.) confounding the results. As such, identification of environmental thresholds and variables that promote triploid performance should be investigated more thoroughly to further improve their production and stability. Comparing the impacts of a single environmental stressor has been useful in other systems, as increased mortality (and appetite) was observed in triploid salmonids when exposed to higher temperatures (Ojolick et al., 1995; Preston et al., 2017) highlighting the needs for these triploids to be reared under

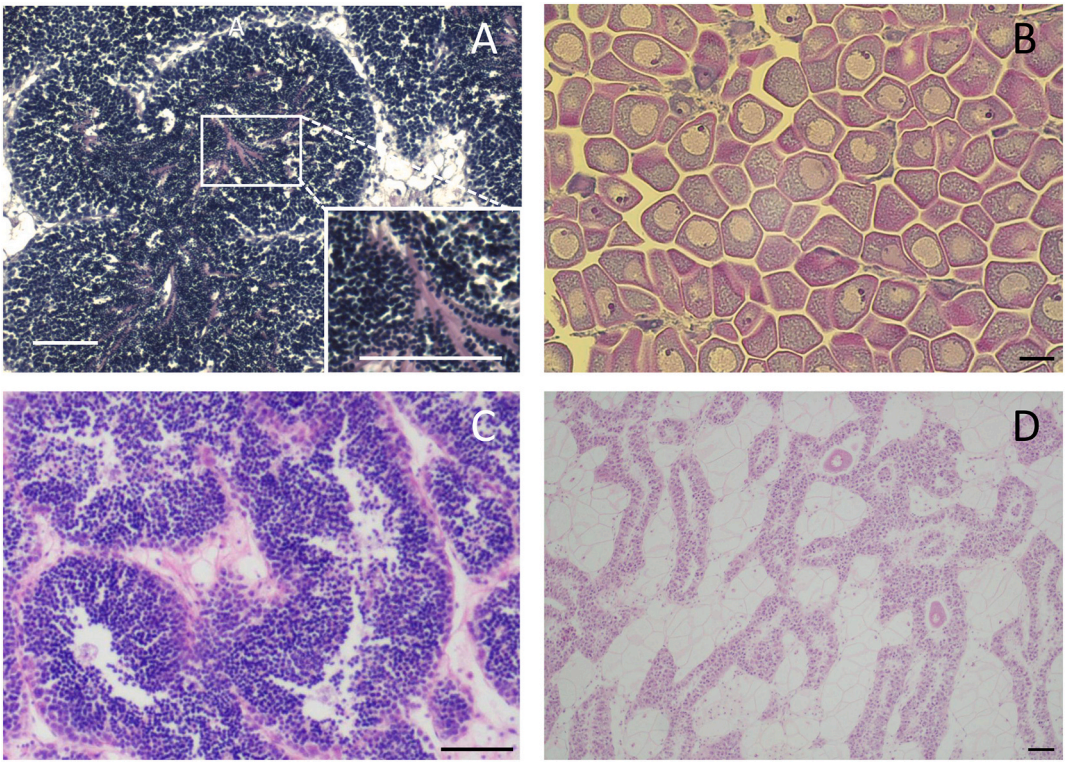

Fig. 3. Histological sections of gonad from diploid and triploid *Crassostrea virginica* during peak reproductive period (hematoxylin and eosin staining). A) Diploid male showing mature spermatozoa (note spermatozoa tails stained in purple-pink in the magnified section). B) Mature diploid female showing numerous well-developed oocytes. C) Male triploid at peak maturity where the follicles are lined with spermatogonia and containing numerous primary spermatocytes, but few to no spermatozoa. D) Oligo female triploid with few oocytes and follicles lined with oogonia. All scale bars are 50 μm. Photo credit for C and D: Joseph Matt. See Matt and Allen Jr (2021) for more information about gamete development in triploid oysters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cooler conditions than diploids to achieve their physiological optima. The variables that influence gonad development in triploids are likely multifaceted with temperature and genetics just part of the story, and as such identifying these variables should be prioritized to limit possible adverse outcomes of gametogenesis in triploids.

For diploid bivalves, gonad development and spawning are known stressors that can reduce the animal's ability to withstand heat stress and reduce immune performance (Li et al., 2007) with certain pathogens most virulent post spawning (De Decker et al., 2011). Although triploids generally display similar survival as compared to their diploid counterparts, this is typically observed in the absence of reproduction (Matt et al., 2020; Matt and Allen Jr, 2021). However, as stated above, sterility is not absolute in triploid oysters (Fig. 3) and when sterility is incomplete some authors have suggested that the benefits of triploids may be forfeited (Duchemin et al., 2007; De Decker et al., 2011; Houssin et al., 2019; Melo et al., 2020). For example, in *C. gigas*, abnormally pronounced gonad development was believed to have been a factor leading to significantly higher mortality among triploids in France during the summers of 2015 and 2016 (Houssin et al., 2019). Similar conclusions were drawn about an abnormal mass mortality event occurring in *C. virginica* from Virginia in the summer of 2014 (Matt and Allen, 2015). Oysters from these stocks were found to have advanced gonad development (without spawning), which corresponded to abnormally high triploid mortality. However, a recent follow up study found that triploid mortality of *C. virginica* did not correspond to gonad development and the authors suggested that a "physiological disorder related to reproduction" could be at fault (Matt and Allen Jr, 2021); albeit data for this study was limited as the authors were only able to collect 30 moribund oysters which had similar gonad morphology to healthy triploids. In fish, male triploids often develop testes similar to diploids but either without spermatozoa production or the production of nonviable sperm, while ovary development in females is severely reduced and does not lead to gamete maturation (see reviews by Tiwary et al., 2004 and Fraser et al., 2012). Overall, gamete development does not appear to influence triploid survivorship in fish, although this topic has not yet been explicitly addressed.

3.3. Food availability and relationship to triploid metabolism

The increased size observed in many triploid species have led some investigators to hypothesize that triploid physiology would require higher respiration rates and metabolic activities to sustain their increased growth rate, possibly resulting in greater susceptibility to stressors such as low food availability. Although logically sound, this theory has not panned out as both diploid and chemically induced triploid *C. virginica* presented equivalent mortality, respiration, and ammonia excretion rates when held at ambient (8–15 °C) and elevated temperatures (30 °C) (Shpigel et al., 1992). Equivalent respiration rates between ploidies were also noted in Sydney rock oysters by Kesarcodi-Watson et al. (2001), with only ammonia excretion rates significantly higher in adult diploids than triploids. In a recent study, Mizuta et al. (2021) contrasted the feeding physiology of diploid and triploid *C. virginica* and *C. gigas* and showed that animals within each species had equivalent activities (clearance rate, filtration rate, rejection rate, organic ingestion rate absorption rate, absorption efficiency, and selection efficiency) with disregard to their ploidy status, excluding absorption efficiency which was higher in triploid *C. gigas* as compared to their diploid counterparts. Analysis of gut contents on *C. gigas* triploids and diploids deployed in both fast growing and slow growing locations also found no difference in diet based on ploidy (McCarthy et al., 2016). Additionally, no significant difference in valve opening or duration of valve opening was observed between diploid and triploid *C. gigas* throughout the year as measured by high-frequency noninvasive (HFNI) valvometry (Payton et al., 2017). In conclusion, the way diploids and triploids allocate energy may vary considerably (growth vs gametogenesis), but the metabolic similarities between the ploidies suggest that the response to food stress should be similarly impactful regardless of ploidy.

3.4. Triploid performance under multi-stressor scenarios

Although exposing animals to single stressors is an important and necessary method to elucidate physiological thresholds, multiple co-occurring stressors are frequently present in nature, possibly resulting in synergistic effects. For instance, triploid salmon and rainbow trout (*Oncorhynchus mykiss*) both display significantly higher mortalities than diploids (Ojolick et al., 1995; Hansen et al., 2015), when exposed to both elevated temperatures (>20 °C) and hypoxic conditions (70% O_2 saturation); even though no ploidy difference was observed in respiratory requirements and mortality rates when both stressors were independently investigated (Benfey and Sutterlin, 1984; Hyndman et al., 2003; Galbreath et al., 2006). Observations such as these have led to the hypothesis that triploid animals may be more vulnerable to co-occurring stress events. Meyers et al. (1991) accidentally found support for this theory as a broken water pump exposed experimental triploid and diploid *C. gigas* to 2 days of desiccation and high temperature stress after which cumulative mortality was greater in triploids (34.3%) than diploids (25.1%), which was believed to be due to the prolonged exposure to hot air. In a more conscious effort to understand how multiple stressors would impact the survival of triploid oysters, Bodenstein et al. (2021) exposed oysters to desiccation and tumbling stress (common farm practices) on two separate occasions during the summer in the Gulf of Mexico. Results of this study also found that triploid oysters were at higher mortality risk from these farm stressors than diploids particularly when they are coinciding. Salinity and temperature extremes have also produced similar results as studies performed at field grow-out sites in Alabama reported that triploid *C. virginica* under prolonged stress from low salinity and high temperature experienced significantly greater mortality than diploids (Wadsworth et al., 2019b). However, in this study it took prolonged exposure (45 days) to rather extreme conditions (salinity <5 ppt and temperature > 28 °C) to lead to differential mortality between the two groups of oysters. Since environmental conditions frequently fluctuate and multiple coinciding stress events are regularly and increasingly encountered in aquaculture (particularly in estuarine environments where bivalve aquaculture is most active), the impact of concurrent multiple stressors on triploid animals deserves greater attention as the possibility of synergistic interactions between stressors may increase vulnerability and could have large implications for the industry.

4. Mortality trends in triploid animals

4.1. Juvenile mortality

Like all animals, different ontogenetic stages in triploids are sensitive to different stressors and can result in periods of differential mortality, and for r-selected animals such as bivalves and many fish species, the stages most prone to mortality are the larvae and juvenile stages. During their developmental stages, problems of increased mortality and deformities (lower jaw deformities, skeletal impairments, and ocular cataracts) have been commonly observed in triploid individuals from several fish species (Peruzzi et al., 2007; Fraser et al., 2012; Benfey, 2016). Some of the deformities acquired during early developmental stages persist within a fish stock leading to abnormal mortality and decreased marketability among the triploids later in life, ultimately reducing the demand for triploid fish stocks (Fraser et al., 2012). Fortunately, slight alterations of standard husbandry practices have shown efficacy in reducing triploid larvae mortality and deformities among salmon (Fraser et al., 2012; Taylor et al., 2015), suggesting that triploids may require slightly different rearing conditions to promote their growth as compared to their diploid conspecifics.

Similar benefits of enhanced triploid larvae survival have also been

observed in bivalves by optimization of husbandry methods, in particular switching from chemical induction to crossbreeding methodologies (Table 1). In *C. gigas*, triploids that were induced by crossbreeding have shown comparable survivorship to spat stages when compared to diploid controls (Guo et al., 1996; Li and Li, 2022), whereas chemically induced triploids present greater mortality, particularly before D stage larvae in several bivalve species (Yamamoto et al., 1988; Matthiessen and Davis, 1992; Guo et al., 1996; Hand, 1998; Brake et al., 2004; Mallia et al., 2006; Wadsworth et al., 2019a). Chromosome stability was also improved through the incorporation of crossbreeding methods as chromosome reversions (leading to aneuploids or "mosaic" organisms) were common among chemically induced triploids (often increased by stress events sometimes exceeding 20%; Nell, 2002). In this respect crossbred triploids are more stable (infrequently reverting), and methods are considered so reliable that the reporting of triploid percentage in studies has become less common over time (Table 1). Although triploid technologies have been around for some time there is still little known about how physiological requirements may differ between diploid and triploid organisms, particularly during early developmental stages. Aspects such as food quality and quantity, optimal rearing densities, and ideal growing temperatures all remain largely unexplored as optimal care is usually assumed to be the same between triploids and their diploid counterparts, though this may not be an accurate assessment. Therefore, understanding the differences in optimal growth and care between triploids and diploids is open for further development and optimization.

4.2. Triploid adult mortality

Among the adult stages of animals in which triploid technologies have been commercially developed, triploidy appears to be relatively well tolerated with the majority of publications reporting equivalent mortality rates between diploids and triploids, such as in the case of oysters (Table 1) (Hand et al., 1999; Smith et al., 2000; Garnier-Géré et al., 2002; Hand et al., 2004; Troup et al., 2005; Dégremont et al., 2012; De Decker et al., 2011; Walton et al., 2013) and fish (see review by Fraser et al., 2012). However, among triploid oysters, results can be variable with some studies reporting superior survival among triploids (Matthiessen and Davis, 1992; Samain and McCombie, 2008; Pernet et al., 2012) and others recently reporting specific mass mortality events occurring in triploid *C. virginica* and *C. gigas* (Guévélou et al., 2019; Houssin et al., 2019; Wadsworth et al., 2019a; Matt et al., 2020). Triploid-specific mass mortality events in Virginia and Alabama have even received the term "triploid mortality" as no obvious cause or etiological agent has been found responsible for these mortality events. Interestingly, some locations appear to be more prone to "triploid mortality", as Matt and Allen Jr (2021) were able to use historical data to predict a location in which "triploid mortality" would occur during their study. Regardless, the variables that may predispose a location to "triploid mortality" are still unknown. Moreover, it should be noted that the term "triploid mortality" appears to originate from a region dealing primarily with triploid monocultures, so this term may be misleading (Guévélou et al., 2019). Even so, mass mortality events of triploid oysters have occurred without any association with known pathogens raising concerns about the broad scale use of the technology without a thorough understanding of factors that may affect animal survivorship and aquaculture production.

The nuances in triploid survival success are exemplified in a field study by Hand et al. (1998) who reported that triploid Sydney rock oysters had significantly higher survival in 6 of 13 sites, lower survival at 1 site, and equivalent survival at the remaining 6 as compared to diploid conspecifics. The variability in survival was concluded to likely be a result of different temperatures and food availability between sites, although specific differences in food availability were not measured. Environment-genotype interactions are well known to impact bivalve aquaculture success, so triploid advantage being location dependent is not surprising and has been frequently observed (Matthiessen and Davis, 1992; Brake et al., 2004; Guévélou et al., 2019; Matt et al., 2020; Melo et al., 2020). When conducting an experiment to evaluate the effect of ploidy on performance and survival, careful consideration should be given to ensure the closest genetic background possible between diploid and triploid animals used in the comparison as genotype-environment interactions may blur signals derived from ploidy differences. To avoid the complication from genotype-environment interactions, incorporation of tetraploids produced using methods outlined in Benabdelmouna & Ledu (2015; discussed above) could be beneficial.

5. Triploid immunological performance

This section of the review will be focused on the innate immune system as it is shared by both fish and bivalves allowing more concise conclusions to be drawn. The immunological capabilities of triploid animals are of great concern as triploid success will result from an animal's ability to survive months or even years of daily exposures to potential pathogens. The primary constituent of the bivalve immune system is represented by hemocytes (the equivalent to leukocytes in vertebrates), which are responsible for wound healing, shell secretion, gamete reabsorption, phagocytosis of pathogens, encapsulation of foreign objects, and production of antimicrobial compounds (Song et al., 2010; Allam and Raftos, 2015; Allam and Pales Espinosa, 2016). Due to the critical functions of hemocytes, hemocyte metrics such as cell counts, proportions of different cell types (e.g., granulocytes vs. agranulocytes), and cell activities (phagocytic activity, production of reactive oxygen species, etc.) are regularly used to assess the immune status of bivalves (Allam and Raftos, 2015; Table 2).

Resulting from their extra set of chromosomes, the nuclei of triploid cells are typically ~50% larger than those of diploid cells (Rasch et al., 1970; Child and Watkins, 1994) causing a total increase in cell volume. This feature, dubbed triploid cell gigantism (Guo and Allen, 1994a), has been observed in numerous triploid animals including several species of fish (Rasch et al., 1970; Small and Benfey, 1987; Budiño et al., 2006; Maxime, 2008; Tolarová et al., 2014), shrimp (Xiang et al., 2006), *Drosophila* sp. (Held, 1979), clams (Guo and Allen, 1994a) and oysters (Haberkorn et al., 2010). The consequences of increased cell sizes are not fully understood, but one thought is that the increased cell volume could aid in key immunological functions such as phagocytosis (Fig. 4). Supporting this idea, triploid tench (*Tinca tinca*) and Atlantic salmon were shown to have higher phagocytic capabilities per cell, however, the triploid fish also had fewer cells in their circulatory system making overall phagocytic activity at an organismal level equivalent between ploidies (Budiño et al., 2006; Chalmers et al., 2016). In bivalves, *C. gigas* has been the most thoroughly studied in terms of triploid immune function (Table 2), however, the benefit of triploidy in regard to phagocytosis remains inconclusive as some studies observed superior phagocytosis rates among triploid hemocytes as compared to diploids (Gagnaire et al., 2006), while others found equal rates between ploidies (Duchemin et al., 2007). Unfortunately, these studies did not report phagocytic activity for different cell types so the discrepancies between the studies may be a result of different hemocyte compositions as Gagnaire et al. (2006) did note a slight but significant increase in granulocytes among triploids, which represent the cell type most responsible for phagocytosis (Allam and Ford, 2006; Jiang et al., 2016). Although the data among bivalves is currently inconsistent (Table 2), work involving fish such as *O. mykiss* (rainbow trout) and *Plecoglossus altivelis (*Ayu) found that nonspecific defense mechanisms (complement proteins, phagocytosis, and neutrophil activity) were equal between ploidies suggesting that susceptibility to disease would be similar (Kusuda et al., 1991; Yamamoto and Iida, 1995).

Beyond phagocytosis, hemocytes and blood cells (in fish) are responsible for many essential functions, and although lower total blood cell counts are frequently observed in triploid organisms (salmon, trout, shrimp) compared to their diploid counterparts, this reduction does not appear to negatively impact the animals. In fact, several experiments

Table 2
Summary of studies (from most recent to oldest) contrasting diploid and triploid oyster immune parameters. When multiple triploid induction methods were tested only the best performing method is presented. Triploid percent rate sometimes changes over time and as such triploid % reports data from the last time point provided. Performance of triploids as compared to their diploid counterparts is presented. Cross: triploid produced by crossing diploid and tetraploid animals. NA: not available. SOD: superoxide dismutase. HSP70: heat shock protein 70. The equal sign (=) indicates statistically similar performance between triploids and diploids.

Study	Location	Field vs Lab study	Species	Stage	Triploid induction	Triploid %	Hemocyte parameters	Antioxidant and stress response
Li et al., 2022	Shandong, China	Lab (heat stress)	*C. gigas*	18 months	Cross	NA	NA	SOD = in gills, Higher in hepatopancreas (excluding 48 h): Lower catalase activity: Higher malondialdehyde (indicative of oxidative stress): HSP70 variable between organs: Metallothionein =
Haberkorn et al., 2010	Morbihan, France	Lab (harmful algae toxin exposure)	*C. gigas*	>20 months	NA	NA	Hemocyte counts =: Greater cell size: Higher reactive oxygen species production: Phagocytosis =	Phenoloxidase increased
Duchemin et al., 2007	Brittany, France	Field	*C. gigas*	1–2.5 years	NA	>99%	Higher hemocyte viability (2/11 months) = (9/11 months): Lower hemocyte counts (March) = (10/11 months): Phagocytosis =	NA
Gagnaire et al., 2006	Charente-Maritime, France	Field	*C. gigas*	>1.5 years	Cross	NA	Hemocyte viability =: Higher percentage of granulocytes: Higher percentage of esterase-positive hemocytes: Higher phagocytosis	Higher percentage of peroxidase-positive hemocytes

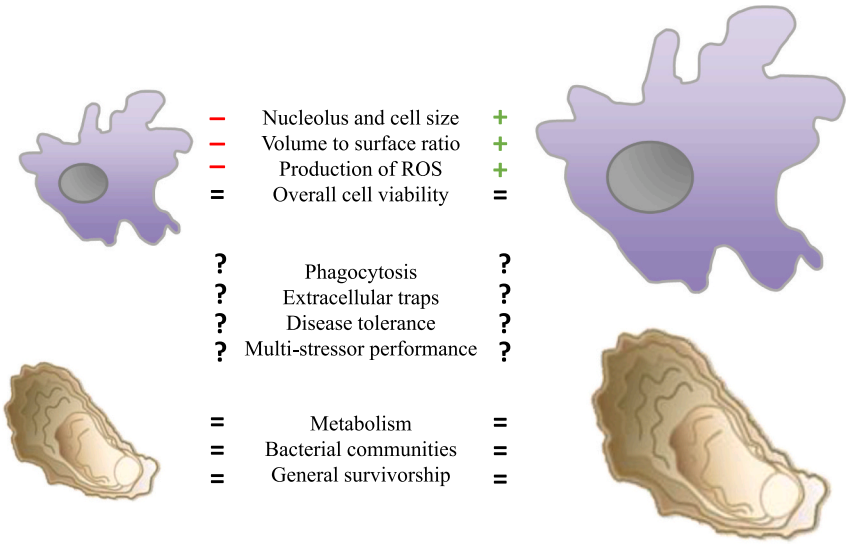

Fig. 4. Representation of organismal and cellular differences typically observed between diploid and triploid oysters. Symbols indicate parameters that are lower (–), higher (+) or equal (=) between diploids and triploids.? signify aspects of oyster biology that have either led to mixed results or topics that have yet to be addressed in the context of ploidy.

involving triploid fish exposed to stress (confinement, handling, and exhaustive), demonstrated that triploid fish responded equivalently to acute stress despite the fact that they had fewer blood cells as compared to diploids (Benfey and Biron, 2000; Sadler et al., 2000). Interestingly, another study showed that triploid trout actually recover from stress more rapidly than diploids (Hyndman et al., 2003) suggesting that the larger cells of triploid animals may compensate for their reduced number. The tradeoff between fewer but more active cells may be beneficial as certain immunological functions, such as oxidative burst (i.e., production of reactive oxygen species or ROS) and esterase production, have been observed to be increased in triploid fish and oysters (Gagnaire et al., 2006; Samain and McCombie, 2008; Haberkorn et al., 2010; Tolarová et al., 2014). These cellular products are critical to pathogen neutralization and as such imply greater resilience to pathogens. Interestingly, even though triploids of many species appear to have a tradeoff of fewer but more active cells, triploid oysters may deviate from this trend by retaining similar cell numbers along with their increased size. Evidence for this was demonstrated in *C. gigas* as diploid and triploid hemocyte counts were observed to be statistically equivalent throughout the year (excluding 1 month, March, when diploid showed higher

counts) (Duchemin et al., 2007), while the larger triploid cells produced more ROS (Haberkorn et al., 2010); however, few examples currently exist, and more work is required to see the breadth and consistency of these observations in bivalves.

Considered to be a general sign of stress, hemocyte mortality is a common feature used to assess stress responses. Seasonal sampling of field deployed *C. gigas* initially showed that triploids and diploids have equivalent rates of hemocyte mortality throughout the year, suggesting that both ploidies respond similarly to environmental stress during these times (Gagnaire et al., 2006; Haberkorn et al., 2010). However, a more resolute examination using monthly hemolymph samples detected a significant increase in the proportion of dead hemocytes in diploid *C. gigas* around gametogenic periods (2 of the 11 months sampled) (Duchemin et al., 2007). No work currently exists contrasting how diploid and triploid oysters immunologically respond to specific stressors in a controlled environment. Work examining key cellular immune parameters would be beneficial for identifying subtle differential stress responses that could impact immune capacities which in turn can translate into major alterations in animal's ability to resist infections under acute or chronic stress exposures.

Overall, the extent and the influence of having fewer but more active cells in triploids remain unknown, and its impact on disease dynamics will likely vary between pathogens as different pathogens can elucidate different immune responses. The influence of triploidization on aspects of the humoral immune system, cell regulation via apoptosis, extracellular traps (etosis), and numerous other aspects of cell biology remain completely unaddressed which is unfortunate as they are likely important for understanding the costs and benefits of this technology. This is particularly true in the case of etosis where DNA extruded from etotic hemocytes (Fig. 5) is clearly involved in antimicrobial processes as part of the innate immune response (von Köckritz-Blickwede and Nizet, 2009), and can also lead to substantial host damage if improperly regulated (Ortmann and Kolaczkowska, 2018). The presence of extracellular traps has now been demonstrated in several vertebrate and invertebrate species including oysters (Robb et al., 2014; Poirier et al., 2014; Fig. 5) although factors that regulate extracellular traps production and antimicrobial activity remain elusive with very limited data available on non-model organisms. This cellular response will likely vary according to the DNA content of each cell, making triploid cells potentially markedly more effective in neutralizing invaders than diploid cells, but also at increased risk of adverse effects from extracellular traps if the etotic response is improperly regulated. Overall, the ability to induce the production of extracellular nets from cells with different DNA contents makes oysters an appealing model for the study of etosis regulation and immune potency.

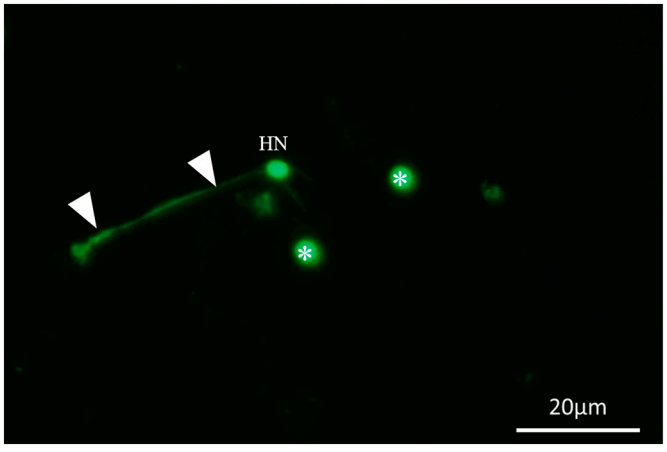

Fig. 5. *Crassostrea virginica* hemocytes in a monolayer stained using the cell impermeable DNA binding stain SYTOX green to visualize decondensed chromatin (extracellular trap) formed during the process of etosis (arrowheads) being extruded from a hemocyte nucleus (HN). Asterisks (*) denote nuclei of necrotic hemocytes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

6. Disease impacts and susceptibility of triploids

6.1. Parasitic infections

Genetic improvements in aquaculture lines (e.g., selective breeding) have had widespread success in reducing numerous parasites present in aquaculture, improving survival rates and yield of bivalves and fish alike (Guo, 2009; Regan et al., 2021). Acting in tandem with selective breeding efforts, the enhanced glycogen content (i.e., energy reserves) present in triploids (Qin et al., 2018) has led to a great deal of optimism about how triploids may further enhance disease resistance in stocks.

One of the first studies to address if triploidy may have any inherent impact on disease development was that of Meyers et al. (1991) who exposed chemically induced triploids of *C. gigas* and *C. virginica* to the protozoan parasite *P. marinus*. In this study, both ploidies of *C. virginica* became 100% infected, while triploid and diploid *C. gigas* had prevalence's of 12% and 20% respectively (statistically insignificant, possibly due to the small sample size). Cumulative mortality of diploid and triploid *C. virginica* reached 100% at 150 days post-infection, with parasite body burdens higher in diploids throughout the experiment. Halfway through the experiment, mortality of diploid *C. virginica* was significantly higher than that of triploids, which the authors hypothesized was due to a short-term energetic advantage due to triploid sterility. This same year, another study by Barber and Mann (1991) confirmed these observations as they found that ploidy had no impact on *P. marinus* prevalence or intensity in *C. virginica* during a field grow-out experiment with both ploidies experiencing high infection prevalence (96%) and intensities ranging from moderate to heavy. The results of these studies led to the conclusion that although the enhanced growth of triploids could be useful for production purposes, the animal's increased size and energy resources did not confer any inherent resistance (ability to prevent infection) against *P. marinus*. However, possible enhanced tolerance (ability to limit the damage of an infection) of triploids to parasitic disease was observed by Matthiessen and Davis (1992) who reported that chemically induced *C. virginica* triploids had consistently higher survival and significantly greater growth even though the triploids were more frequently infected with MSX as compared to their diploid controls (~2× more infected). It should be noted, however, that different spawning stocks were used for each ploidy and that the triploids in this study were from a selectively bred MSX resistant stock, possibly skewing the results. Additionally, notable mortality (~90%) of triploid larvae was experienced during this experiment (due to the use of cytochalasin B), which could have unintendedly selected for more tolerant individuals before the field phase of the study.

Since the initial observations concerning triploid performance (disease resistance, growth and mortality), crossbreeding methods (also called genetic) for triploid production have largely replaced chemical induction methods warranting a reassessment of these initial observations. Attempting to address this knowledge gap Dégremont et al. (2012) and Wadsworth et al. (2019b) deployed triploid and diploid *C. virginica* in the Chesapeake Bay and Alabama estuaries, respectively, then monitored the animals for nearly two years. Results of the Chesapeake Bay study corroborated the observations made by Matthiessen and Davis (1992), as the triploids in this study maintained significantly enhanced growth (152% larger), equivalent parasite prevalence, along with consistently less mortality (albeit not significant due to high variability) compared to diploids (Dégremont et al., 2012). In contrast, the Alabama study found mixed results based on location and time of year, with triploids tending to have heavier infections (Wadsworth et al., 2019b). Such results would be expected if triploid oysters had enhanced tolerance leading to prolonged survival with an infection allowing heavier infection intensities to be more prevalent. Similarly, triploid Sydney

rock oysters were observed to be 30% heavier (whole weight) and had a third the mortality of sibling diploids in sites impacted by *Bonamia roughleyi* (previously known as *Mikrocytos roughleyi,* or winter mortality) while mortality was equivalent in sites without the parasite (Hand et al. 1998). Overall, the influence of traits such as polyploidy, or sterility on disease tolerance needs further investigations to evaluate, for example, whether excess ("free") energy available by the lack of reproductive effort may allow triploid oysters to better cope with (i.e., tolerate) infections as the implications can be far-reaching for both basic and applied science.

6.2. Bacterial and viral infections

Bacterial pathogens represent a dual threat to the aquaculture industry impacting both people (via consumption) and animal stocks, making their relationship to ploidy a concern for the industry. Among people, bacteria from the *Vibrio* genus can cause serious harm as foodborne pathogens, with the risk of gastrointestinal damage and possibly death increasing as bacterial concentration exceeds 10^3 CFU per gram of consumed seafood tissue (World Health Organization, 2005). Addressing human consumptive needs, Walton et al. (2013) monitored diploid and triploid *C. virginica* grown in Alabama estuaries and observed that neither ploidy offered an advantage in reducing bacterial risk. Both diploid and triploid oysters had an equally high prevalence of human pathogens, *Vibrio parahaemolyticus* and *V. vulnificus* (100% and 81% respectively), with slightly fewer colony forming units per gram of triploid tissue. A more exhaustive sampling strategy in which samples were collected throughout the summer over a two-year period in Virginia also failed to detect any differences between pathogenic *V. parahaemolyticus* and *V. vulnificus* concentrations between 2 diploid and triploid oyster pairs (Audemard et al., 2023).

Oysters suffer from both specific and opportunistic bacterial pathogens in which virulence can be enhanced by stressful environments, ultimately leading to mortality events. In *C. gigas* coinciding spawning events, high temperatures, and high bacterial abundances have been shown to act additively leading to "Summer Mortality Syndrome" (SMS), which is capable of causing mortality events >50% (Wendling and Wegner, 2013). Being that reproduction is believed to be a primary factor in SMS, the possible use of triploidy to abate the virulence of *Vibrio* pathogens involved in SMS was hypothesized and evaluated using *C. gigas* by De Decker et al. (2011) via multiple bacterial injections throughout the year. The results showed that both triploid and diploid oysters responded similarly to infection with equivalent mortality rates overall. In this study, temporal differences were apparent showing triploids to have lower mortality during reproductive periods and higher mortality during non-reproductive periods (De Decker et al., 2011). Supporting these observations, an extensive field study by Pernet et al. (2012) observed similar trends with both ploidies of *C. gigas* having equal survivorship in winter and early spring, but with the mortality rate of diploids doubling that of triploids during summer and early fall. The observed mortality was also associated with increased detection of *Vibrio splendidus* and Ostreid herpesvirus-1 (OsHV-1). Interestingly, triploids in this study were observed to have enhanced elimination of viral DNA. Similar observations of better antiviral mechanisms were made in triploid Atlantic salmon exposed to Salmonid alphavirus subtype 1 (SAV1) where a slower accumulation of viral prevalence (Moore et al., 2017) and lower total qPCR copy numbers of viral RNA were detected as compared to diploid salmon (although mortality was equivalent) (Herath et al., 2017). Little is known about how triploidy influences intracellular viral dynamics making this an open area for further investigation.

The previous examples of how ploidy interacts with bacterial and viral pathogens were all conducted using crossbred triploid oysters, and while this is the primary method of triploid induction in *C. virginica* and *C. gigas*, the method only produces half-siblings with different paternal sources (tetraploids used for triploid induction are primarily male) which adds genetic variability between comparisons. In an attempt to remediate these differences, *C. gigas* diploids and chemically induced full-sibling triploids were induced and exposed to *V. aestuarianus* and or OsHV-1. Results showed that triploids were significantly more susceptible to *V. aestuarianus,* primarily during the spat stages (Azéma et al., 2016) and offered no increased protection to OsHV-1 regardless of age and size (Azéma et al., 2016; Dégremont et al., 2016). These results are somewhat surprising as other work has indicated that larger oysters are more tolerant to the OsHV-1 (Dégremont, 2013; Pernet et al., 2016) but the improved growth rate observed in these triploids apparently did not alter viral dynamics in these exposure experiments. There remains a dearth of information on how triploidy may alter individual cellular responses to pathogens and how these changes scale up to an entire animal, making further studies critical to understanding the mechanisms that have led to the conflicting results prevalent among triploid disease studies.

7. Conclusion

Triploidy is quickly becoming an industry standard and although it may never entirely replace more traditional methods, triploid technologies are all but guaranteed to play a significant role in the future of aquaculture. Although these animals offer some benefits compared to their diploid counterparts, current and future stressors such as marine disturbances induced by climate change, ocean acidification, and emerging diseases are likely to persist and continue to impact aquaculture. Our current understanding of the ability of triploids to withstand most stressors is still fragmented and largely based on observational studies, so if triploid animals are to persist as an important food source, a great deal of research is necessary to evaluate any inherent weaknesses in these animals, particularly under multi-stressor scenarios. Investigations using proteomic and transcriptomic approaches are also in dire need as this data is extremely limited and may enlighten mechanistic processes associated with triploid responses to environmental and biological stressors.

Beyond applied research, the increased DNA content and cell size of triploid animals represents a useful tool for basic scientific research. DNA derived extracellular traps (Fig. 5) have been shown to be a highly conserved and potent antimicrobial mechanism as well as a considerable cause of inflammatory disease (Delgado-Rizo et al., 2017), however numerous knowledge gaps exist in this field due to the breadth, and complexity of this response. In this regard triploid animals may serve a useful purpose allowing the investigation of how a 50% increase in DNA content influences formation, efficacy, and autoreactivity of extracellular traps in an otherwise healthy animal. Extracellular traps have been discovered in numerous invertebrate species including oysters (Robb et al., 2014; Poirier et al., 2014; Bachère et al., 2015; Fig. 5) making further investigation into triploid extracellular traps an area ripe for further research.

To ensure the longevity of triploid use in aquaculture and reduce the traditional risky "try-and-fail" approaches that are predominant in aquaculture development strategies, further research on the comparative immunity of diploid and triploid bivalves is a necessity. Such investigations will need to carefully consider the genetic background of investigated stocks to reduce or completely eliminate variations related to genotype-environment interactions. The research surrounding triploid animals is largely sporadic with few consistencies between studies. As such, further investigations are required to not only support aquaculture production, and by extension farmers and consumers, but to also bring insight into fundamental understanding of cellular and molecular processes, and how changes in DNA content can influence an animal.

Author contributions

CB drafted the initial manuscript with guidance from BA. Both authors edited and approved the manuscript before submission.

Declaration of Competing Interest

Authors declare no conflicts of interest.

Data availability

No data was used for the research described in the article.

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