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Research Article

Carry-Over Effects of Broodstock Conditioning on the Salinity Tolerance of Embryos of the New Zealand Geoduck (*Panopea zelandica*)

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The New Zealand geoduck (*Panopea zelandica*) has seen considerable interest from the NZ aquaculture industry. A major bottleneck in culturing *P. zelandica* is early life stages mortality (e.g., embryo). Therefore, in this study, we investigated the embryonic performance and their transition to the first feeding larval stage (D-veliger) under different salinities (26, 30, 32, and 35 ppt) of four different offspring groups generated from broodstock being fed different ratios (25 : 75, 50 : 50, 60 : 40, and 75 : 25) of the haptophyte *Tisochrysis lutea* (formerly *Isochrysis galbana*) (ISO) and the diatom *Chaetoceros muelleri* (CM) during gametogenesis. Broodstock within all diet ratio treatments successfully conditioned, producing viable embryos. Average egg size ranged between 75 and 80 μm and was not affected by the diet ratios of the broodstock. Survival 48 hr postfertilization, D-veliger larvae yield, and incidence of abnormalities depended on both the embryo rearing salinity and broodstock feeding ratios. The combined salinity of 32–35 ppt and a feeding ratio of 50 : 50 and 60 : 40 (ISO:CM) had the highest survival of embryos (56.0%–77.5%), highest production of D-veliger larvae (>65%), and lowest incidence of abnormalities within D-Veliger (<47%). The size of the larvae decreased with decreasing salinities, with the largest found at 35 ppt ($101.22 \pm 0.49\,\mu$m in shell length). Embryos and larvae did not survive at salinity 26 ppt. These results suggest that diet during gametogenesis can play a role on the offspring ability to cope with environmental stressors at least during the critical first few days after fertilization. These findings provide important information on transgenerational effects due to broodstock diet, especially during the early life stages.

1. Introduction

The New Zealand geoduck (*Panopea zelandica*) is a large sessile clam found in both the North and the South Islands of New Zealand. This species has received considerable attention from the New Zealand aquaculture industry [1, 2], with a significant projection to contribute to an overall aquaculture export sector of NZ$3 billion by 2035 [3].

While this species has excellent potential to achieve a high commercial value, there are several obstacles that need to be overcome before reliable production and markets can be established. Unlike other aquaculture bivalve species in New Zealand (e.g., Pacific oyster (*Crassostrea gigas*), Greenshell™ mussel (*Perna canaliculus*)), geoduck spat (seed) cannot currently be sourced from the wild [2]. Thus, the development of a geoduck aquaculture industry would necessitate a reliable source of suitable quantities of high-quality hatchery-produced spat [2]. A challenge associated with spat production of *P. zelandica* is that adults are difficult to obtain from the wild and maintain in captivity. In addition, current hatchery protocols and practices which are based on other *Panopea* species are not ideal for *P. zelandica*. Therefore, new protocols are being developed to establish an effective and efficient aquaculture industry for *P. zelandica*.

In general, for broadcast spawning bivalves, such as geoduck, parental investment (i.e., gamete quality) is a crucial determinant of larval success in terms of development, growth, and survival. Previous studies have shown that bivalve fecundity is species-specific and can be influenced by diet

(i.e., microalgal quantity and quality) [4, 5]. In hatcheries, cultured microalgae are the main source of polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) for cultured bivalves [6]. With the high diversity in nutritional composition (e.g., levels of lipids and PUFAs) among microalgal species, there is no single species of microalgae that can provide all the nutrients needed for broodstock conditioning during gametogenesis [7, 8]. For practical purposes, broodstock conditioning of bivalve species has tended to use microalgal species that are both readily available and have shown some success in supporting gonad development and spawning. One such diet combination is that of *Chaetoceros* sp. (rich in DHA) and *Isochrysis* sp. (rich in EPA), which has been used successfully with geoducks *P. generosa* [9] and *P. zelandica* [10] previously.

However, even when broodstock successfully spawn, the development of embryos and larvae might not be "optimal." Indeed, the energy required during early embryonic development is fully dependent on the resources provided for in the egg [11]. Also, the development of embryos into the first feeding larvae (i.e., D-veliger) is a process of intense cellular activity during which any impairment within a series of biochemical and physiological mechanisms can result in malformed larvae [12]. Thus, broodstock history tends to have a significant effect beyond gametogenesis and into embryonic development [11]. During development, embryos are more sensitive to environmental stressors compared to the juvenile and adult stages, especially with regard to salinity and temperature [13, 14]. Indeed, even minor deviations from optimal temperature ranges have been known to cause reduced developmental rates and increased abnormalities of embryos and larvae of *P. zelandica* [15]. As for salinity, pacific geoduck (*P. japonica*) embryos were shown to have a low salinity tolerance with both embryos and larvae unable to survive at salinities below 26 ppt [16]. High salinity susceptibility was also seen in scallop (*Argopecten irradians irradians*) embryos, which have a narrow salinity range for normal development [17]. There is little to no information about the optimal salinity range for development of *P. zelandica* embryos. In addition, it is likely that *P. zelandica* may have a distinct salinity tolerance range since, unlike other *Panopea* species which have a larger spatial distribution range extending to the intertidal zone, *P. zelandica* is completely restricted to subtidal environments between 5 and 25 m [18].

The present study was therefore designed to assess the efficacy of a range of practical microalgal mix ratios in the conditioning of geoduck broodstock through carry-over effects of their offspring (i.e., embryos and larvae), in terms of survival, D-veliger metamorphosis, incidence of abnormalities, and size of D-veligers after exposure to four different salinity regimes.

2. Methods and Materials

2.1. Parental Broodstock and Source of Gametes. Broodstock *P. zelandica* (116.41 ± 6.49 mm shell length, 669.92 ± 92.30 g wet weight) were collected from Golden Bay (South Island, New Zealand) and were conditioned at the Cawthron Institute's Aquaculture Park (Nelson, New Zealand) as a part of an ongoing geoduck research program since 2013. Briefly, four groups of 10 geoducks (a total of 40 animals) were housed in 100 L tanks connected to a flow through seawater system (Figure 1(a), blue lines). Each broodstock group was fed one of four different microalgal diet ratios, mixing the haptophyte *Tisochrysis lutea* (formerly *Isochrysis galbana*; "ISO") and the diatom *Chaetoceros muelleri* ("CM") (i.e., 25% : 75% = treatment code BSFR1, 50% : 50% = BSFR2, 60% : 40% = BSFR3, 75% : 25% = BSFR4, ISO:CM proportions; Figure 1(a), green lines). The concentration of microalgal cells in the feeding tanks was kept constant by keeping the outflow cell concentration at 40 cells/μL. All geoducks were fed for a period of 90 days, at which point all animals were induced to spawn, providing the gametes necessary to evaluate salinity tolerance in their offspring during embryonic development (i.e., 48 hr after fertilization).

2.2. Spawning and Fertilization. All broodstock were induced to spawn by adding an excess of microalgae directly into the holding tanks following current best practice available for this species. Once the animals began to spawn, they were separated and placed in individual 4 L containers ($0.3 \times 0.3 \times 0.45\ m^3$). This was done to ensure that premature fertilization did not occur. The number of spawned females and males in each feeding treatment were one female and three males BSFR1; three females and two males BSFR2; two females and three males BSFR3; and two females and two males BSFR4. The collected eggs were checked for shape and size, whereas sperm were checked for concentration and motility. All the gametes from all the individuals appeared normal and none were rejected. All gametes were then stored at 4°C. The females that spawned within each feeding treatment had their eggs pooled together after being checked. The sperm from all males across feeding treatments were pooled to standardize paternal effects. The eggs from individual treatments were suspended in 1,000 mL beakers of filtered seawater and fertilized with sperm at a concentration of 500 : 1 (sperm:egg ratio), following previous observations of high fertilization ratios and low polyspermy [19]. Eggs and sperm were left to fertilize for 30 min at 34 ppt salinity and an ambient temperature of 17°C, after which excess sperm was washed away through a 43 μm mesh with FSW (Figure 1(b)). The fertilized eggs were concentrated in 1 L beakers and adjusted to get a final concentration of 2,000 fertilized eggs mL^{-1}. Fertilization success was measured by observing the formation of polar bodies under an Olympus Omax compound microscope at 20x magnification.

2.3. Embryo Challenge Setup and Procedure. The challenge experiment was designed to evaluate embryonic development (obtained from the four different broodstock groups) under four different salinities in tissue culture dishes (TCD's). The salinities selected were 26, 30, 32, and 35 ppt and were created by adding different volumes of reverse osmosis (RO) filtered freshwater to 1 μm filtered UV-treated seawater (Table 1) at 15°C. Eggs obtained from each broodstock diet group and salinity combination (4×4) had six 4 mL TCD replicate

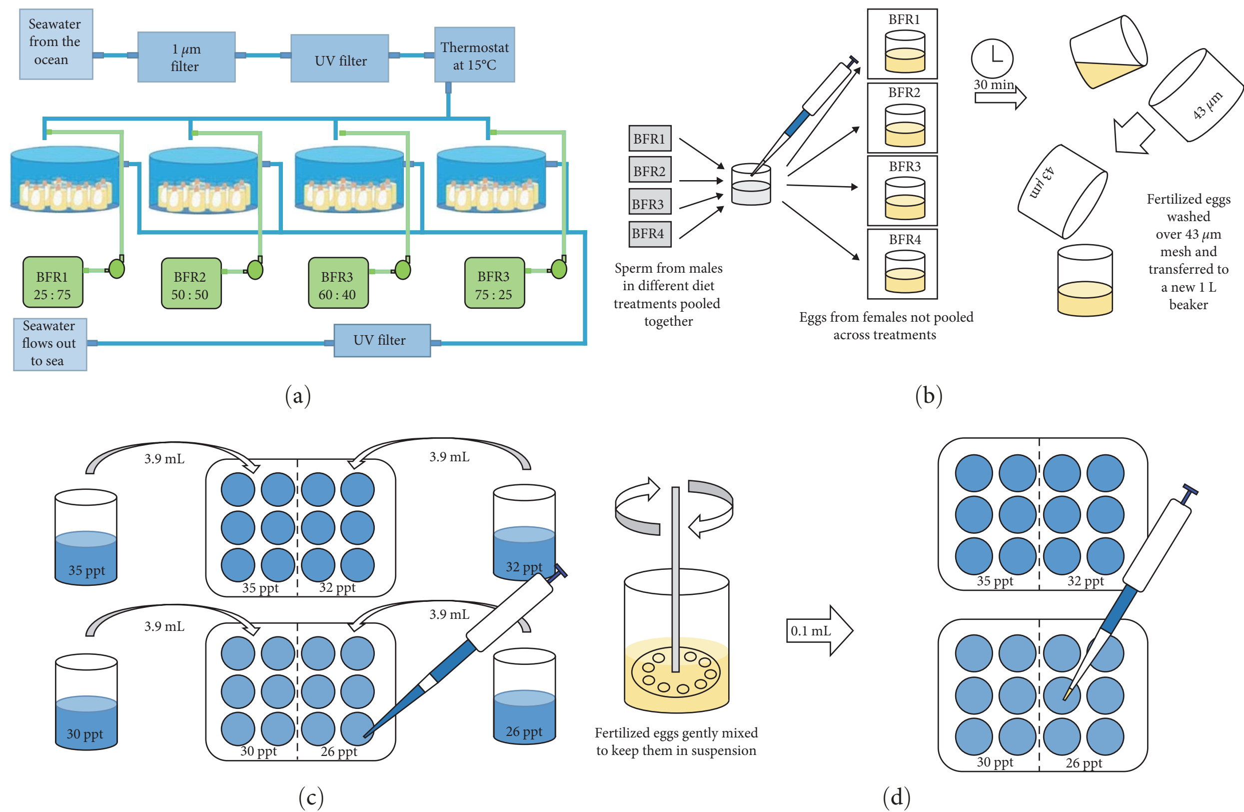

Figure 1: Experimental design and methods: (a) flow-through system (blue) connected to the geoduck broodstock housing tanks showing the pathway of seawater. Each housing tank was connected to its own algal tank with a pneumatic pump (green lines) that pumped premixed concentrations into the housing tanks, (b) spawning and fertilization procedure, (c) making of the salinities and setting up the TCD for the fertilized eggs, and (d) transfer of embryos into TCDs (*Note*. This was repeated for all broodstock feeding ratio treatments).

Table 1: Salinity treatment preparation.

Seawater (mL) 35 ppt		RO freshwater (mL) 0 ppt	Final volume (mL) and salinity
1,000	+	0	1,000 (35 ppt)
906.25	+	93.75	1,000 (32 ppt)
833.33	+	166.67	1,000 (30 ppt)
653.85	+	346.15	1,000 (26 ppt)

wells, resulting in a total of 96 wells used for evaluation (Figure 1(c)).

Each TCD well had 3.9 mL of the salinity solution to which 0.1 mL of the concentrated fertilized eggs from each broodstock group was added, giving a final volume of 4 mL with 200 embryos in each well (Figure 1(d)). Embryos were then incubated at 17°C in a humidity-controlled environment for 48 hr.

2.4. Sampling Procedure. After 48 hr, 0.5 mL 4% formalin was added to each TCD well to fix the embryos and larvae, which then progressively sank to the bottom of each TCD well. After 10 min, the embryos and/or larvae were observed under an Olympus Omax compound microscope at 10x magnification. All the samples were observed/processed within 24 hr and preserved in 70% ethanol for analysis under a scanning electron microscope. There were four different parameters evaluated after 48 hr including: 1—survival; 2—total proportion of D-larvae present (abnormal and normal); 3—proportion of abnormally developing D-larvae; and 4—size of normally developing D-veligers.

Survival, which included embryos, trochophores, and D-veligers, was measured by counting the individuals that were visible, regardless of developmental stage and abnormalities, in each TCD well for each feeding ratio and salinity treatment. Different early development stages were identified by descriptions provided by Le et al. [19] and by Sharma et al. [15]. Survival percentages were calculated using the following formula:

$$\text{Survival } (\%) = \frac{\text{Total number of embryos and larvae visible}}{200 \text{ (total number of fertilized eggs initially added)}} \times 100. \quad (1)$$

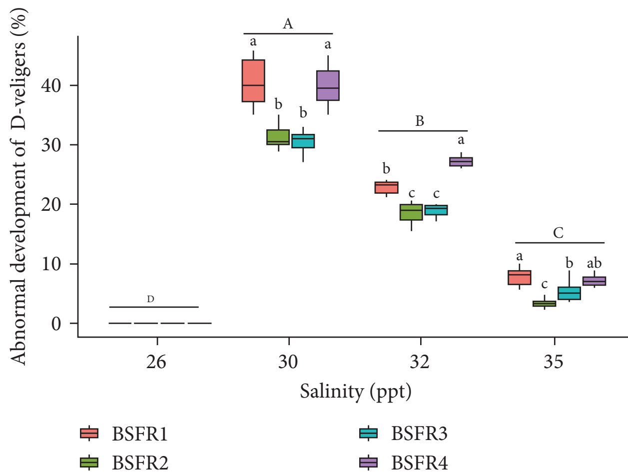

FIGURE 2: Percentage boxplots of D-veliger larvae showing abnormalities, reared at different salinities for 48 hr after fertilization for each broodstock feeding ratio treatment. *Note*. Boxplots with different letters denote significant differences (uppercase—between salinity treatments; lowercase—within each salinity treatment), $n = 200$. Broodstock feeding treatments, including *Tisochrysis lutea* and *C. muelleri* at cell ratios of 25 : 75 (BSFR1), 50 : 50 (BSFR2), 60 : 40 (BSFR3), and 75 : 25 (BSFR4).

The D-veliger stage was chosen as an endpoint as it is a transition from a nonfeeding phase to a feeding larval state. The proportion of normally developed D-veliger larvae was estimated as follows:

$$\text{D} - \text{veliger}\ (\%) = \frac{\text{Number of normally developed D larvae}}{200\ (\text{total number of fertilized eggs initially added})}. \tag{2}$$

Incidence of abnormalities in D-veligers and the fine-scale nature of these abnormalities were determined under a scanning electron microscope. Abnormalities were recorded as any deformity in the shell structure of the D-veligers (Figure 2). The percent incidence of abnormality at this stage was then calculated using the following formula:

$$\text{Abnormality}\ (\%) = \frac{\text{Number of abnormally developed D} - \text{veligers}}{\text{Total number of D} - \text{veligers}} \times 100. \tag{3}$$

Size of D-veligers was recorded during observations under the scanning electron microscopy. This was done by taking the average measurement for 50 normally developing D-veligers for each larval salinity treatment combination. The D-veligers were measured along the longest axis of the shell.

2.5. Scanning Electron Microscopy (SEM). Preserved D-veligers were prepared following the method described by Le et al. [20], washed with phosphate buffer (138 mM NaCl, 2.7 nM KCL, 10 nM Na_2HPO_4, 1.8 mM KH_2PO_4; pH = 7.4) for 5 min, then rinsed with 1 min with deionized water. D-veligers were then dehydrated through an ascending series of analytical grade ethanol 50%, 60%, 70%, 80%, 90%, and 100% for 15 min each. After dehydration, samples were soaked in 98% chloroform for 30 s, and then dried for 12 hr in a desiccator. Dried samples were placed on adhesive carbon disks and mounted on aluminum stubs. Samples were then sputter coated with carbon for 40 s using an ion sputter coater (Hitachi E-1045) and then imaged via SEM at 5.0 kV.

Abnormalities in D-veligers were characterized based on observations by His et al. [21], Saidov and Kosevich [22], and Lasota et al. [23]. It is important to note that not all abnormally developing D-veligers had a singular abnormality and thus, during characterization, larvae were grouped based on the most prominent abnormality type.

2.6. Statistical Analysis. Egg sizes were analyzed using one-way ANOVA, comparing the effects of Broodstock feeding ratio as a factor (four levels: BSFR1, BSFR2, BSFR3, and BSFR4), and egg diameter (μm) as the dependent variable.

Survival of larvae was analyzed using two-way ANOVA with Broodstock feeding ratio (four levels: BSFR1, BSFR2, BSFR3, and BSFR4) and rearing salinities (four levels: 26, 30, 32, and 35 ppt) as factors. Proportion of population attaining D-stage, incidence of abnormalities, and size of D-veligers were analyzed using two-way ANOVAs, with Broodstock feeding ratio (four levels: BSFR1, BSFR2, BSFR3, and BSFR4) and rearing salinities (three levels: 30, 32, and 35 ppt) as factors. All data were checked for normality and homoscedasticity using the Shapiro–Wilk and Levene's tests, respectively [24].

For all analyses, Tukey pairwise comparisons were used to examine the significant differences among the factor levels. All statistical analyses were conducted using the R Studio software R-4.1.0; and the significance was taken at $p < 0.05$.

3. Results

3.1. Egg Size. There were no significant differences (ANOVA, $p = 0.89$) observed within the egg sizes from females in the different broodstock feeding ratio treatments (Figure 3).

3.2. Survival. Survival 48 hr after fertilization was high in all broodstock conditioning treatments when embryos were raised at 32 and 35 ppt salinities (56.0%–75.7%; Figure 4), but overall survival at 30 ppt was below 30% regardless of broodstock feeding ratio. There were no survivors at salinity 26 ppt.

Significant differences in survival were based on both broodstock feeding ratios (two-way ANOVA, $F_{(3,80)} = 22.80$, $p < 0.001$) and rearing salinities (two-way ANOVA, $F_{(3,80)} =$ 1,050, $p < 0.001$). There was also a significant (two-way ANOVA, $F_{(9,80)} = 6.26$, $p < 0.001$) interaction between broodstock feeding ratio and rearing salinity.

Overall, mean survival in different salinities showed no significant differences between 32 and 35 ppt salinity exposures. The survival from different broodstock feeding treatments

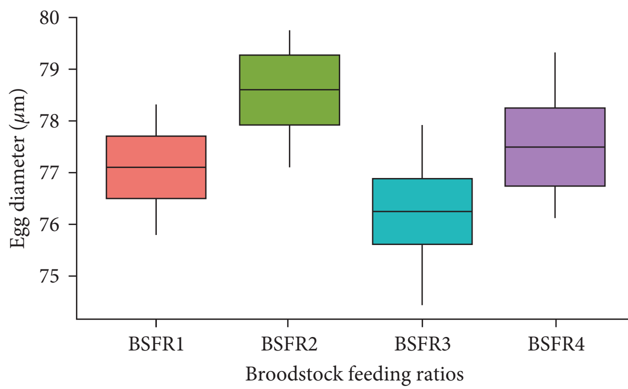

FIGURE 3: Egg diameter boxplots obtained from spawned females in feeding treatments, including *Tisochrysis lutea* and *C. muelleri* at cell ratios of 25 : 75 (BSFR1), 50 : 50 (BSFR2), 60 : 40 (BSFR3), and 75 : 25 (BSFR4) treatment; ($n = 50$ eggs for each group).

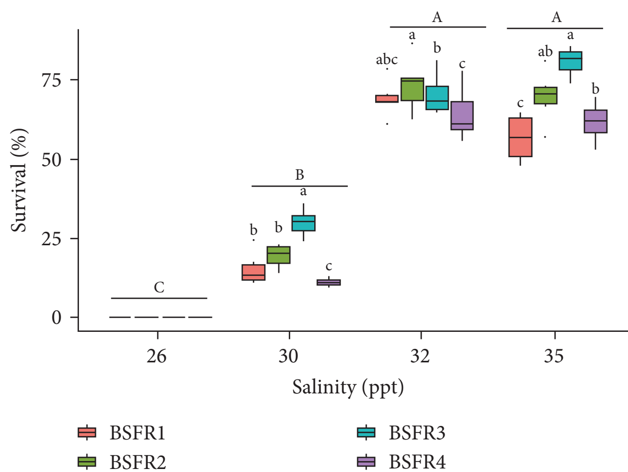

FIGURE 4: Survival boxplots at different salinities 48 hr postfertilization for each broodstock feeding ratio treatment. *Note*. Letters above boxplots (uppercase—salinity; lowercase—individual diet treatments) denote significant differences. Broodstock feeding treatments, including *Tisochrysis lutea* and *C. muelleri* at cell ratios of 25 : 75 (BSFR1), 50 : 50 (BSFR2), 60 : 40 (BSFR3), and 75 : 25 (BSFR4).

did, however, differ significantly within salinity exposure, with BSFR2 and BSFR3 treatments tending to support better survival (Figure 3) in salinities 30 ppt and over.

3.3. Development to D-veligers. After 48 hr postfertilization, more than 65% of the surviving embryos in salinities 35 and 32 ppt had transitioned into D-veligers (Figure 5). The transition of D-veligers in the 30 ppt salinity was below 30%.

The percentage of individuals transitioning to D-veligers was significantly different among broodstock feeding treatments (two-way ANOVA, $F_{(2,80)} = 111.11$, $p < 0.001$). There was also a significant difference in the transition to D-veligers among embryos reared in different salinities (two-way ANOVA, $F_{(2,80)} = 4{,}216.30$, $p < 0.001$), and a significant interaction between both broodstock feeding and embryo rearing salinities (two-way ANOVA, $F_{(6,80)} = 26.95$, $p < 0.001$).

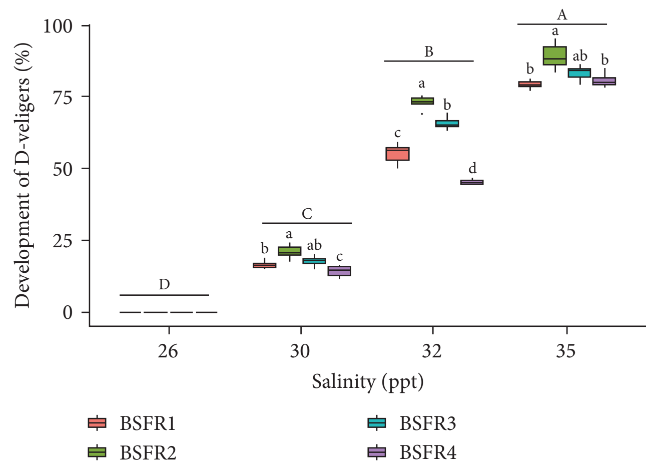

FIGURE 5: Proportion boxplots of normally developed D-veligers reared at different salinities for 48 hr after fertilization for each broodstock feeding ratio treatment. *Note*. Boxplots with different letters denote significant differences (uppercase—between salinity treatments; lowercase–within each salinity treatment), $n = 200$ individuals for each treatment. Broodstock feeding treatments, including *Tisochrysis lutea* and *C. muelleri* at cell ratios of 25 : 75 (BSFR1), 50 : 50 (BSFR2), 60 : 40 (BSFR3), and 75 : 25 (BSFR4).

Overall, embryos obtained from BSFR2 treatment had a higher percentage of transition into D-veligers when compared to larvae obtained from the other treatments (Figure 5).

3.4. Incidence of Abnormal D-Veliger Development. The overall morphological abnormalities ranged from minor deformations on the D-veliger to total distortion of the shell. The different kinds of abnormalities that were observed are shown in Figure 6. Minor abnormalities included deformities on the D-veliger shell, such as indentations and minor ripples on the surface of the shell (Figure 6(b)), in and around shell edge/margins (Figure 2(c)), and hinges causing concavity of the shell hinge (Figure 6(d)). Major deformities included apparent helical whirling of the D-veliger shell (Figure 6(e)) and eversion of the soft tissues.

Incidence of abnormally developing D-veligers was significantly affected by both broodstock diet (two-way ANOVA, $F_{(2,80)} = 46.85$, $p < 0.001$) and embryo rearing salinity (two-way ANOVA, $F_{(2,80)} = 1{,}041.37$, $p < 0.001$). There was also a significant interaction between broodstock feeding and rearing salinity of the embryos (two-way ANOVA, $F_{(6,80)} = 5.83$, $p < 0.001$).

The incidence of D-veliger abnormalities increased with decreasing salinity exposure. The percentage of abnormally developing D-veligers was between 4% and 11% at 35 ppt, 15% and 47% at 32 ppt, and 37% and 55% at 30 ppt (Figure 2). There were no larvae observed at the lowest salinity of 26 ppt.

Overall, regardless of the rearing salinity, the incidence of abnormalities within the D-veligers was lower in the BSFR2 (50 : 50) and BSFR3 (60 : 40) treatments (Figure 2).

3.5. Size of D-Veligers. The size (shell length) analysis of D-veligers was restricted to normally developing larvae at 35, 32, and 30 ppt salinities (Figure 7).

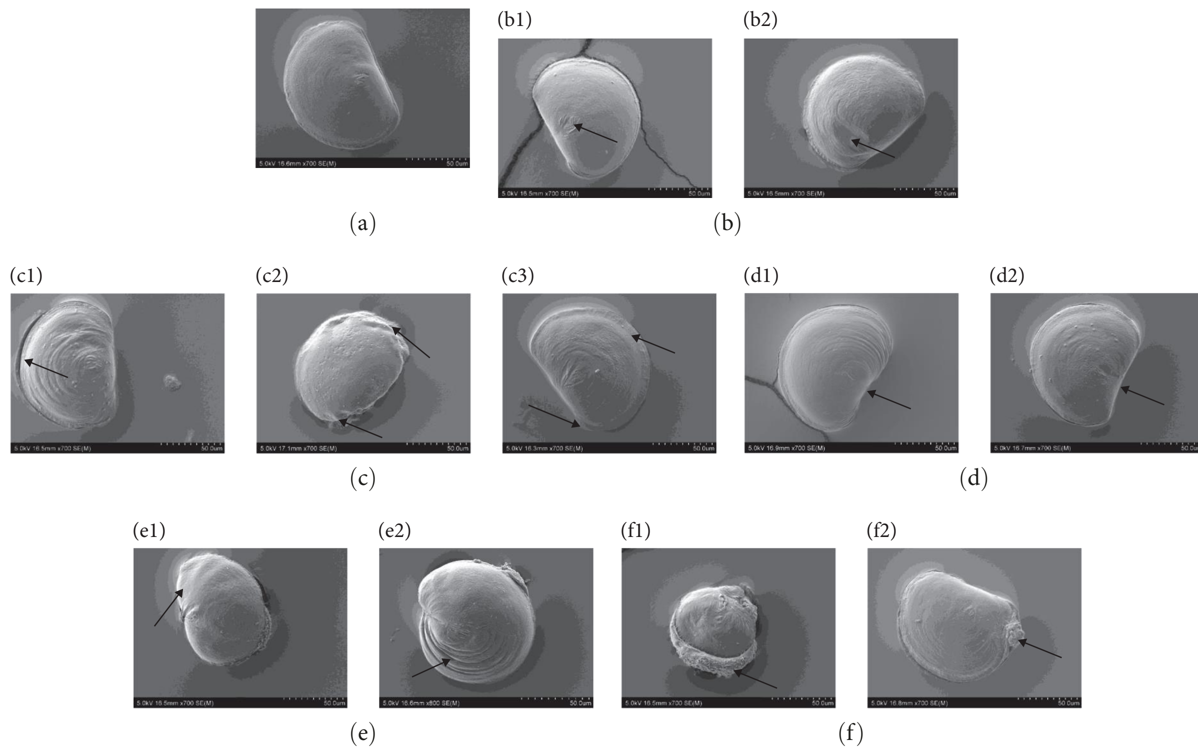

Figure 6: Abnormalities identified in D-veligers developed from embryos reared at 35 and 32 ppt salinity: (a) normally developing D-veliger for reference. Abnormalities included (b) (i, ii) indentation of the shell—exemplified in two individuals, (c) (i–iii) developmental issues around the shell margins—exemplified in three individuals, (d) (i, ii) concaving of the D-veliger shell hinge—exemplified in two individuals, (e) (i, ii) helical deformation of the shell—exemplified in two individuals, and (f) (i, ii) eversion of soft tissues—exemplified in two individuals. Black arrows indicate the locations of shell distortions.

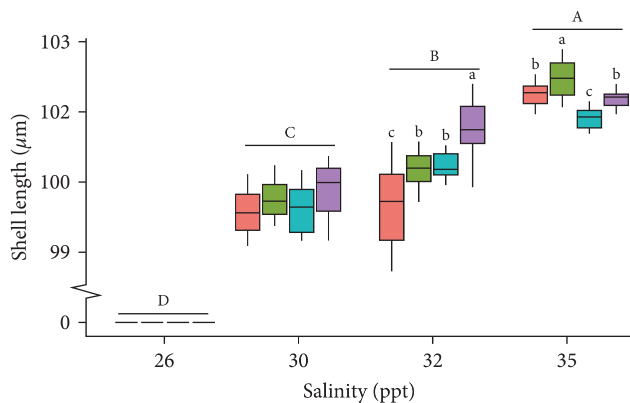

Figure 7: Size boxplots of normally developing D-veligers, reared at different salinities for 48 hr after fertilization for each broodstock feeding ratio treatment. *Note*. Boxplots with different letters denote significant differences between salinity treatments (uppercase—between salinity treatments; lowercase—within each salinity treatment). Broodstock feeding treatments, including *Tisochrysis lutea* and *C. muelleri* at cell ratios of 25 : 75 (BSFR1), 50 : 50 (BSFR2), 60 : 40 (BSFR3), and 75 : 25 (BSFR4).

There were significant effects of both broodstock feeding ratio (two-way ANOVA, $F_{(2,704)} = 46.85$, $p < 0.001$) and embryo rearing salinity (two-way ANOVA, $F_{(2,704)} = 1{,}041.37$, $p < 0.001$) on the size of the newly formed D-veligers. The interaction between salinity and feeding ratio was also significant (two-way ANOVA, $F_{(6,6704)} = 5.83$, $p < 0.001$).

The size of D-veligers decreased with decreasing salinities. The average sizes of the D-veligers were $101.22 \pm 0.49\,\mu$m at 35 ppt, $99.90 \pm 0.57\,\mu$m at 32 ppt, and 99.57 ± 0.54 at 30 ppt (Figure 7). Even though there were differences seen within the larvae from different broodstock feeding ratios, the overall effect was not as pronounced as seen with the rearing salinity.

4. Discussion

In the present study, broodstock from all microalgal feeding treatments completed gonad maturation and spawned eggs and sperm. Differences in the microalgal ratios during conditioning (i.e., gametogenesis) had important carry-over implications on embryo survival and transition into normally developed D-veligers when being reared at different salinities. The lower limit for the survival of *P. zelandica* embryos appears to be between 26 and 30 ppt salinity; as no fertilized eggs survived at 26 ppt, and only <30% survivors were recorded after 48 hr. However, the embryos obtained from broodstock that were conditioned with similar proportions of *Tisochrysis lutea* and *C. muelleri* (ISO:CM 50 : 50 or 60 : 40) had an overall higher survival, greater number of larvae transitioning into D-veligers, and reduced incidence of abnormalities within the D-veligers at lower salinities, potentially indicating a wider salinity tolerance.

The influence of diet on the reproductive development of the females was not immediately obvious, with females in all

feeding ratio treatments successfully spawning and producing viable embryos. This might be in part due to the extended conditioning period of 90 days provided as previous broodstock conditioning data on *P. zelandica* suggest that 73 days is sufficient in gonad maturation [10]. The extended conditioning period would have given the broodstock the opportunity to accumulate the necessary lipids and proteins for gonad maturation. Indeed, the ability of broodstock to acquire the necessary resources due to extended conditioning period was also seen in *P. generosa* [25]. Marshall et al. [25] suggested that a minimum amount of time for broodstock conditioning is crucial to acquire enough resources for gonad maturation, when *P. generosa* is fed with different levels of *Isochrysis* sp. and CM (at 1 : 1 ratio) for different amounts of time. Similarly, García-Esquivel et al. [26] found that gonad development in *P. globosa* could be achieved on a single-species diet of *Tisochrysis lutea* if sufficient time was provided. However, in both of the previous studies [25]; [26], the quality of the gametes produced was not studied in detail.

The egg diameters of *P. zelandica* from all feeding ratio treatments were well within the range reported by Le et al. [10] and Gribben and Hay [18]. The low variability in egg diameters within *P. zelandica* could be attributed to the species-specific nature of eggs in some bivalve species, e.g., mussels [27]. Even though the egg sizes were similar among treatments, it is possible that the biochemical composition (e.g., lipids, proteins) could have differed among treatments. For example, when mussels (*M. galloprovincialis*) broodstock were conditioned on different ratios of *Chaetoceros calcitrans* and *Pavlova lutheri*, spawned individuals produced similar sized eggs, but the composition within the eggs and the viability of the developing larvae greatly reflected the nutritional content of the algal diet [27].

Viability of *P. zelandica* embryos was clearly affected by the broodstock conditioning. In the present study, equal amounts of CM (rich in DHA) and ISO (rich in EPA) in the diet may have provided the best nutritional balance for the subsequent viability of embryos and larvae. Indeed, diet quality of broodstock and viability of offspring have been studied for the oysters *Ostrea chilensis* [28] and *O. edulis* [29], the scallops *Argopecten purpuratus* [30] and *A. nucleus* [31], and the clam *Ruditapes decussatus* [5, 32]. For example, when the diet of the scallop *Nodipecten nodous* is enriched with DHA and EPA during the conditioning period, the resultant veliger larvae have a greater survival [33]. This agrees with a study on mussels, *M. galloprovincialis*, which also found that diet composition of the broodstock had a significant effect on larval viability. This was attributed to the inability for the broodstock to successfully deposit glycogen (simple sugars) and sterols during the conditioning period [34]. In the present study, since the nutritional content of microalgae provided have very different amounts of EPA and DHA; feeding ratios either lacking in DHA or EPA tended to produce *P. zelandica* embryos and/or larvae with reduced viability under low salinity stress. Similarly, Huo et al. [16] found that lower salinities caused a reduction in the viability of *P. japonica* embryos. This was suggested to be the result of gradual swelling and final rupture of the embryos. However, in the present study, embryos obtained from females fed ratios with equal amounts of ISO:CM were more resilient to the changes in salinity. Based on these results, it is likely that providing broodstock with a balanced ISO:CM ratio may result in embryos with better osmotic regulation and improved resilience to osmotic stress.

A decrease in rearing salinity of the embryos greatly reduces the D-veliger yield. This negative effect of low salinity on larval yield has been well-documented in a number of marine bivalves, such as the oysters *Crassostrea belcheri* [35], *C. iredalei* [36], and *C. rhizophorae* [37]; the mussels *Perna viridis* [38], *Mytilus edulis*, and *M. trossulus* [39]; the scallop *Pinctada imbricata* [40]; and the clams *Katelysia rhytiphora* and *Anadara trapezia* [41], including the geoduck *P. japonica* [16]. However, broodstock conditioned on microalgal ratios with similar proportions of ISO:CM overall tended to have a higher proportion of individuals transitioning into D-veligers, regardless of the rearing salinities.

There was a clear effect of both broodstock feeding rations and embryonic salinity exposure on the incidence of abnormal development of the D-veligers, which increased with decreasing salinities. This is in agreement with other studies on oysters (*C. rhizophorae* [37] and *C. iredalei* [36]) and clams (*P. japonica* [16]). For *P. japonica*, the authors suggested that abnormalities, in the case of irregular cleaveages in the D-veligers, were attributed to lower salinities which resulted in osmotic changes during early embryonic development. In contrast, broodstock that were provided with similar feeding ratios of ISO:CM had on average less incidence of abnormal development within the D-veligers at lower salinities and also a reduced baseline abnormal development in ambient seawater (35 ppt).

The shell length of the D-veligers measured at 48 hr post-fertilization was affected more by the rearing salinity of the embryos than the feeding ratio of the broodstock. There was a decrease in overall size of the D-veligers with decreasing salinities. Indeed, Tan and Wong [35] also found that in *Crassostrea belchri*, a decrease in salinity negatively affected D-veliger size. The overall decrease in D-veliger size was suggested to be influenced by the salinity at which the broodstock was conditioned [40, 42, 43]. Broodstock used in this study which were conditioned at 35 ppt salinity and the largest-sized D-veligers were also recorded at salinity 35 ppt compared to other salinities. Further investigations are needed to determine whether the salinity tolerance of *P. zelandica* embryos and larvae is an after effect of broodstock conditioning salinity.

5. Conclusion

The results of the present study indicate that *P. zelandica* broodstock can be successfully conditioned with different microalgal ratios of ISO and CM, producing viable gametes. Embryos of *P. zelandica* were observed to be highly sensitive to changes in salinity. Rearing embryos in decreasing salinity from 35 ppt had a negative impact on embryonic survival, D-veliger yield, an increase in incidence of abnormalities in the D-veligers, and an overall reduction of the size of

D-veligers. The lower limit for survival of *P. zelandica* embryos is between 26 and 30 ppt. The *P. zelandica* embryos obtained from broodstock that were kept on similar proportions of ISO and CM (50 : 50 and 60 : 40) tended to have a higher baseline survival, D-veliger yield, and a decrease in abnormalities of the D-veligers. This suggested that having similar proportions ISO and CM during the conditioning period produces embryos that are more tolerant to the changes in salinity.

Data Availability

Data were collected by the authors who take responsibility for their integrity and accuracy of analysis. Data presented in this paper will be made available upon request.

Ethical Approval

This research was conducted ethically, and all experiments were performed in accordance with the relevant institutional and national guidelines for the care and use of laboratory animals.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] A. C. Alfaro, A. G. Jeffs, and N. King, "Enabling and driving aquaculture growth in New Zealand through innovation," *New Zealand Journal of Marine and Freshwater Research*, vol. 48, no. 3, pp. 311–313, 2014.

[2] P. E. Gribben and K. G. Heasman, "Developing fisheries and aquaculture industries for *Panopea zelandica* in New Zealand," *Journal of Shellfish Research*, vol. 34, no. 1, pp. 5–10, 2015.

[3] J. M. E. Stenton-Dozey, P. Heath, J. S. Ren, and L. N. Zamora, "New Zealand aquaculture industry: research, opportunities and constraints for integrative multitrophic farming," *New Zealand Journal of Marine and Freshwater Research*, vol. 55, no. 2, pp. 265–285, 2021.

[4] P. Lubet, "Ecophysiologie de la reproduction chez les mollusques lamellibranches," *Haliotis*, vol. 7, pp. 49–55, 1976.

[5] S. D. Utting and P. F. Millican, "Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability," *Aquaculture*, vol. 155, no. 1–4, pp. 45–54, 1997.

[6] S. Li, J. Xu, J. Chen, J. Chen, C. Zhou, and X. Yan, "The major lipid changes of some important diet microalgae during the entire growth phase," *Aquaculture*, vol. 428-429, pp. 104–110, 2014.

[7] R. Gouda, E. Kenchington, B. Hatcher, and B. Vercaemer, "Effects of locally-isolated micro-phytoplankton diets on growth and survival of sea scallop (*Placopecten magellanicus*) larvae," *Aquaculture*, vol. 259, no. 1–4, pp. 169–180, 2006.

[8] S. Hemaiswarya, R. Raja, R. Ravi Kumar, V. Ganesan, and C. Anbazhagan, "Microalgae: a sustainable feed source for aquaculture," *World Journal of Microbiology and Biotechnology*, vol. 27, pp. 1737–1746, 2011.

[9] R. Marshall, R. S. McKinley, and C. M. Pearce, "Effect of temperature on gonad development of the Pacific geoduck clam (*Panopea generosa* Gould, 1850)," *Aquaculture*, vol. 338–341, pp. 264–273, 2012.

[10] D. Le, A. C. Alfaro, and N. King, "Broodstock conditioning of New Zealand geoduck (*Panopea zelandica*) within different temperature and feeding ration regimes," *New Zealand Journal of Marine and Freshwater Research*, vol. 48, no. 3, pp. 356–370, 2014.

[11] C. G. Waters, S. Lindsay, and M. J. Costello, "Factors relevant to pre-veliger nutrition of tridacnidae giant clams," *Reviews in Aquaculture*, vol. 8, no. 1, pp. 3–17, 2016.

[12] D. Leverett and J. Thain, "Oyster embryo-larval bioassay (revised)," in *ICES Techniques in Marine Environmental Science (TIMES)*, ICES, 2013.

[13] B. L. Bayne, "The biology of mussel larvae. Marine mussels: their ecology and physiology," pp. 81–120, 1976.

[14] O. R. Chaparro, V. M. Cubillos, Y. A. Montiel, K. A. Paschke, and J. A. Pechenik, "Embryonic encapsulation and maternal incubation: requirements for survival of the early stages of the estuarine gastropod *Crepipatella dilatata*," *Journal of Experimental Marine Biology and Ecology*, vol. 365, no. 1, pp. 38–45, 2008.

[15] S. S. Sharma, A. C. Alfaro, N. L. C. Ragg, and L. N. Zamora, "Effects of temperature on early development of the New Zealand geoduck *Panopea zelandica* (Quoy & Gaimard, 1835)," *Aquaculture Research*, vol. 51, no. 2, pp. 751–760, 2020.

[16] Z. Huo, H. Guan, M. G. Rbbani et al., "Effects of environmental factors on growth, survival, and metamorphosis of geoduck clam (*Panopea japonica* A. Adams, 1850) larvae," *Aquaculture Reports*, vol. 8, pp. 31–38, 2017.

[17] S. T. Tettelbach and E. W. Rhodes, "Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop *Argopecten irradians irradians*," *Marine Biology*, vol. 63, pp. 249–256, 1981.

[18] P. E. Gribben and B. E. Hay, "Larval development of the New Zealand geoduck *Panopea zelandica* (Bivalvia: Hiatellidae)," *New Zealand Journal of Marine and Freshwater Research*, vol. 37, no. 2, pp. 231–239, 2003.

[19] D. V. Le, T. Young, A. C. Alfaro et al., "Practical fertilization procedure and embryonic development of the New Zealand geoduck clam (*Panopea zelandica*)," *Journal of the Marine Biological Association of the United Kingdom*, vol. 98, no. 3, pp. 475–484, 2016.

[20] D. V. Le, T. Young, A. C. Alfaro, E. Watts, and N. King, "Effect of neuroactive compounds on larval metamorphosis of New Zealand geoduck (*Panopea zelandica*)," *Aquaculture Research*, vol. 48, no. 6, pp. 3080–3090, 2017.

[21] E. His, M. N. L. Seaman, and R. Beiras, "A simplification the bivalve embryogenesis and larval development bioassay method for water quality," *Water Research*, vol. 31, no. 2, pp. 351–355, 1997.

[22] D. M. Saidov and I. A. Kosevich, "Rehabilitation of *Mytilus edulis* larvae abnormalities induced by $K_2Cr_2O_7$ in short-term experiments," *Ecotoxicology*, vol. 30, pp. 1242–1250, 2021.

[23] R. Lasota, K. Gierszewska, F. Viard, M. Wolowicz, K. Dobrzyn, and T. Comtet, "Abnormalities in bivalve larvae from the Puck Bay (Gulf of Gdansk, southern Baltic Sea) as an indicator of environmental pollution," *Marine Pollution Bulletin*, vol. 126, pp. 363–371, 2018.

[24] J. P. Queen, G. P. Quinn, and M. J. Keough, *Experimental Design and Data Analysis for Biologists*, Cambridge University Press, 2002.

[25] R. Marshall, R. S. McKinley, and C. M. Pearce, "Effect of ration on gonad development of the Pacific geoduck clam, *Panopea generosa* (Gould, 1850)," *Aquaculture Nutrition*, vol. 20, no. 4, pp. 349–363, 2014.

[26] Z. García-Esquivel, E. Valenzuela-Espinoza, M. I. Buitimea, R. Searcy-Bernal, C. Anguiano-Beltrán, and F. Ley-Lou, "Effect of lipid emulsion and kelp meal supplementation on the maturation and productive performance of the geoduck clam, *Panopea globosa*," *Aquaculture*, vol. 396, pp. 25–31, 2013.

[27] J.-A. Fearman, C. J. S. Bolch, and N. A. Moltschaniwskyj, "Energy storage and reproduction in mussels, *Mytilus galloprovincialis*: the influence of diet quality," *Journal of Shellfish Research*, vol. 28, no. 2, pp. 305–312, 2009.

[28] J. A. Wilson, O. R. Chaparro, and R. J. Thompson, "The importance of broodstock nutrition on the viability of larvae and spat in the Chilean oyster *Ostrea chilensis*," *Aquaculture*, vol. 139, no. 1-2, pp. 63–75, 1996.

[29] R. Gonzalez Araya, C. Mingant, B. Petton, and R. Robert, "Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development," *Aquaculture*, vol. 364-365, pp. 272–280, 2012.

[30] I. Martínez-Pita, C. Sánchez-Lazo, and F. J. García, "Influence of microalga lipid composition on the sexual maturation of *Mytilus galloprovincialis*: a hatchery study," *Aquaculture Nutrition*, vol. 22, no. 1, pp. 202–216, 2016.

[31] L. Velasco and J. Barros, "Potential for hatchery broodstock conditioning of the Caribbean scallops *Argopecten nucleus* and *Nodipecten nodosus*," *Aquaculture*, vol. 272, no. 1–4, pp. 767–773, 2007.

[32] A. S. A. Abbas, E. El-Wazzan, A. R. Khafage, A.-F. M. El-Sayed, and F. A. Abdel Razek, "Influence of different microalgal diets on gonadal development of the carpet shell clam *Ruditapes* decussatus broodstock," *Aquaculture International*, vol. 26, pp. 1297–1309, 2018.

[33] S. Sühnel, F. Lagreze, G. Zanette, A. R. M. Magalhães, and J. F. Ferreira, "Effect of the fatty acid EPA and DHA in the conditioning of the scallop *Nodipecten nodosus* (Linné 1758)," *Aquaculture*, vol. 330–333, pp. 167–171, 2012.

[34] A. K. Pettersen, G. M. Turchini, S. Jahangard, B. A. Ingram, and C. D. H. Sherman, "Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus galloprovincialis*) larvae," *Aquaculture*, vol. 309, no. 1–4, pp. 115–124, 2010.

[35] S.-H. Tan and T.-M. Wong, "Effect of salinity on hatching, larval growth, survival and settling in the tropical oyster *Crassostrea belcheri* (Sowerby)," *Aquaculture*, vol. 145, no. 1–4, pp. 129–139, 1996.

[36] A. N. P. Fang, T. C. Peng, P. K. Yen, Z. Yasin, and A. T. S. Hwai, "Effect of salinity on embryo and larval development of oyster *Crassostrea iredalei*," *Tropical Life Sciences Research*, vol. 27, no. supp 1, pp. 23–29, 2016.

[37] A. E. Dos Santos and I. A. Nascimento, "Influence of gamete density, salinity and temperature on the normal embryonic development of the mangrove oyster *Crassostrea rhizophorae* Guilding, 1828," *Aquaculture*, vol. 47, no. 4, pp. 335–352, 1985.

[38] S. Tan, "Effect of salinity on hatching, larval growth, and survival in the green mussel *Perna viridis* (Linnaeus)," *Cell*, vol. 1050, no. 1, pp. 2–5, 1996.

[39] J.-W. Qiu, R. Tremblay, and E. Bourget, "Ontogenetic changes in hyposaline tolerance in the mussels *Mytilus edulis* and *M. trossulus*: implications for distribution," *Marine Ecology Progress Series*, vol. 228, pp. 143–152, 2002.

[40] W. A. O'Connor and N. F. Lawler, "Salinity and temperature tolerance of embryos and juveniles of the pearl oyster, *Pinctada imbricata* Röding," *Aquaculture*, vol. 229, no. 1–4, pp. 493–506, 2004.

[41] J. A. Nell, W. A. O'Connor, M. P. Heasman, and L. J. Goard, "Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshayes)," *Aquaculture*, vol. 119, no. 2-3, pp. 149–156, 1994.

[42] S. J. Nowland, W. A. O'Connor, S. S. Penny, M. W. J. Osborne, and P. C. Southgate, "Water temperature and salinity synergistically affect embryonic and larval development of the tropical black-lip rock oyster *Saccostrea echinata*," *Aquaculture International*, vol. 27, pp. 1239–1250, 2019.

[43] L. M. Yaroslavtseva and E. P. Sergeeva, "Adaptability of the bivalve mollusk *Crenomytilus grayanus* larvae to short- and long-term changes of salinity," *Bulletin of the Russian Far Eastern Malacological Society*, vol. 14, pp. 30–40, 2010.