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Optimal conditions for pressure shock induction of triploidy in turbot and a comparison of induction efficiency with cold shock

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ABSTRACT

The mass production of triploid turbot *Scophthalmus maximus* is expected to enhance the economic benefits of aquaculture due to its sterility, growth, and survival advantages. Among the methods for inducing triploidy in teleost, pressure shock has been considered a more consistent and reliable approach. In this study, the optimal parameters for inducing triploidy in turbot through hydrostatic pressure shock were investigated in a series of trials, including the intensity (55–75 MPa), timing (3.5–8.5 min after fertilization, maf) and duration (4–12 min). The ploidy level was determined by flow cytometry analysis. Under a water temperature of 14.5 ± 0.5 °C, treatment optima for pressure shock were determined to be 4.5–5.5 maf with 60 MPa for 6 min, resulting in 100% triploidy rate. A comparison of induction efficiency between pressure shock using this combination and cold shock initiated at 6.5 maf in −2 °C sea water for 25 min was carried out using eggs from three females. The higher hatching rates and triploidy rates, and lower abnormality rates were investigated with pressure shock treatment compared with cold shock treatment. The optimized parameters were successfully applied to three large batches of eggs (~ 250, 300 and 330 mL) for mass production of triploid turbot. The total length and body weight of triploids were significantly lower than those of diploid counterparts at 2 months after hatching (mah), however, they exhibited a significant increase at 8 and 11 mah, respectively, and maintained this higher level thereafter. Additionally, the survival rates remained similar from 2 to 12 mah. The results of this preliminary study indicate that pressure shock rather than cold shock is more beneficial for the commercial production of triploid turbot under farming conditions.

1. Introduction

The turbot, *Scophthalmus maximus*, is a main species of marine fish cultured in Europe, China and Chile due to its high growth rate, low food coefficient, high resistance to disease, high stocking-density capacities and high consumer acceptability (Ruyet et al., 1991; Ruyet, 2002; Lei et al., 2012). The growth rate of triploid turbot has been demonstrated to exhibit significantly higher growth rates with an 8% increase in survival rate compared to diploid turbot at a given age, particularly following the attainment of sexual maturity (Cal et al., 2006). Large-scale production of triploid turbot would therefore improve the economic benefits for industrial farming. In recent years, turbot has been considered as one of the best candidate species for offshore marine farming in China. Consequently, establishing triploid population for offshore farming is imperative not only to prevent genetic pollution but also to mitigate potential invasions by alien species resulting from accidental escape of farmed fish in this aquaculture model.

The induction of triploidy in teleost is typically achieved through mechanical treatment or interploidy crossing between tetraploid and diploid individuals. Artificial induction of tetraploid in turbot had been carried out in several studies, aiming to produce tetraploid broodstock that can yield triploid progenies via interploidy crossing (Wu et al., 2014; Zhu et al., 2017; Wu et al., 2019; Meng et al., 2021). However, induced autotetraploid seems to be intolerated by turbot as tetraploid

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juveniles can not survive past the first year of life (Wu et al., 2019; Meng et al., 2021). Alternatively, triploidy can be directly induced through temperature (cold/heat) or pressure shocks shortly after fertilization to prevent the extrusion of the second polar body (Arai, 2001; Hulata, 2001). The induction of triploidy in turbot by cold shock has been established and applied in large-scale production practice (Piferrer et al., 2000; Piferrer et al., 2003; Terrones et al., 2003; Cal et al., 2010; Hernández-Urcera et al., 2017). However, this method has generally been characterized by the lower hatching rate and unstable triploid incidence in practice (Piferrer et al., 2003; Aydın and Okumuş, 2017; Domingues et al., 2019; Aydın et al., 2021; Aydın et al., 2022). The reason for this might be due to the difficulty in controlling the cold baths, an uneven distribution of the eggs and minor variations in temperature, which affected the triploidy induction efficiency.

In this regard, pressure shock is known to be a more consistent and reliable technique than temperature shock as it consists of an abrupt, transient increase in pressure (Piferrer et al., 2009). Pressure is applied equally to all the eggs under treatment and at the same temperature as fertilization. A shorter exposure time minimizes the risk of physical damage to the embryos, especially during triploid induction by cold shock in turbot, where the temperature should be controlled below −1°C for more than 25 min (Piferrer et al., 2003). The method has been used commercially in several cultured species including rainbow trout (*Oncorhynchus mykiss*) (Chourrout, 1984), Nile tilapia (*Oreochromis niloticus*) (Hussain et al., 1991), European sea bass (*Dicentrarchus labrax*) (Peruzzi and Chatain, 2000), Arctic charr (*Salvelinus alpinus*) (Gillet et al., 2001), brown trout (*Salmo trutta* L.) (Preston et al., 2013), and mandarin fish (*Siniperca chuatsi*) (Bi et al., 2020). However, there have been no reports on triploid induction by pressure shock in turbot till now.

Therefore, this study aimed to investigate the application of hydrostatic pressure shock treatment for triploid induction in turbot. The objectives were as follows: (1) to optimize the three key parameters (intensity, timing and duration) of pressure shock treatment under a strictly controlled temperature; (2) to compare the relative induction efficiency between hydrostatic pressure shocks using the optimized treatment parameters and cold shocks using previously established parameters; and (3) to evaluate survival and growth rates in juvenile triploid turbot induced by pressure shock in comparison with their diploid counterparts.

2. Materials and methods

2.1. Broodstock management and gamete collection

The broodstock of turbot consisted of 30 females and 40 males (body weight 1.5–4.0 kg, 4 years old), which were obtained from domesticated juveniles and maintained at Tianyuan Aquaculture Co., Ltd (Yantai City, Shandong province, China). The broodstock was evenly distributed in two 36000 L concrete tanks in mixed groups for more than 2 months under the conditions of controlled photoperiod (16 h light:8 h dark) and water temperature (12–14 °C) to regulate gonad maturation. The biomass density in both tanks was approximately 3 kg m^{-2}. Mature females received an injection of Gonadotropin-releasing hormone (GnRH) and Luteinizing Hormone Releasing Hormone (LHRHa) at 500 U/kg and 10 μg/kg body weight, respectively. Ovulated eggs were obtained by exerting gentle abdominal pressure between 48 and 72 h (depending on gonad development) following hormone injection. The eggs from a single female were collected in a 1000 mL glass beaker and maintained in a water bath of 14.5 °C for less than two hours. Oocyte diameter, membrane clarity, oil globule shape and ovarian fluid pH were used to determine the quality of each batch of eggs before fertilization (Kjørsvik et al., 2003). Running males were recognized by gentle abdominal pressure. Milt from one male was collected into a plastic straw, motility checked, diluted 10 times with modified Ringer's-200 solution (4.33 g/L NaCl, 2.01 g/L KCl, 0.54 g/L $CaCl_2$, 0.23 g/L $MgCl_2{\cdot}6\ H_2O$, 0.28 g/L NaH_2PO_4, 0.20 g/L $NaHCO_3$ and 1.00 g/L Glucose) and stored at 4 °C until used.

2.2. Fertilization and egg incubation

The eggs from one female were divided into approximately equal groups of selected volumes, held in 1000 mL beaker, fertilized with diluted sperm at a ratio of 1:20 (V_{milt}/V_{eggs}), and activated by adding sea water with twice the gamete volume at 14.5°C. Take the moment of activation as time zero for embryonic development. Sea water was further added to a total volume of 1000 mL at 2 min after fertilization (maf). The fertilized eggs were left undisturbed until further treatment. Before pressure shock, floating eggs were collected, rinsed and transferred to plastic vials with perforated mesh for shocking. The eggs of the control group (unshocked samples) and the shocked groups were subsequently incubated in net cages (15 L or 100 L according to the number of fertilized eggs), respectively, which were suspended in 3000 L fiberglass reinforced plastic tanks with a thermos-regulated incubator system at 14.5 ± 0.5 °C.

2.3. Optimal parameters of pressure shock treatment

A commercial high capacity Hydrostatic Pressure Chamber (FH-200 M, Qingdao Starfish Instrument Co., Ltd, China) with a volume of 1500 mL pressure cell was used for triploid induction experiments. After the cylinder had been sealed with a screw cap and purged of air, the required pressure level inside was elevated in less than 15 s and maintained at that level. The pressure was decompressed instantaneous at the end of the treatment. The hydrostatic press was placed in the room with the controlled temperature of 14–16 °C, and the pressure chamber was pre-cooled with 14.5 °C seawater before treatment to avoid temperature fluctuations caused by the press.

Three experimental series were designed, and in each series one shock parameter (timing, intensity and duration, respectively) was tested, while the remaining two were kept constant in order to optimize the parameters under the strictly controlled pre-shock temperature (14.5 ± 0.5 °C). According to the results of the triploidy induction in turbot by cold shock (Piferrer et al., 2000), the experimental series were designed as follows: intensities were tested at 55, 60, 65, 70 and 75 MPa, respectively, while the timing and duration were fixed at 6.5 min after fertilization (maf) and 6 min; timings were tested at 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 maf while the intensity and duration were fixed at 60 MPa and 6 min; and durations were tested for 4, 6, 8, 10 and 12 min while the intensity and timing were fixed at 60 MPa and 5.5 maf. In each experiment, eggs from one female were divided into equal samples (~ 5 mL); one sample was assigned as the control group, and the other samples were shocked with hydrostatic pressure and thereafter moved to a small net (15 L) for hatching as described in Section 2.2. All experiment series were replicated up to three times using eggs from different females, for a total of nine females.

Data on the fertilization rate at 4 h after fertilization (haf), hatching rate at 125 haf, abnormality rate at 125 haf and the triploidy rate at 1–2 days after hatching in each experiment were collected and analyzed to determine the pressure treatment optima. The hatching rate was recorded as the percentage of the total swimming larvae out of the number of fertilized eggs. The abnormality rate was recorded as the percentage of abnormal larvae (thick, short or curved bodies) out of the total number of hatched larvae. The triploidy rate was recorded as the percentage of triploid larvae out of the total examined larvae. Triploid yield, the percentage of triploid larvae per total number of fertilized eggs, was calculated for each treatment as the product of hatching rate and triploidy (%) divided by 100.

2.4. Ploidy determination

Thirty randomly selected swimming larvae (1- to 2-day-old larvae at

14.5 ± 0.5℃) in each experimental series were sampled, individually placed into disposable tubes and stored deep frozen at − 80℃ until analysis. The ploidy level was determined using flow cytometry following the protocol described by Meng et al. (2021). In brief, a single larva was thawed in 0.5 mL of 4′,6-diamidino-2-phenylindole (DAPI) (Solabrio, China) solution and then desegregated, vortexed, filtered through a 30 μm Partec CellTrics filter before being tested within 30 min. Prior to this, the voltage value was adjusted using control group samples to ensure that the fluorescence peak of FL4 channel reached the point of fluorescence channels of 100, which was regarded as the diploid standard. Subsequently, with fixed receiving channel settings, the voltage value and the appropriate injection speed, the dyed samples from the treatment groups were tested individually. Triploidy was recognized as the relative DNA content 1.5 times higher than that of the diploid larvae (Nascimento et al., 2020).

2.5. Comparison between pressure and cold shock

Cold shocks for triploid induction were involved subjecting the eggs in an incubator with a mixture of seawater and ice at − 2.0℃. Water flow was maintained via a pump to ensure the consistent temperature throughout the treatment.

Three independent experiments were conducted to compare the triploid induction efficiency between pressure and cold shock using eggs from three different females. For each experiment, eggs from one female were divided into three equal groups (~10 mL). One group was fertilized and left untreated to create the diploid control group (Ctr). The second group was fertilized and shocked with pressure using the optimal parameters to create the pressure shock induced triploid group (PST). The third group was fertilized and cold shocked at 6.5 maf for 25 min as described by Piferrer et al. (2003) and Meng et al. (2013) to create the cold shock induced triploid group (CST). After treatment, the eggs of the three groups were immediately transferred to the 15 L net cages for hatching. Data on the fertilization rate, hatching rate, abnormality rate and triploidy rate were compared among the three groups.

2.6. Production of triploid juveniles

Approximately 430, 350 and 400 mL of eggs were obtained from three females, respectively. Milt from four males was collected and mixed in each large scale fertilization experiment. The eggs from each fish were divided into two groups for normal fertilization and triploid induction, respectively: about 100 mL of eggs from each female were fertilized and left untreated to produce the diploid control, and the remaining eggs (~ 330, 250 and 300 mL) were fertilized and pressure shocked to produce the triploid turbot. After treatment, the eggs in both groups were immediately transferred to separate net cages with a volume of 100 L for hatching. The larvae were reared according to established protocols outlined in the culture of turbot handbook (Ruyet et al., 1991). Diploid and triploid juveniles labeled with distinct fluorescent colors were mix cultured in the same indoor tanks from 90 days after hatching (dah) under water temperature of 19 ± 1 °C. The juveniles were fed on commercial dry feed (Xingyuan, Qingdao Tuny Star International Trading Co., Ltd) by an automatic bait casting machine.

The total lengths and body weights of diploids and triploids were measured on monthly (n = 30), while the survival rates were assessed at 2 and 12 months after hatching (mah) respectively. Ploidy level was determined at 6 mah.

2.7. Statistical analysis

The data are expressed as mean ± SD. Percentage data on the fertilization rates, hatching rates, abnormality rates, triploidy rates and triploidy yields in the experiments were analyzed with Tukey's honest significant difference test (ANOVA) (Section 2.3 and Section 2.5) and two independent-sample t-test (Section 2.6), respectively. Growth differences of total lengths and body weights between ploidies were evaluated using the Student's t-test. SPSS 17.0 software was used for data analysis with a significance level of 0.05.

3. Results

3.1. Optimal parameters of pressure shock treatment

No significant differences were detected by ANOVA analysis in fertilization rates among the nine control groups, despite each batch of eggs being obtained from different females (supplemental data). Furthermore, the pressure intensity, timing and duration of post-fertilization shock had no significant impact on the fertilization rates in the shocked groups of the parameter experiments, which ranged from 76.7% to 92.5%, respectively.

The effects of pressure intensity, timing and duration on hatching rates and triploidy rates are shown in Fig. 1. The ploidy status of larvae in the treatment groups was analyzed individually with the diploid control serving as the reference standard. As expected, the DNA content of triploid was 1.5 times higher than that of diploid (Fig. 2). Pressure shocks at all six intensities examined (55, 60, 65, 70 and 75 MPa) were capable of producing viable triploid larvae when initiated at 6.5 maf and lasted for 6 min (Fig. 1-A). However, the groups shocked at an intensity of 60 MPa exhibited significantly higher hatching rate compared to other groups ($F = 104.87$, $p < 0.05$). The triploidy rate was 89.4% ± 4.8% in the group shocked at 55 MPa, and was almost 100% in groups shocked above or equal to 60 MPa. The triploid yield was highest in the group shocked at 60 MPa (13.0% ± 2.0%, $F = 99.23$, $p < 0.05$).

The six examined timings (3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 maf) were fell within the period for inhibiting the extrusion of the second polar body, when the pressure intensity and duration were fixed at 60 MPa and 6 min (Fig. 1-B). However, the hatching rates of the groups initiated at 4.5 and 5.5 maf were significantly higher than that of the other four groups ($F = 14.07$, $p < 0.05$). Triploidy rates in groups initiated later than 4.5 maf approached almost 100%, while it was 70.6% ± 9.2% in the group initiated at 3.5 maf. The triploid yields were 18.5% ± 6.6% and 13.5% ± 2.1% in groups initiated at 4.5 maf and 5.5 maf, respectively, which was also significantly higher than that of the other groups ($F = 14.02$, $p < 0.05$). Therefore, the optimal timing for triploidy induction in turbot by pressure shock was 4.5–5.5 maf under controlled pre-shock water temperature of 14.5 ± 0.5℃.

The hatching rates in groups lasting for 4 and 6 min (28.1% ± 4.9% and 33.6% ± 5.1%, respectively) were significantly higher than that of the other three groups (8, 10 and 12 min) when the pressure intensity and timing were fixed at 60 MPa and 5.5 maf (Fig. 1-C, $F = 53.80$, $p < 0.05$). However, the triploidy rate in the group lasting for 4 min was 95.6% ± 5.9%, while that in groups receiving shocks lasting more than 6 min were almost 100%. The triploid yield of 6 min duration (33.6% ± 5.1%) was significantly higher than that of 4 min duration (27.4% ± 4.6%) ($F = 56.03$, $p < 0.05$). Therefore, the optimal shock duration was recommended to be 6 min.

3.2. Comparison between pressure and cold shock

The comparison of hatching, abnormality and triploidy rates among the control (Ctr), pressure shock (PST) and cold shock (CST) groups induced from three females is presented in Table 1. Compared with the Ctr, both the PST and the CST showed significantly decreased hatching rates ($F = 46.52$, $p < 0.05$). Although the difference was not significant, the average hatching rate in the PST (48.4% ± 6.7%) was 23.9% higher than that in the CST (39.0% ± 4.5%). In addition, the abnormality rate in the PST (10.0% ± 1.2%) was significantly lower than that in the CST (19.5% ± 2.9%) ($F = 27.90$, $p < 0.05$), and similar to that in the Ctr (6.7% ± 2.1%). Results of flow cytometric ploidy analysis of the swimming larvae (n = 30 per group) in PST and CST induced from three females indicated that the triploidy rates in PST were relatively stable

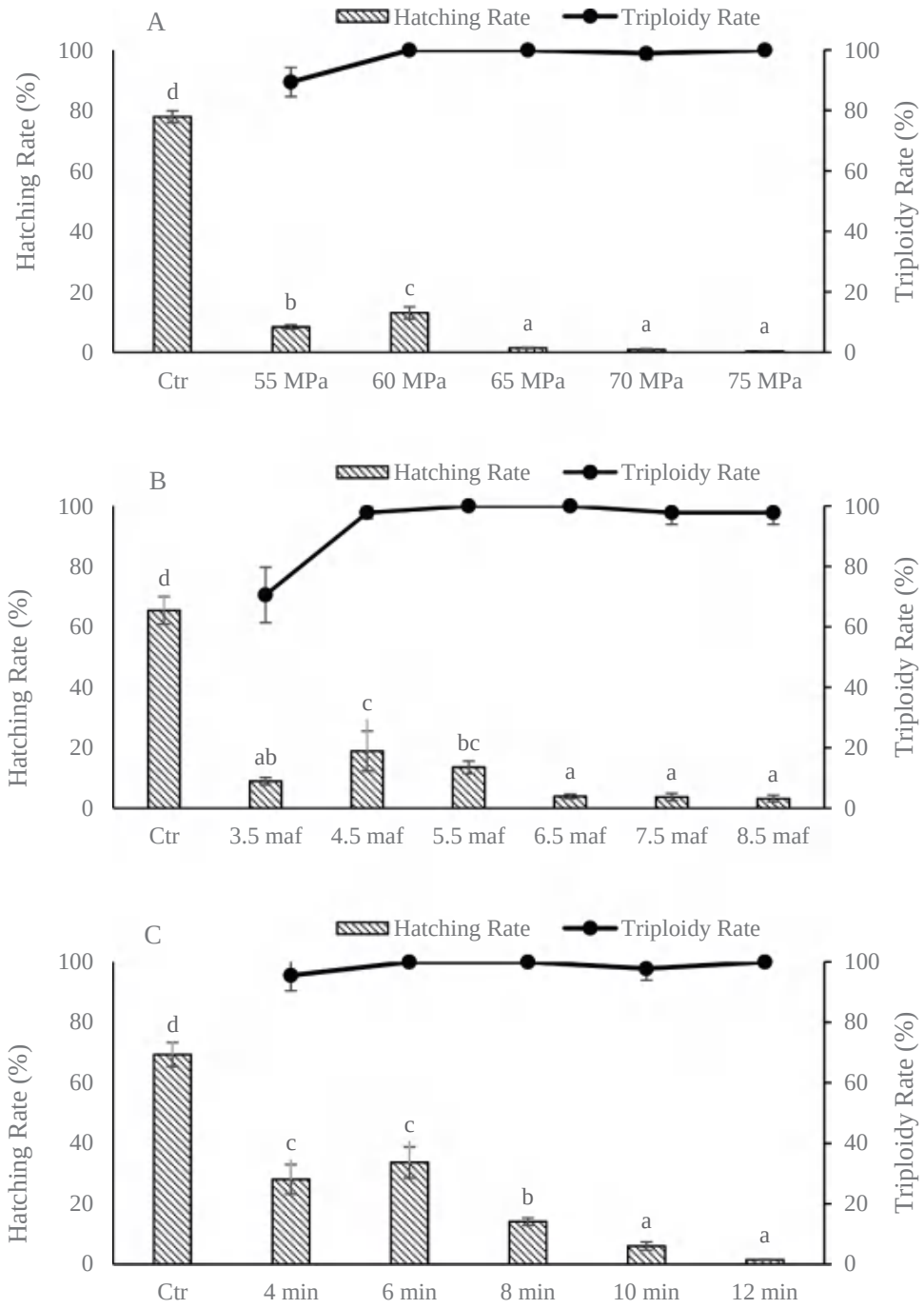

Fig. 1. Effects of intensity, timing and duration of pressure shock on the hatching rate of eggs and the triploidy rate of larvae for triploid induction in turbot. The fertilized eggs were pressure shocked at: different intensities of 55, 60, 65, 70 and 75 MPa with a fixed timing of 6.5 min after fertilization (maf) for 6 min (Fig. 2-A); different timings of 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 maf with a fixed intensity of 60 MPa for 6 min (Fig. 2-B); different durations of 4, 6, 8, 10 and 12 min with a fixed intensity of 60 MPa initiated at 5.5 maf (Fig. 2-C). Data are presented as means and standard errors of the raw data from three replicated experiments. Different lowercase letters above the column indicate significant differences in the hatching rate between groups with Tukey's honest significant difference test (ANOVA) ($p < 0.05$).

and high, 100% in two batches and 96.7% in one batch compared with 90.0% in two batches and 100% in one batch of CST.

3.3. Mass production of triploid juveniles

Table 2 presents the results of three batches of large-scale triploid induction and their diploid control counterpart, using the optimal pressure shock parameters (intensity of 60 MPa, timing at 5.5 maf and duration for 6 min). The fertilization rates were similar between the control groups and triploid groups. The hatching rate was significantly lower in the triploid groups (36.5% ± 4.3%) compared to the control groups (65.5% ± 4.6%) ($p = 0.001$), in accordance with previous observations (Table 1). Although there was a slightly lower survival rate in the treatment groups (14.5% ± 2.8%) during the period 1–60 dah as compared to the control groups (16.1% ± 1.9%), no statistically significant differences were detected. Thereafter, survival rate of the progenies from the 1st female was similar between the control (93.2%) and triploid (94.7%) group until 12 months after hatching (mah). The

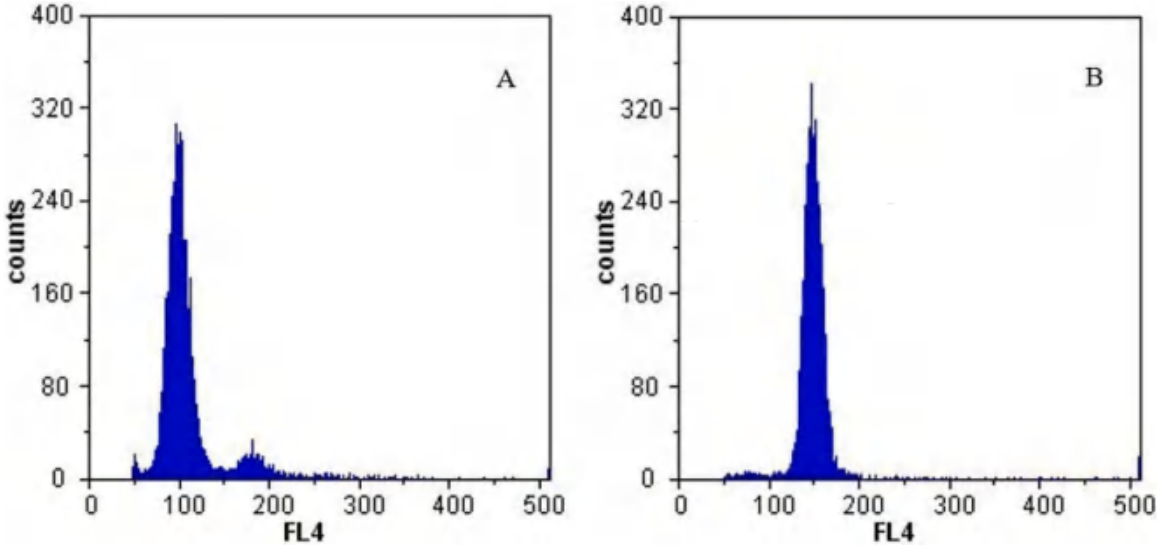

Fig. 2. Ploidy identification of larvae in diploid control (A) and triploid (B) turbot by flow cytometry analysis. Thirty individuals were randomly selected and tested in each group of the parameter experiments. X-axis reports fluorescence (FL) values on an arbitrary scale.

Table 1
Hatching rates, abnormality rates and triploidy rates among the normal control (Ctr), pressure shock induced triploid (PST) and cold shocked induced triploid (CST) eggs from three females (F1, F2 and F3, respectively). Different lowercase letters in the average line indicate significant differences between groups with Tukey's honest significant difference test (ANOVA) ($p < 0.05$).

Groups	Hatching rate (%)				Abnormality rate (%)				Triploidy rate (%)			
	F1	F2	F3	Ave	F1	F2	F3	Ave	F1	F2	F3	Ave
Ctr	76.5	73.3	79.2	76.3 ± 3.0^a	6.1	9.0	5.0	6.7 ± 2.1^a	-	-	-	-
PST	42.7	46.7	55.7	48.4 ± 6.7^b	11.40	9.04	9.54	10.0 ± 1.2^a	100	96.7	100	98.9 ± 1.9^a
CST	36.8	36.1	44.2	39.0 ± 4.5^b	16.82	19.05	22.56	19.5 ± 2.9^b	90	90	100	93.3 ± 5.8^a

Note: Ave represents the average of equivalent data from the three females (Mean ± SD).

Table 2
Mass production of triploid in turbot. Fertilization, hatching, survival and ploidy level characteristics of triploid as compared to control diploid turbot.

Variable	Control diploid	Triploid	Significance level
Weight of eggs used (g) (F1–3)	100/F	330, 250, 300	NA
Approximate total number of eggs used	1.2×10^5	$3.0–4.0 \times 10^5$	NA
Volume (mL) of sperm used after being diluted 1:10	5	15	NA
Fertilization rate (%)	89.0 ± 1.8	87.4 ± 5.7	NS
Hatching rate at 1 dah (%)	65.5 ± 4.6	36.5 ± 4.3	$p = 0.001$
Survival rate in the period 1–60 dah with respect to the total hatched larvae (%)	16.1 ± 1.9	14.5 ± 2.8	NS
Survival rate in the period from 2 to 12 mah (%)[a]	93.2	94.7	NS
Triploidy rate at 6 mah ($n = 30$)[a]	-	100%	NA

Note: F1-3, eggs used from three females. F, female. [a] analysis applied on progenies of Female 1. dah, days after hatching. NA, not applied. NS, not significant. Data as mean ± SD.

triploidy rate was determined to be 100% in this treatment group.

At 2 and 3 mah, the total length of triploid juveniles was significantly lower than that of the control group ($p = 0.02$ for both cases, respectively, Fig. 3-A). However, no significant difference in total length was observed among 4–7 mah. Subsequently, starting from 8 mah, triploids exhibited an accelerated growth rate, resulting in a significantly higher total length compared to diploid counterparts except at 10 mah.

In terms of body weight comparisons between triploids and diploids, it was observed that at 2 mah, the body weight of triploid juveniles was significantly lower than that of diploids ($p = 0.02$, Fig. 3-B). However, from ages ranging from 3 to 7 mah, although slightly lower in weight compared to diploids, no statistically significant difference was detected. Subsequently, a rapid increase in body weight among triploids was noted with significant differences observed at both 11 and 12 mah ($p = 0.04$ and 0.01 respectively). Notably, at 12 mah, the average body weight for triploids reached approximately 565.8 ± 61.8 g which represented a substantial increase by approximately10.7% when compared to their diploid counterparts (511.1 ± 59.5 g).

4. Discussion

In the present study, the optimal parameters of pressure intensity, timing and duration were determined by hydrostatic pressure shock induction of triploidy in turbot under a strictly controlled temperature. These parameters were then scaled-up to mass produce triploid juveniles with large volumes of eggs. Pressure shocks seemed to be more effective than cold shocks, allowing higher hatching rate, lower abnormality rate and relatively stable triploidy induction rate. The total length and body weight of triploids were significantly lower than their diploid counterparts at 2 mah, but showed a significant increase at 8 and 11 mah, which was maintained thereafter.

The high triploidy rates and yields obtained in this study confirmed the effectiveness of hydrostatic pressure shock treatment in inhibiting the second polar body extrusion during meiosis II in turbot eggs, as reported in other teleost (Benfey and Sutterlin, 1984; Huergo and Zaniboni-Filho; 2006; Bi et al., 2020). Various theories exist regarding the mechanism underlying the retention of the second polar body by pressure shock treatment. These generally include pressure acting on the microtubule depolymerization of the meiotic spindle, inhibition of

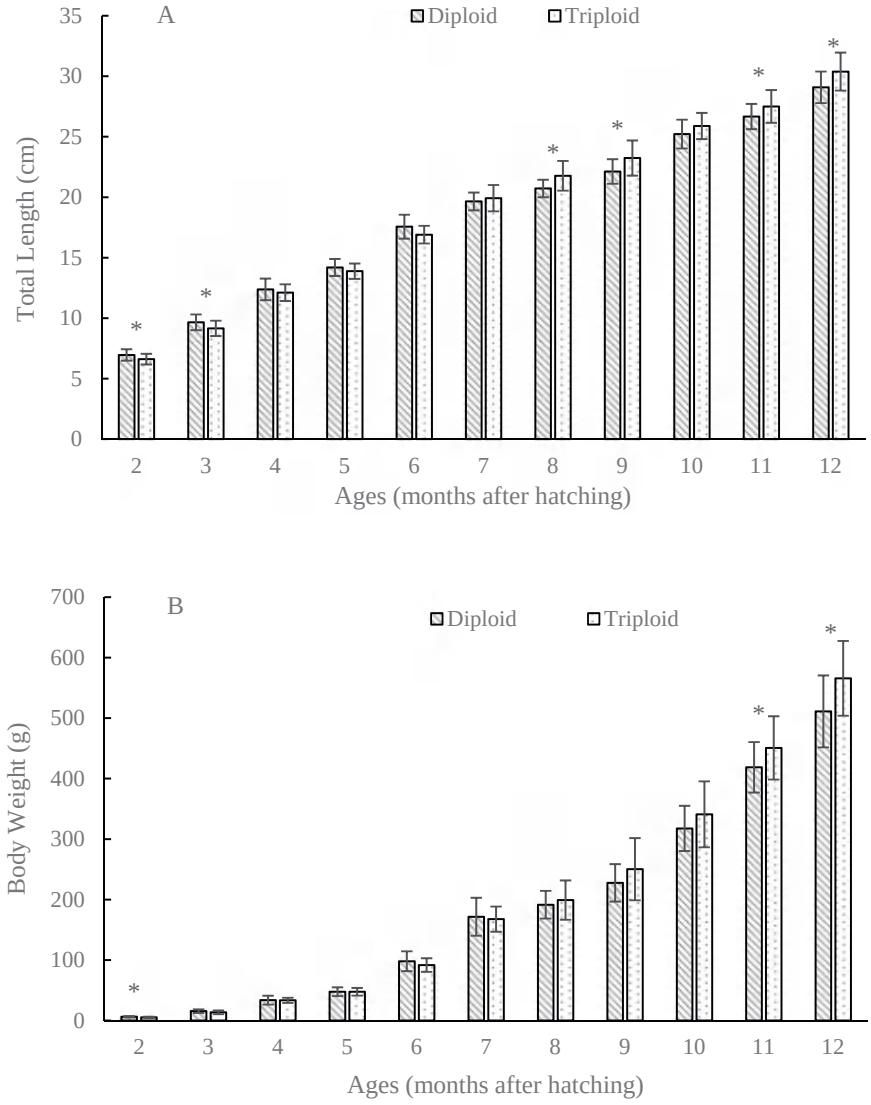

Fig. 3. The comparison of the total length (A) and body weight (B) between triploid juveniles of turbot and the diploid counterparts at 2–12 months after hatching (mah). Thirty individuals were randomly selected and measured for each mah. Data were expressed as mean ± SD. An asterisk (*) above the column indicate significant differences in the total length or body weight between groups with the Student's t-test ($p < 0.05$).

nucleation capacity of the centrosome, and cytoplasmic density changes, resulting in retention of the second polar body (Salmon, 1975; Zhu et al., 2007; Gao et al., 2018). Although further investigation is required to elucidate the precise mechanism involved in the current study, optimizing the timing, intensity and duration of pressure shocks is crucial for enhancing triploidy induction rate and successfully inducing viable triploids.

Based on the results, an optimal pressure intensity of 60 MPa was recommended in this study as it achieved the highest triploidy rate (100%) and triploid yield (13.0%). This optimal pressure intensity is consistent with previous studies on teleosts, where triploid induction typically achieved within a range of 60–65 MPa (ranging from 34 to 85 MPa) (Piferrer et al., 2009). Moreover, these optimal pressure intensities showed no significant correlation with egg diameters (~1–7 mm) (Malison et al., 1993; Felip et al., 2001; Kozfkay et al., 2005; Huergo and Zaniboni-Filho, 2006; Piferrer et al., 2009; Sierra-Flores, 2009). However, it is noteworthy that the optimal pressure intensity required for triploid induction was found to be lower than the previously reported value of 75 MPa for tetraploid induction in turbot (Meng et al., 2021), suggesting potential variations in the underlying mechanisms responsible for inhibiting meiosis II and mitosis I through pressure shock. Consequently, further investigations focusing on cytological analysis of microtubule organization and nucleus alterations are warranted to elucidate the precise mechanism by which pressure inhibits the second polar body extrusion.

The timing for triploid induction in teleost is considered to be associated with the process of meiosis II of the fertilized eggs, especially during the metaphase time window for spindle formation (Hussain et al., 1991; Piferrer et al., 2009). In turbot eggs, under the hatching water temperature of 15.5 °C, the formation of spindle apparatus during metaphase of meiosis II was observed at 4–8 maf (Sun et al., 2005). As the hatching temperature in this study is 14.5 °C, which is 1 °C lower compared to the aforementioned study, it can be inferred that the formation of spindle apparatus during meiosis II may occur slightly later. Therefore, it is likely that the optimal timing for triploid induction falls within the range of 4.5–5.5 maf and corresponds to the spindle formation under a lower hatchery temperature. Pressure shock treatment before or after this critical period probably affected certain cytological processes of meiosis II, resulting in reduced triploidy rates and triploid yields, respectively.

The optimal timing for triploid induction with pressure shock treatment in teleost is usually narrowed down to a precise moment or a

narrow time range shortly after fertilization under the strictly control of pre-shock water temperature. For example, it was recommended to induce triploidy at 6 maf for European sea bass at 13ºC (Peruzzi and Chatain, 2000), 5 maf for *Paralichthys olivaceus* at 16–18ºC (You et al., 2001) and 7 maf for barfin flounder at 7.2–8.5ºC (*Verasper moseri*) (Mori et al., 2004). In this study, the optimal timing for pressure shock treatment induced triploidy in turbot was determined to be 4.5–5.5 maf which is quite similar to those of the above fish species. The similarity in egg characteristics and hatching water temperature may account for the similar timing of these fish. The optimal timing might fluctuate in a certain range with the small change of pre-shock fertilization / treatment water temperature, which has been stressed in teleost (Purdom, 1972; Piferrer et al., 1994; Peruzzi and Chatain, 2000). The strictly controlled temperature (14.5 ± 0.5ºC) before treatment is a critical constant for the parameters validity and the retention of the second polar body in this study. However, the optimal timing for suppressing meiosis II with pressure shock treatment was somewhat earlier than that with cold shock treatment in turbot (Piferrer et al., 2003; Meng et al., 2013), although timing earlier than 6.5 maf were not tested in cold shock treatment in this study. The minor differences in pre-shock water temperature (14.5ºC and 14.0ºC) and fertilization with heterologous inactivated sperm in gynogenesis induction (sperm of *Pagrus major*) (Meng et al., 2013) might be responsible for the above results. Similar results were reported in the study on the induction of triploidy in ictalurid catfish (*Ictalurus punctatus*) (Lilyestrom, 1989). The results might indicate that cold shock treatment can be initiated more or less later than pressure shock but still be effective as it can slow down the rate of meiosis II division.

In terms of duration, the majority of fish tested exhibited successful results with a range of 2–6 min (Peruzzi and Chatain, 2000; Fetherman et al., 2015; Lahnsteiner and Kletzl, 2018; Bi et al., 2020). Pressure shock lasting for 4 min resulted in a high hatching rate but reduced the triploidy rate, while durations exceeding 8 min produced 100% triploidy rates but decreased the triploid yield in this study. The choice of pressure shock treatment duration of 6 min aligns with the developmental time from metaphase to anaphase during meiosis II in turbot eggs (Sun et al., 2005). The duration of pressure shock is significantly shorter than an equivalent cold shock for triploid induction in teleost. The results can also be attributed to the deceleration of meiotic division events caused by cold shock.

Compared to the diploid control, the hatching rates of triploidy induced by pressure shock and cold shock decreased significantly. This decline can be attributed partly to physical damage inflicted on the embryos due to the shock treatment or the presence of an additional chromosome set in triploids (3 n) (Hulata, 2001; Piferrer et al., 2009). However, in the present study, pressure shock treatment resulted in higher hatching rates and triploid yields compared to cold shock treatment. Additionally, it led to lower abnormality rates and relatively stable triploidy rates. The reason for this may be that all treated eggs were subjected to abrupt and equivalent pressure intensity, and a shorter exposure time reduced the risk of physical damage and temperature variation by pressure shock treatment. This result is consistent with the comparative study of pressure shock and temperature shock in other fish, such as, Nile tilapia (*Oreochromis niloticus*) (Hussain et al., 1991) and European sea bass (Peruzzi and Chatain, 2000). In practice, achieving 100% tripoidy induction is important, even if some mortality occurs since, as mortality in the induction cycle is less important than later mortality from an economic standpoint (Arai, 2001; Felip et al., 2001; Piferrer et al., 2009).

The performance of triploids is species-specific and has been extensively documented in laboratory-scale studies, but there is limited information available regarding their performance at the production scale (Piferrer et al., 2009). In this study, the survival rate of triploid turbot was slightly lower than that of diploid individuals during the larvae stage; however, similar survival rates were observed between triploids and diploids from 2 to 12 months after hatching (mah). Similar findings have also been reported for tench (*Tinca tinca* L.) (Flajšhans et al., 2004) and European seabass (Felip et al., 1999), where triploids exhibited lower early survival compared to diploids probably due to reduced viability of eggs, developing embryos, and hatched larvae up until the first feeding stage. Thereafter, their survival rates tended to be similar to those of diploids during the juvenile growout stage. The total length and body weight of triploids were significantly lower than those of diploid counterparts at 2 mah, however, they exhibited a significant increase at 8 and 11 mah, respectively, and maintained this higher level thereafter. Nevertheless, it should be noticed that the communal rearing of diploid and triploid juveniles might not allow for an accurate evaluation of the growth performance of triploids due to potential behavioral factors between the two ploidy group. Nonetheless, the results obtained from pressure shock induced triploids agree with previous findings described for triploid turbot induced by cold shock treatment, where superior growth and survival were demonstrated after reaching the first sexual maturity (~ 24 months) (Cal et al., 2006).

In conclusion, this study showed that triploid turbot can be induced by pressure shock at an intensity of 60 MPa initiating at 4.5–5.5 maf and lasting for 6 min under pre-shock water temperature of 14.5 °C, and confirms the higher induction efficiency and relatively stable induction rate by pressure shock treatment compared with cold shock treatment. Despite exhibiting lower hatching rates compared to their diploid control counterparts, triploid turbot demonstrated comparable survival rates while displaying significantly enhanced somatic growth rates at 11 and 12 mah. Further studies on the effect of pressure shock induced triploidy on growth, morphology, hematology, endocrinology, behavior, energetics, gonad development, gene interactions and gene expression patterns are required to evaluate the biological and economic performance capacity of triploids with respect to diploids in turbot. In any case, from a practical viewpoint, it is evident that triploidy induced by hydrostatic pressure treatment, rather than via cold shock, may benefit the commercial production of triploid turbot.

Ethics approval

Not applicable or not required for this study.

CRediT authorship contribution statement

Zhen Meng: Conceptualization, Methodology, Writing, Supervision and Funding acquisition; **Bangyin Zhang**: Investigation; **Chuer Song**: Investigation; **Lin Yang**: Investigation; **Xinfu Liu**: Supervision and Writing – review & editing; **Yudong Jia**: Validation and Funding acquisition; **Zhi Yang**: Resources; **Hesen Zhang**: Resources.

Declaration of Competing Interest

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

Data availability

All relevant data are within the paper, and those are available from the corresponding author.

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Code availability

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101775.

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