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Research Article

The impact of triploidy on vertebral deformities in snow trout (*Schizothorax richardsonii*)

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Background: Triploidy induction is widely employed in aquaculture to produce sterile fish, offering potential benefits such as enhanced growth and improved market control. However, induced triploidy is frequently associated with skeletal deformities, which pose a threat to fish welfare and reduce commercial viability. Optimizing induction protocols is essential to mitigate these negative effects.

Methods: Triploidy was induced in fertilized eggs of *Schizothorax richardsonii* using hydrostatic pressure shocks of 5000, 6000, and 7000 psi, applied for either 3 or 5 minutes at 12 minutes post-fertilization. Ploidy status was verified through cytogenetic analysis. At 75 days post-hatching, vertebral deformities were assessed via morphological examination. Deformity rates were statistically compared across treatment groups and against untreated diploid controls.

Results: The treatment of 5000 psi for 5 minutes produced the highest triploidy induction rate. All triploid groups exhibited significantly higher vertebral deformity rates compared to diploid controls. The maximum recorded deformity rate was $3.5 \pm 0.13\%$ in the 7000 psi / 5 minute group, whereas diploid controls showed no deformities (0.0%). Our findings revealed that deformities increased with rising pressure intensity or prolonged exposure.

Conclusion: Triploidy induction in *S. richardsonii* has significant potential for enhancing aquaculture production, but it also increases the risk of skeletal deformities. These deformities can adversely affect fish survival, marketability, and production costs. To improve the commercial feasibility and ethical standards of triploid fish farming, further research should focus on optimizing induction parameters and investigating alternative methods to minimize deformities.

Keywords: *triploidy, pressure shock, vertebral deformities, Schizothorax richardsonii, aquaculture, fish health*

Introduction

Schizothorax richardsonii, commonly known as Snow trout, is a significant coldwater fish species with considerable aquaculture potential in India, particularly in the Central Himalayan region (Kamalam et al., 2019). These fish are typically found in snow-fed torrential streams, preferring rapids and pools with water temperatures ranging between 8°C and 22°C (Sharma, 1989), although they are capable of tolerating a broader range from 0°C to 32°C (FRC, personal communication). In addition to flowing rivers and streams, the species is also reported from land-locked lakes (Pradhan, 1982). Members of the genus *Schizothorax* belong to the family Cyprinidae, commonly referred to as snow trout. This family encompasses over 15 genera and more than 100 species globally (Mirza, 1991). In India, *Schizothorax* species are distributed across various cold water habitats from Jammu and Kashmir to the Eastern Himalayas, including Assam, Sikkim, and Bhutan typically occurring at elevations between 1,180 and 3,000 meters

above sea level (Jhingran, 1982). These fish exhibit short migration cycles, generally moving upstream with rising water temperatures and downstream when temperatures decline. Triploid organisms possess three sets of chromosomes, distinguishing them genetically and physiologically from diploids (Tiwary et al., 2004). Triploidy induction involves disrupting the normal extrusion of the second polar body shortly after fertilization, typically through physical or chemical shocks (Peruzzi et al., 2000). Triploidy results in sterility, which not only serves genetic containment purposes but also favors the production of larger females, as indicated by Zúñiga-Panduro et al. (2014). A triploid zygote forms through the fusion of three haploid nuclei (Tave, 1990). Various methods are employed to induce triploidy, including thermal (hot or cold), hydrostatic pressure, chemical agents, anesthetics, and electric shock. Among these, temperature shock remains widely used due to its cost-effectiveness and feasibility for large-scale application (Silva et al., 2022; Nascimento et al., 2021; Rozy et al., 2022). However, it is less consistent in producing uniform results, likely due to the challenge of maintaining uniform temperature exposure across egg batches. For instance, Vasconcelos et al. (2022) reported 100% triploidy in a species using a 4°C cold shock applied for 5 minutes after 25 minutes post-fertilization. Hydrostatic pressure shock has also demonstrated efficacy in several aquatic species such as coho salmon, oysters, mussels, and mandarin fish (*Siniperca chuatsi*) (Bi et al., 2020; Teskeredžić et al., 1993). In *Megalobrama amblycephala*, pressure treatment at 7,877 psi for 3 minutes post-fertilization resulted in an 85.92% triploid rate (Zheng et al., 2023). Similarly, high hydrostatic pressure (9000 psi for 5 minutes) successfully induced triploidy in grayling eggs (Hliwa et al., 2022). Atlantic salmon eggs subjected to 8000 psi pressure treatment exhibited survival rates ranging from 26.1% to 36.8%, with triploid genotypes being dominant (Glover et al., 2020). In pikeperch, hydrostatic pressure yielded comparable triploidization outcomes (Káldy et al., 2021). Despite the aquaculture advantages of triploids, such as sterility and improved growth in some cases, a major concern is the higher prevalence of skeletal deformities, particularly vertebral and jaw anomalies, compared to diploids (Fraser et al., 2012a). These deformities may be influenced by genetic and environmental factors, including temperature (Wargelius et al., 2005). Notably, several studies have documented skeletal anomalies such as lower jaw deformities and opercular shortening in triploid Atlantic salmon, particularly in later developmental stages (Amoroso et al., 2016; Taylor et al., 2012). Although opercular deformities can occur in both diploids and triploids, their impact on growth, welfare, and marketability is significant (Boglione et al., 2013). Temperature has also been shown to affect skeletal development, with higher temperatures correlating with increased deformity rates and reduced survival (Amoroso et al., 2016). One hypothesis is that rapid growth at elevated temperatures leads to increased muscle mass that may not be adequately supported by under-mineralized bone (Fjelldal et al., 2006). The commercial application of triploidy in aquaculture remains limited due to these deformities, which reduce survival and performance (Amoroso et al., 2016). For instance, Whitt et al. (1972) observed higher jaw deformity rates in hybrid sunfish than in parental species. Therefore, considering both performance and welfare concerns, it is important to investigate skeletal deformities in triploid *S. richardsonii*, a species with limited prior documentation on this topic. This study aims to evaluate the extent of vertebral deformities in triploid Snow trout compared to diploids, thereby contributing to the understanding of their suitability for commercial aquaculture.

Materials and methods

Broodstock rearing

Adult male (n = 20) and female (n = 30) broodstock, aged 2–3 years, were collected using nets and traps from the Sirodi stream, Bhowali (District Nainital, Uttarakhand, India). The brooders were transported live in oxygenated polythene bags to the ICAR–Directorate of Coldwater Fisheries Research (DCFR), Bhimtal. Upon arrival, males and females were maintained separately in tanks to prevent unwanted natural spawning. The broodstock were fed a formulated diet containing 50% crude protein. The diet was composed of mustard oil cake (40%), goat liver (30%), rice polish (15%), wheat flour (10%), and fish oil (5%). The feed was pelletized into 4 mm pellets and offered twice daily at a rate of 4–5% of body weight. Water quality parameters were monitored regularly according to standard methods (APHA, 1998).

Triploid Induction

Mature brooders were selected and induced to spawn using the dry stripping method. Eggs from three females were fertilized with milt from four males. Both diploid (control) and triploid (treated) groups were derived from the same broodstock. Triploidy was induced using hydrostatic pressure shock at three pressure levels: 5000, 6000, and 7000 psi, for durations of either 3 or 5 minutes. The shock was applied 12 minutes post-fertilization. For each group, 10,000 eggs were used: half were subjected to pressure shock and half remained untreated (control). All fertilized eggs were incubated in a flow-through incubation system. Following hatching, the fry was reared in FRP (fiber-reinforced plastic) tanks under controlled conditions. Key water quality parameters during the rearing period were as follows: dissolved

oxygen 7.02 ± 0.43 mg/L, free carbon dioxide 0.73 ± 0.37 mg/L, temperature 18.2 ± 2.21°C, pH 7.10 ± 0.01, and total alkalinity 72.7 ± 1.12 mg/L.

Triploidy verification

To confirm triploidy, ten fingerlings from each group were sampled, and twenty chromosomal slides were prepared per group. Fin clips were collected from each specimen and smeared on pre-cleaned slides with a few drops of 50% acetic acid. The slides were air-dried at room temperature. Silver nitrate ($AgNO_3$) staining was performed following the method of Howell & Black (1980) to visualize nucleolar organizer regions (NORs).

Vertebral deformity assessment

A total of 100 larvae from each group were examined for vertebral deformities at 75 days post-hatching using an Olympus CKX53 inverted microscope at 20×, 40×, and 100× magnifications. Deformities were documented photographically using Magma-DC 10 camera with Magvision software. The deformity rate was calculated using the following formula:

Deformity (%) = Number of deformed larvae/Total larvae observed×100.

Results

Triploidy induction through pressure shock

Triploidy was successfully induced in *Schizothorax richardsonii* using hydrostatic pressure shocks of 5000, 6000, and 7000 psi, applied for 3 and 5 minutes. Among all treatments, the highest triploidy induction rate of 94% was achieved with a pressure shock of 5000 psi for 5 minutes**.** The corresponding hatching rate and survival rate under this condition were 70.6% and 76.9%**,** respectively.

Ploidy verification using silver staining

The number of nucleolar organizer regions (NORs) per cell varied from one to three in both diploid and triploid individuals. Diploid tissues exhibited 50–52% of cells with one nucleolus and 42.6–49% with two nucleoli. In contrast, triploid tissues showed a more diverse distribution: 16.5–30.5% of cells with one nucleolus, 30.2–40.3% with two, and 34.8–47.3% with three nucleoli (Figure 1). The presence of a higher proportion of cells with three nucleoli in triploids served as confirmation of successful triploidy induction.

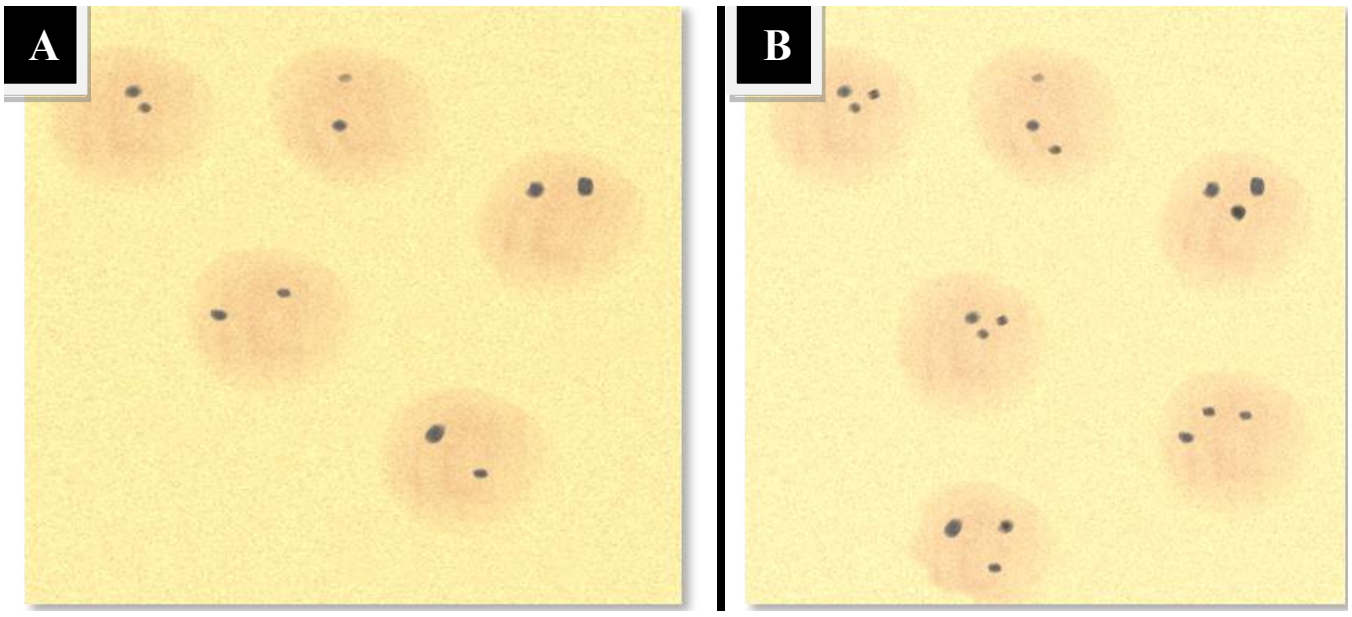

Figure 1. (A) Diploid group showing one and two interphase NORs. (B) Triploid group showing two and three interphase NORs

Vertebral deformity

Triploid groups exhibited a higher frequency of vertebral deformities compared to the diploid control. No deformities were observed in the control group (0.0 ± 0.00%). Deformity percentages increased with both pressure intensity and exposure time. At 5000 psi, deformity rates were 0.5 ± 0.10% (3 min) and 1.5 ± 0.10% (5 min). At 6000 psi, deformities were 0.75 ± 0.03% (3 min) and 2.2 ± 0.15% (5 min). The highest deformity rates were observed at 7000 psi: 2.8±0.15 (3 min) and 3.5±0.13 (5 min) (Table 1).

Table 1. Comparative vertebral deformity percentages in diploid (control) and triploid snow trout under different pressure shock conditions

Pressure (psi)	Exposure time (min)	Deformity %
5000	3	0.5±0.10
	5	1.5±0.10
6000	3	0.75±0.03
	5	2.2±0.15
7000	3	2.8±0.15
	5	3.5±0.13
Control	-	0.0±0.00

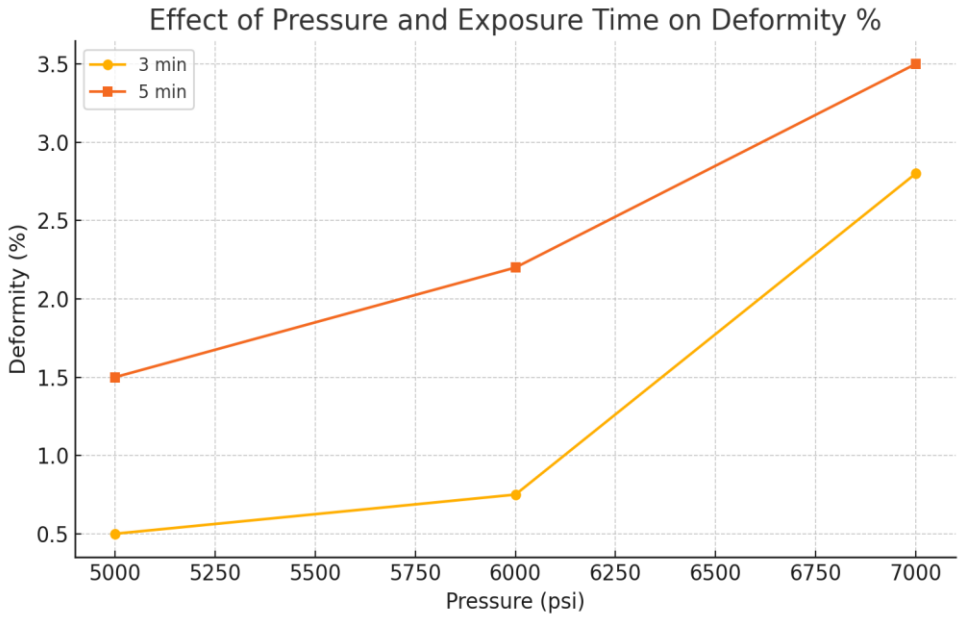

Figure 2. Line graph showing the effect of pressure and exposure time on deformity percentage, deformity increases with both higher pressure and longer exposure time

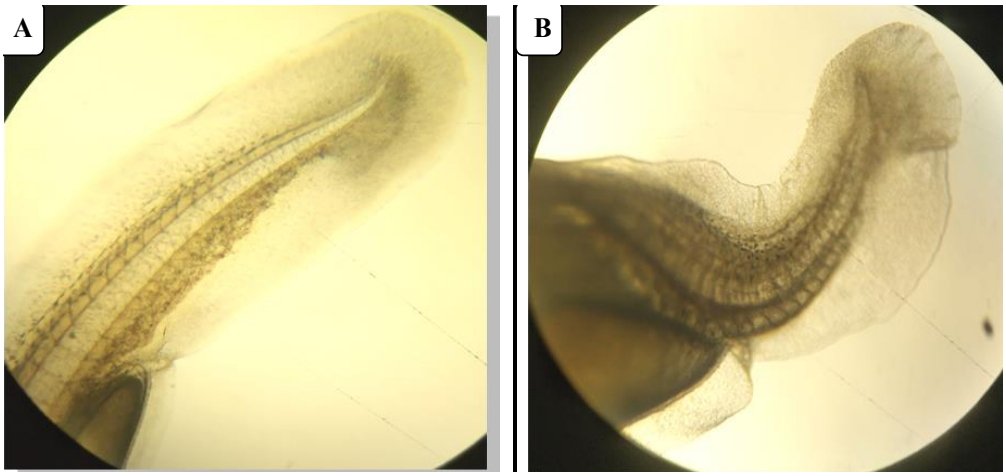

Figure 3. Comparison of vertebral deformities between diploid (A) and triploid (B) Schizothorax richardsonii at 10× magnification

Discussion

The efficacy of triploidy induction in teleost fishes is influenced by several critical factors, particularly the magnitude and duration of hydrostatic pressure shock (Felip et al., 2001). Previous studies have reported effective induction within pressure ranges of 4267 to 10,667 psi applied for 1 to 5 minutes (Meng et al., 2012). In the present study, a 94% triploidy success rate was achieved when a 5000 psi pressure shock was applied 12 minutes post-fertilization for duration of 5 minutes. This result not only falls within the effective range suggested in earlier reports but also supports the notion that lower pressure with extended duration can be more successful than higher pressures. Conversely, higher pressure intensities (6000 and 7000 psi) resulted in reduced induction efficiency, aligning with findings in Nile tilapia where increased pressure compromised fertilization and viability (Hussain et al., 1991). Similar results were observed by Zheng et al. (2023) in *Megalobrama amblycephala*, where the optimal induction was reported at 85.92% triploidy using 7797 psi (55 MPa) pressure for 3 minutes, initiated 3 minutes post-fertilization. Triploidy confirmation in the current study was performed through silver staining of the nucleolar organizer regions (NORs) as per the method of Howell & Black (1980). Diploid individuals displayed predominantly one to two NORs per cell (Figure 1A), whereas triploid individuals exhibited up to three NORs per cell (Figure 1B). These findings are in agreement with previous observations in various fish species (Sato et al., 2020). Moreover, Kim et al. (2017) endorsed silver staining as a practical and reliable technique for ploidy identification in *Oncorhynchus mykiss*, especially in farm settings lacking sophisticated cytogenetic tools. Skeletal deformities, particularly vertebral malformations, are a recurring concern in triploid fish. While some studies have reported low deformity rates (<2%) without any significant influence of ploidy (Taylor et al., 2012). In the present investigation, vertebral deformities were significantly higher in triploid *S. richardsonii* compared to diploids ($p < 0.0001$), especially under increased pressure and prolonged exposure conditions (Figure 2A & B). Triploidy-induced skeletal abnormalities have been well documented in various salmonids such as Atlantic salmon (*Salmo salar*), rainbow trout (*O. mykiss*) (Weber et al., 2014), brown trout (*Salmo trutta*) (Preston et al., 2013), and Arctic char (*Salvelinus alpinus*) (Fraser et al., 2022). Notably, vertebral deformities were observed in this study just below the dorsal fin region, which is a common site reported for skeletal issues in juvenile fish (Grotmol et al., 2003 (Figure 3). The underlying mechanisms for higher deformity rates in triploids may be attributed to altered bone metabolism and mineral requirements, especially phosphorus. Fjelldal et al. (2016) demonstrated that increased dietary phosphorus improves skeletal integrity in triploid Atlantic salmon during early developmental stages. This corresponds with the hypothesis that organisms with larger genomes such as triploids require greater nucleic acid and consequently higher phosphorus levels (Neiman et al., 2012; Sambraus et al., 2020). Our results corroborate earlier reports of higher deformity incidence in triploid Atlantic salmon and other species. However, some studies contradict these findings (Taylor et al., 2012), suggesting that deformity prevalence may also be influenced by strain, nutrition, and rearing conditions.

Conclusion

The present study demonstrates that both increased hydrostatic pressure and prolonged exposure time lead to a higher incidence of deformities in treated fish, with the highest deformity percentage observed at 7000 psi for 5 minutes. These findings highlight the critical need to optimize induction parameters to balance effectiveness with minimal developmental anomalies. Future studies should focus on refining pressure shock protocols by integrating molecular and histological assessments to better understand the underlying causes of deformities. Additionally, exploring the long-term survival, growth performance, and reproductive capacity of treated fish will be essential to establish the practical applicability of these methods in aquaculture systems.

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Author contributions

Toshibaa- Fish Sampling and Measurement, Preparation and Analysis of silver staining of Nucleolar Organizer Region, Writing – Review, and Editing. H.C.S. Bisht- Conceptualization and Experimental Design, N.N. Pandey- Triploidization Induction through Pressure Shock. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

In this work, we strictly adhered to the guidelines of Institutional Animal Care and Use Committee of ICAR-Directorate of Coldwater Fisheries Research and ARRIVE (Animal Research: Reporting of In Vivo Experiments) for the care and maintenance of the experimental animals.

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