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Research Article

A comparative analysis of air and manual stripping techniques in female rainbow trout (*Oncorhynchus mykiss*) broodstocks

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Abstract

Numerous techniques are used in aquaculture to stripped eggs from fish broodstocks. The most common is the manual method, where the abdomen of the fish is gently massaged to extract the eggs. Alternatively, injecting gas pressure into the abdominal cavity can facilitate the release of eggs with potentially fewer negative impacts on the welfare and egg quality. This study compared the pneumatic stripping methods and the manual spawning method in rainbow trout (*Oncorhynchus mykiss*) spawners. The study focused on the effects of these techniques on the quality of the harvested eggs, relative fertility, stripping duration, and pH of the ovarian fluids. The findings revealed that maintaining a gas flow rate at 1.5 L min^{-1} during stripping is crucial to prevent post-stripping mortality of the broodstocks. The pneumatic method demonstrated a shorter duration of egg retrieval (39.5 s) compared to the manual method (42 s). Notably, there were no significant differences observed in ovarian fluid pH across the various methods (p>0.05). Overall, pneumatic egg extraction leads to higher-quality eggs with a greater hatching rate compared to the manual method. Considering the positive results of the pneumatic stripping and its high level of repeatability and efficiency, it can be used instead of the traditional method in rainbow trout hatcheries.

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) holds significant prominence as a cold-water fish species in Iran, which is cultivated as a vital source of protein through commercial farming. Over the years, its production substantially increased, from 1,928 tons in 1996 to 189932 tons in 2021, approximately 34.16% of the overall aquaculture production (Iranian Fisheries Statistical Yearbook, 2022). The Iranian Fisheries Organization reports a total of 6,500 fish farms in this country, employing 21,000 individuals. However, a considerable number of these farms remain inactive due to challenges such as insufficient producers and an inadequate supply of quality eggs.

In aquaculture, as in the reproductive phase of other animals, the provision of high-quality eggs and sperm is crucial for maintaining the life cycle and ensuring the survival of future generations (Migaud *et al.*, 2013; Bobe, 2015). Due to the considerable expenses linked with broodstock breeding, any changes in the quantity or quality of generated gametes can exert a notable impact on the competitiveness and sustainability of fish farms and aquaculture ventures (Bromage *et al*., 1992). Consequently, effective control over the production of high-quality gametes stands as a critical concern in aquaculture, providing important economic implications (Migaud *et al.*, 2013).

The manual method is widely employed in fish breeding farms as the primary approach for stripping. This technique involves securely but gently holding the broodstock fish from the head and tail, positioning the head at a 45° upward angle. Gently massaging the abdominal area in the direction of the anus with the thumb and forefinger promotes the release and extraction of eggs. It is crucial to apply minimal pressure on the abdominal region and avoid exerting excessive force, as this can potentially harm internal organs such as the spleen and liver of the fish (Hoisty *et al.*, 2012). Furthermore, it is recommended to cease stripping before the final eggs are expelled from the abdomen through this method. This precaution is essential, as the thin abdominal wall carries the risk of the eggs breaking and the pH of the ovarian fluid decreasing due to the dispersion of the yolk sac. These factors have a detrimental effect on the fertilization of the eggs (Dietrich *et al*., 2007).

In recent years, the pneumatic stripping method has emerged as an alternative to the conventional method and is used in the breeding of various fish species This technique involves injecting gas into the female fish's abdominal cavity, leading to the release of eggs under gas pressure. It was firstly utilized in Australia in 1957 and has since been investigated in rainbow trout and *Salmo Trutta Morpha fario* (Kowalski *et al*., 2018), *Coregonus lavaretus* (Cejko, 2016), and wild species such as the *northern pike* (Kowalski *et al*., 2020). The pneumatic method provides several advantages, including ease of use for both the operator and the fish, higher percentages of fertilization, greater eyed eggs and hatching rate, improved quality of the obtained gametes, preservation of ovarian fluid pH, and higher egg survival rates in various growth stages, and reduced risk of damage to the fish's skin and mucus (Guardiola *et al*., 2015; Tacchi *et al.*, 2015).

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Consequently, it promotes better fish welfare compared to the traditional manual method of stripping.

The present study aims to compare the traditional manual method with the pneumatic method, specifically involving oxygen and nitrogen injection, for stripping in rainbow trout. The efficacy of these techniques was evaluated based on parameters such as egg weight, fertilization rate, hatching success, collection time, and ovarian fluid pH. In other words, we aimed to investigate whether the pneumatic method of egg collection is a beneficial tool for the reproduction of rainbow trout.

Materials and methods

Experimental design

The study was conducted during the winter of 2022 at the Research Center for Genetics and Breeding of Coldwater Fish, Yasouj, Iran. A total of 30 four-year-old female broodstock (their hand stripping was more difficult due to the higher weight of 4-year-old broodstock) were determined to be ready for spawning through weekly examinations. According to the objective of this study, different methods of stripping methods and the effectiveness of gas (oxygen and nitrogen) pressure in the pneumatic method were evaluated. The female fish with an average weight of 3783±503 (g) were divided into three groups based on the stripping method, including the traditional method, stripping with oxygen injection, and stripping with nitrogen injection. Various parameters such as stripping duration, the weight of extracted eggs, ovarian fluid pH, fertilization percentage, eyed eggs, hatching success, broodstock mortality, and relative fertility were recorded and analyzed.

Extraction of eggs

To perform stripping using the pneumatic method, a specialized table was designed and constructed. The table allowed the broodstock fish to be positioned at an angle of 30-45° (Kowalski *et al*. 2018). The table was equipped with a chamber at the bottom to collect the eggs efficiently.

The female broodstocks were anesthetized using clove powder at a concentration of 150 ppm for stripping, following the method of Moradyan *et al*. (2013). One group of females (consisting of 10 individuals) underwent stripping using the manual method, involving gentle abdominal massage. The other two groups underwent ovulation with the assistance of gas injection (oxygen and nitrogen) into the abdominal cavity. The gas introduction was accomplished by inserting a 0.8 mm needle under the ventral fin of the broodstocks (Fig. 1).

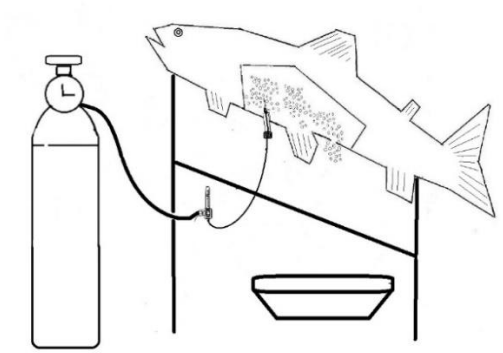

Figure 1: Schematic of egg stripping in fish by pneumatic method.

The needle was connected to a gas capsule via a syringe, and a narrow tube connected to a flowmeter and pressure gauge was employed (Kowalski *et al*., 2018). Both the

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manual and pneumatic methods were performed by a skilled operator. To monitor the survival of the broodstocks, individual ones were tagged before stripping using a tagging device. Before the experiment, air pressure and flow were tested and adjusted to achieve optimal efficiency and prevent fish mortality (by trial and error method). Different needle diameters were also evaluated for gas injection into the abdominal cavity.

Fertilization

To minimize the influence of sperm variation, a mixture of sperm from six fertile males was used to fertilize all the eggs. Three minutes after mixing sperm and eggs, each batch of eggs was washed three times in succession with incubation water.. The obtained eggs were then placed in separate trays. The water temperature in the incubation facility was maintained at approximately 10±1°C. After approximately 18 days, the eggs eyed and subsequently underwent the hatching process.

Parameters calculation

Stripping duration: This parameter refers to the period commencing from the insertion of the needle into the abdominal region of the fish until the complete removal of the needle from the fish's belly (Kowalski *et al*., 2020).

$$\text{Relative fish fertility} = (\text{egg weight}/\text{fish weight}) \times 100$$

Ovarian fluid pH: This parameter was calculated using a pH meter WTW (model PH 340i /SET (Germany))

$$\text{Fertilization percentage} = (\text{Fertilized eggs number}/\text{ all eggs number}) \times 100$$

(Bromage and Cumarantaunga, 1998)

$$\text{Eyed eggs percentage} = (\text{eyed eggs number}/\text{ fertilized eggs number}) \times 100$$

(Aas *et al.*, 1991)

$$\text{Hatching percentage} = (\text{Larvae number})/(\text{eyed eggs number}) \times 100$$

(Billard and Gillet, 1981)

Statistical analysis

All statistical analyses were conducted at a significance level of 0.05 using SPSS 16.0 for Windows. The data were subjected to repeated measures of one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons (Wharton and Hui, 2010).

Results

The average weight of the broodstock used for the manual stripping method was 3608±359 g, while it was 3958±648 g for the pneumatic method. The air pressure applied was maintained below 1, and the gas flow rate was set at 1.5 L min^{-1}. The rate of broodstocksloss within 24 hours after stripping was found to be 87% for the manual method, 84% for the pneumatic method with oxygen injection, and 78% for the pneumatic method with nitrogen injection.

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Effect of stripping method on stripping time, average egg weight, and relative fertility

Irrespective of the method used, the duration of stripping was calculated to be less than 1 minute. The average extraction times for the pneumatic method with oxygen, nitrogen, and the manual method were 42±2.90 seconds, 37±89 seconds, and 46±2.09 seconds, respectively. A significant difference was observed between the extraction times (p<0.05) (Fig. 2 A). The average weights of the stripped eggs in the pneumatic method with oxygen, nitrogen, and the traditional manual method were 499.8 g, 494.3 g, and 345 g, respectively (Fig. 2 B). To account for the variation in the weight of the tested broodstock, the egg weight-to-reproductive weight ratio was calculated. This ratio exhibited a significant difference (p<0.05), with values of 13.67% for the pneumatic method with oxygen injection and 9.55% for the traditional method (Fig. 2C).

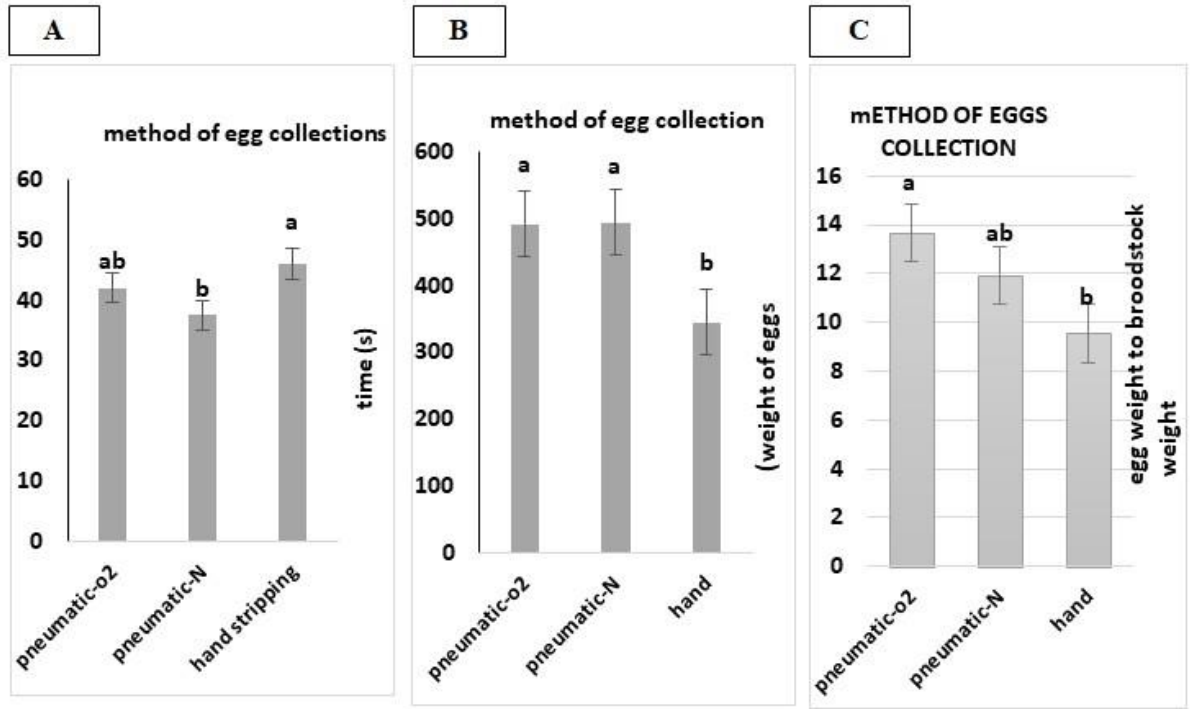

Figure 2: Comparison of stripping methods (pneumatic method with oxygen, aerobic with nitrogen, and traditional) with the duration of egg release (A), the weight of extracted eggs (B), and percentage of egg weight to broodstock weight (C). Values marked with the same letters show no significant difference, while different letters show a significant difference.

Stripping method effect on ovarian fluid pH

No significant difference was observed among the different stripping methods (p>0.05). As depicted in Figure 3, the average pH values of the ovarian fluid in the pneumatic method with oxygen and nitrogen injection, and the traditional manual method were determined to be 7.65±0.59, 8.00±0.06, and 7.84±0.12, respectively.

Stripping methods affect percentages of fertilization, eyed eggs, and hatching

According to the results, fertilization rates were significantly higher in eggs obtained through the pneumatic method, compared

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to the manual method (p<0.05) (Fig. 4A). Specifically, the percentage of fertilization was estimated to be 98% for the pneumatic method with oxygen injection, 97% for the pneumatic method with nitrogen injection, and 92% for the manual method.

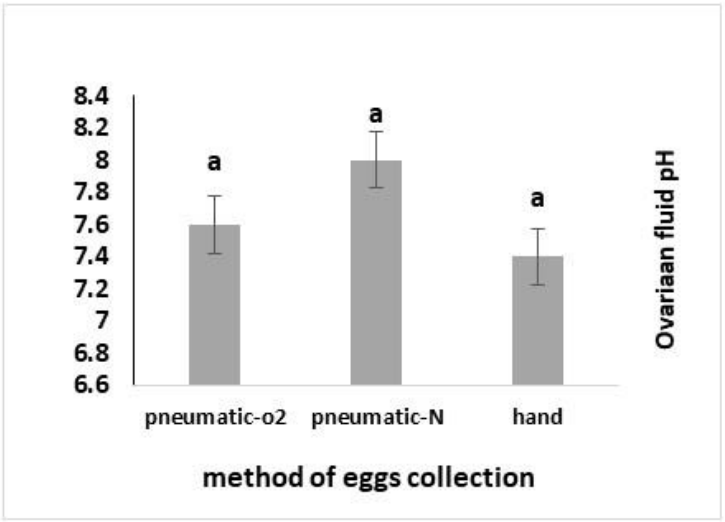

Figure 3: Comparison of stripping methods (pneumatic with oxygen, pneumatic with nitrogen, and traditional) with ovarian fluid pH. The same letters indicate no significant difference.

Moreover, the percentage of eyed eggs was significantly higher in eggs collected through the pneumatic method compared to manual eggs (p<0.05) (Fig. 4B). Additionally, the percentages of hatching differed significantly among eggs obtained through all three methods (p>0.05) (Fig. 4C).

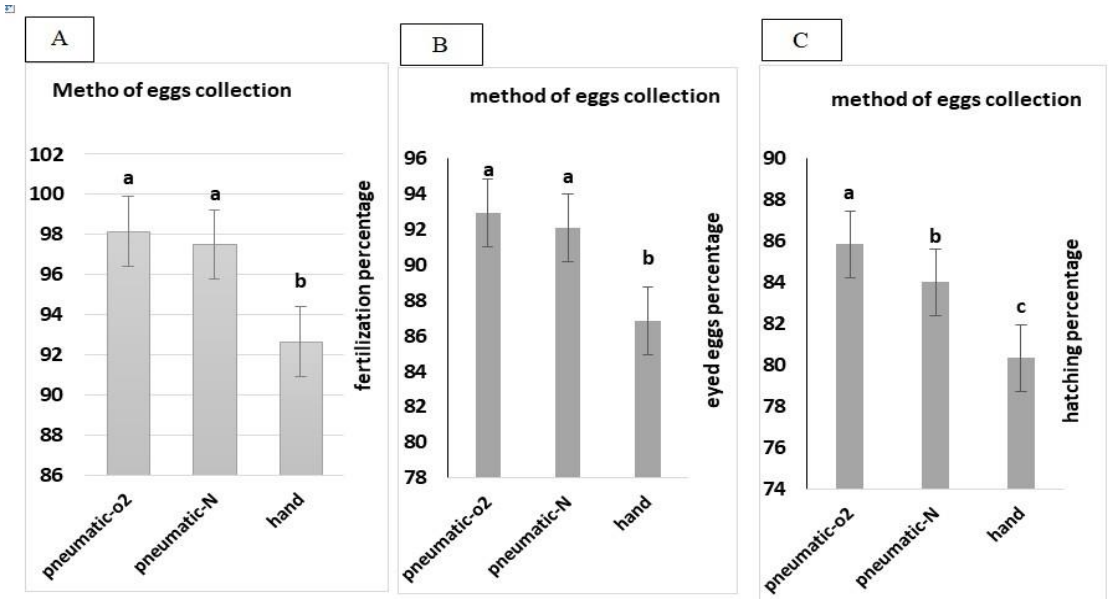

Figure 4: Comparison of the effect of stripping methods (pneumatic with oxygen, pneumatic with nitrogen, and traditional) on fertilization percentage (A), eyed eggs percentage (B), and hatching percentage (C). The same letters show no significant difference, whereas different letters show a significant difference.

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Discussion

Our results highlighted the efficacy of the pneumatic method for stripping rainbow trout eggs in fish breeding facilities, regardless of the specific gas used. The optimal gas flow rate was determined to be 1.5 L min^{-1}, ensuring the complete extraction of eggs from the fish's abdomen while minimizing any potential harm or adverse effects on the fish. It was observed that higher gas flow rates led to abdominal distension in the fish, while lower rates prolonged the anesthesia period, both of which resulted in fish mortality. These results align with a study conducted by Kowalski *et al*. (2018) on rainbow trout and brown trout (*Salmo trutta morpha fario*). It should be noted that the optimal air flow rate for the pneumatic stripping method in other fish species, such as *Esox lucius*, was reported to be 0.5 L min^{-1} (Cejko *et al*., 2016), and 0.5 L min^{-1} for *Coregonus lavaretus* breeding (Kowalski *et al*., 2020). This discrepancy in gas flow rates may be attributed to differences in egg size among different fish species (*Coregonus lavaretus*<*Oncorhynchus mykiss*<*Esox lucius*) (Bonislawska *et al*., 2001), or variations in the location of oviposition (body cavity or ovary cavity) in different species.

As expected, the pneumatic stripping method exhibited a shorter average time to remove the eggs from the fish's body than the manual method. Specifically, the pneumatic method (with both oxygen and nitrogen) had an average of 39.75 s (42 s with oxygen and 37.6 s with nitrogen), while the manual method took about 46 s. This indicates that the pneumatic method allows for faster stripping. In the manual method, the stripping speed is highly influenced by the expertise and experience of the operator. More experienced operators can achieve higher rates of reproduction within a specific period. Conversely, in the pneumatic method, individuals with partial training can perform fish reproduction by simply adjusting the gas pressure and release speed. This makes it easier to manage the reproduction of a large number of broodstocks in the hatchery, as the stripping duration can be approximately estimated. In whitefish (*Coregonus lavaretus*) propagation, the average stripping duration of the pneumatic method with nitrogen was reported as 17 s, while the manual method took 25 s (Kowalski *et al*., 2020). In rainbow trout and brown trout (*Salmo trutta morpha fario*), the manual stripping method took approximately 10 s, which was shorter than the air stripping method (p<0.05). Notably, the manual method exhibited more variation in the stripping duration (Kowalski *et al*., 2018).

This study also revealed that the relative fertility rate is influenced by the stripping method (p<0.05). The average weight of stripped eggs through the pneumatic method (both methods) was 477.03 g, compared to 345 g in the manual method. This difference can be attributed to the complete releasing of eggs in the pneumatic method, in addition to the weight of the broodstocks. In the manual method, approximately 50 g of eggs remain in the abdominal area of the fish, necessitating further reproductive procedures in the following days. These approaches include fish anesthesia, abdominal massage, and

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removal of mucus from the skin surface (Hashemian *et al*., 2020).

The findings of this study indicated that the different stripping methods had no significant effect on the pH of the ovarian fluid. The average pH values in the pneumatic method with oxygen injection, nitrogen injection, and manual method were 7.65, 8.00, and 7.84, respectively. However, the eggs quality, as measured by the percentage of fertilization, eyed eggs, and hatching percentage, was significantly influenced by the used stripping method. In other words, the pneumatic method resulted in a higher percentage of fertile eggs and hatched larvae compared to the traditional manual method. Notably, in the manual stripping method, there is a higher possibility of creating eggs with varying qualities. This can be explained by the fact that, in addition to the individual characteristics of the broodstock female (which are consistent across both methods), the pressure exerted by human hands during stripping process can affect the eggs and may harm them (Kowalski *et al*., 2020). The experience and speed of the operator also play a significant role in this regard. Additionally, the ovarian fluid volume, which acts as a protective buffer against excessive pressure during manual stripping, is an important factor. A lower volume of ovarian fluid makes stripping more challenging, requiring more energy from the operator to extract the eggs, increasing the possibility of egg damage and subsequent decreases in hatching rates (Dietrich *et al*., 2007). The results showed that the effect of stripping methods on the ovarian fluid pH in this study are consistent with the findings of similar studies on the artificial propagation of fish species such as *Esox lucius* and brown trout (Cejko *et al*., 2016; Kowalski *et al*., 2020). Studies on rainbow trout, brown trout, duck fish (bombil), and whitefish have also reported that the stripping method can influence fertilization and spawning rates (Cejko *et al*., 2016; Kowalski *et al.*, 2018, 2020).

Generally, the results of fish stripping employing the pneumatic method, irrespective of the gas source, compared to the manual method, exhibited superior egg quality, easier operation for the breeder, preservation of fish mucus and avoidance of the need for reproducing broodstock.

Conclusions

Overall, the study demonstrates that the air stripping method, irrespective of the type of gas used, can yield high-quality eggs in rainbow trout. The optimal conditions for this method include adjusting the gas flow to 1.5 L min^{-1}, maintaining a gas pressure of 0.8 bar, and utilizing a needle with a thickness ranging from 0.8 to 1.2 mm.

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Conflicts of interest

The author declares no conflict of interest.

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