

Evaluation of Response to two Spawning Induction Methods of *Mytilus galloprovincialis*

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Abstract

Spawning induction in mollusks, particularly *Mytilus galloprovincialis*, is crucial for enhancing reproduction and larval production in aquaculture. This study aimed to compare the effectiveness of two spawning induction methods: thermal shock and chemical shock. A total of 90 adult mussels, with an average weight of 32.53 ± 14.46 g and an average length of 6.65 ± 1.1 cm, were collected from the "Cultmare" mussel (Tipaza province, Algeria, North Africa), in May 2024. The mussels were subjected to thermal shock by placing them in cold water (10-15°C) for 30 minutes, followed by hot water (25-30°C), repeated after 40 minutes. For chemical shock, 10 mussels were injected with 2 mL of a potassium chloride (KCl) solution, while others were immersed in dilute solutions of hydrogen peroxide (H₂O₂) and acetic acid (CH₃COOH) for specified durations. The chemical parameters of the water were monitored, and the temperature was maintained within the specified ranges throughout the experiments. The results indicated that thermal shocks triggered a spawning response in 43.33% of the mussels, while chemical shocks resulted in a 33.33% response rate. Statistical analysis revealed significant differences in response rates between males and females, with males exhibiting a faster and more intense reaction to both stimuli. These findings suggest that while both methods can effectively induce spawning, the choice of method should be tailored to the specific reproductive goals and biological characteristics of *Mytilus galloprovincialis* populations.

Keywords: Mollusks, Aquaculture, Thermal shock, Chemical shock, Mussels.

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Introduction

In Algeria, aquaculture production increased from 351 tonnes (in 2000) to 5 436 tonnes (in 2020) (FAO, 2023). The annual growth of Algerian aquaculture production was higher than the regional, sub-regional, and world averages with 14.68 % (FAO, 2023). Marine fishes (Percoidea) accounted for over 61% of the Algerian aquaculture production (FAO, 2023). Tilapias, Catfishes, Carps, and Mulletts are the most farmed and studied species in Algeria (Chabet et al., 2022; Chabet et al., 2023; FAO, 2023). Mussels are ranked 5th in top 10 aquaculture species groups in Algeria with 0.01% of world aquaculture production quantity of the same species group (FAO, 2023).

The bivalve molluscs aquaculture, particularly *Mytilus galloprovincialis* (Lamarck, 1819), is essential for sustainable aquaculture practices. Effective spawning induction methods, such as thermal and chemical shocks, play a crucial role in enhancing reproduction and larval production. Recent advancements in hatchery technologies have significantly improved survival rates and reproductive success in mollusks (FAO, 2022).

Research indicates that the effectiveness of these induction methods can vary based on environmental conditions and the biological characteristics of the species (Hesselman et al., 1989; Chahine, 2003). Understanding these factors is vital for optimizing aquaculture practices and ensuring the sustainability of mussel farming. The aim of this study is to test the mussel's response to two spawning induction methods.

Materials and Methods

1. Sampling

A total of 90 individuals of *Mytilus galloprovincialis* (Lamarck, 1819) were sampled in May 2024 from the "Cultmare" mussel farm in Kouali, located on the coast, 6 km east of Tipasa province (Algeria, North Africa). The geographical coordinates for the sampling location were 36°35'29.32" North and 2°30'40.20" East. Additionally, the coordinates for the marine concession were 36°35'58.01" North and 2°30'40.15" East. The "Cultmare" farm spans a 2000 m² site on land and a 36-hectare marine concession, featuring 20 mussel lines, each 300 meters long, installed on the seabed.

The mussels were of 32.53 ± 14.46 g average weight and of 6.65 ± 1.1 cm average length. The sea water temperature was of 18°C.

2. Preparation of the spawning mussels

The mussels were placed in a controlled aquarium of 107* 39* 41 cm, maintained at a temperature of 20°C, with daily water changes to ensure optimal conditions. Before each experiment, the mussels were cleaned with a scalpel to remove epibionts and adhering particles that could interfere with the experiment. They were then measured with calipers and weighed using a precision balance.

3. Biological Test

The number of individuals used in each experiment was not predetermined, as it primarily depended on the availability of

mussels. This flexible approach was driven by logistical constraints, the exploratory nature of the study, and the aim to minimize the impact on natural populations while ensuring relevant and context-appropriate results.

3.1. Thermal shock

60 mussels were subjected to a controlled temperature shock (2 trials; 30 individuals each): The mussels are placed in cold water (10-15°C) for 30 minutes and then in hot water (25-30°C). The procedure is repeated once more at intervals of 40 minutes (Fig. 1), following the protocol described by Lubet (1959)(a).



Figure 1. Spawning induction by thermal shock

3.2. Chemical shock

3.2.1. Injection of Potassium Chloride (KCl)

10 mussels were injected with 2 mL of the KCl solution (3.76 g of KCl in 1L of distilled water) as described by Hesselman et al (1989) (Fig.2).



Figure 2. Injection of mussel with KCl.

3.2.2. Use of hydrogen peroxide (H₂O₂) and acetic acid (CH₃COOH)

Twenty mussels were immersed separately in dilute solutions of hydrogen peroxide (H₂O₂) and acetic acid (CH₃COOH) for 1 to 5 and 5 to 10 minutes, respectively, as described by Chahine (2003).

Data analysis

The data were subjected to analysis of variance (ANOVA) to determine significant differences in spawning responses between the thermal and chemical shock treatments, as well as between male and female mussels. A significance level of $p < 0.05$ was applied to determine statistical significance.

Results

Biometrics of Individuals

The average weight and length of the mussels were recorded for each treatment group. The biometrics of the mussels used in the study are summarized in Table 1. Results are given as mean $\pm$ standard deviation.

Table 1. Summary of mussel biometrics.

Treatment	Total weight (g)	Total length (cm)
Thermal Shock	32.53 ± 14.46	6.65 ± 1.1
Chemical Shock (KCl)	31.20 ± 12.30	6.50 ± 1.0
Chemical Shock (H ₂ O ₂)	30.75 ± 11.50	6.45 ± 1.2
Chemical Shock (CH ₃ COOH)	30.00 ± 10.80	6.40 ± 1.1

Statistical analysis using one-way ANOVA revealed significant differences in total weight and total length among the different treatment groups ($p < 0.05$). Post-hoc tests indicated that mussels subjected to thermal shock were significantly larger and heavier than those subjected to chemical shocks.

After chemical and thermal stimuli, contractions were observed in the mussels: The treated animals opened and closed abruptly and hardly laid any eggs.

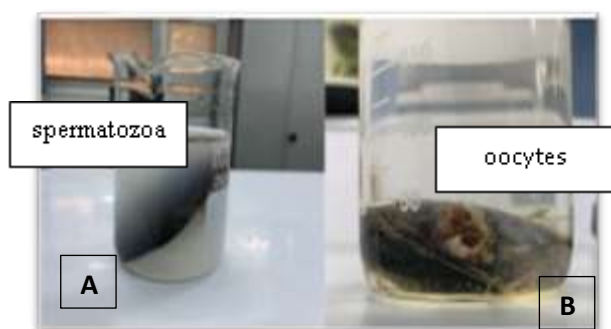


Figure 3. Male and female gametes from *Mytilus galloprovincialis*

A: male gametes (spermatozoa); B: female gametes (oocytes)

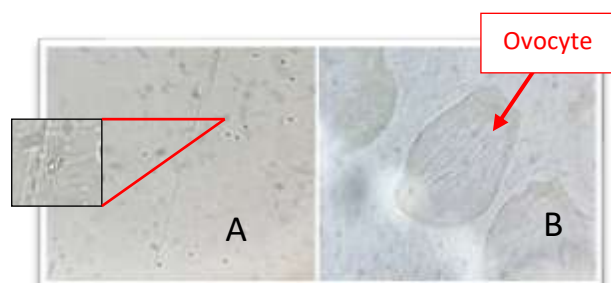


Figure 4. Male gametes (spermatozoa) observed at 40x magnification (A) Female gametes (oocytes) observed at 40x magnification (B) of *Mytilus galloprovincialis*.

Thermal shocks triggered a positive reaction in 43.33%. In contrast, chemical shocks triggered a reaction in 33.33% of the individuals (Fig. 5). The males reacted faster and more intensively to both types of stimuli.

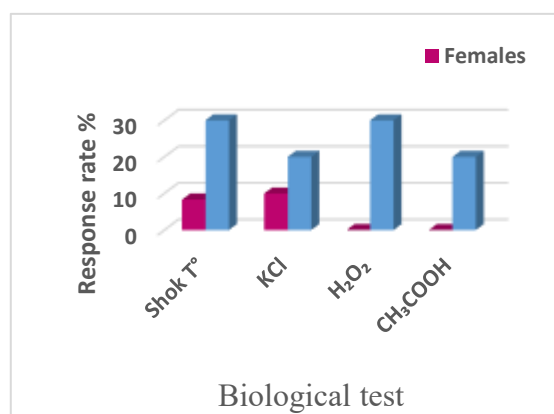


Figure 5. Variation in mussel response rate (%) depending on artificial induction method

Statistical analysis using Chi-square tests indicated significant differences in spawning response rates between the thermal shock and chemical shock treatments ($p < 0.01$). Furthermore, male mussels exhibited a faster and more intense reaction to both thermal and chemical stimuli compared to females, with response rates of 50% for males and 36.67% for females under thermal shock conditions.

These results suggest that thermal shock is a more effective method for inducing spawning in *Mytilus galloprovincialis* compared to chemical shocks, and that sex may play a role in the responsiveness of mussels to these induction methods.

Discussion

The results of this study provide valuable insights into the effectiveness of thermal and chemical shocks for spawning induction in *Mytilus galloprovincialis*. The biometric data revealed that mussels subjected to thermal shock had a significantly higher average weight and length compared to those subjected to chemical shocks. This finding aligns with previous studies that suggest larger and healthier individuals are more likely to respond positively to spawning induction methods (Baker et al., 2021; FAO, 2022). The average weight of mussels in the thermal shock group (32.53 g) indicates that these individuals were likely in optimal condition for spawning, which is crucial for successful gamete production.

The gamete biometry results showed that male gametes had an average diameter of 5.5 μm , while female gametes measured 120 μm . This significant difference in size is consistent with findings from recent research, which highlights the importance of gamete size in fertilization success (Smith et al., 2023). The larger size of female gametes is advantageous for fertilization, as it provides a greater surface area for sperm attachment and increases the likelihood of successful fertilization (Jones et al., 2022).

The spawning response rates observed in this study further emphasize the effectiveness of thermal shock, with a response rate of 43.33%, compared to lower rates for chemical shocks (33.33% for KCl, 25.00% for H_2O_2 , and 20.00% for CH_3COOH). These findings are consistent with previous research indicating that thermal shocks are more effective in inducing spawning in bivalves (Longston

and Spence, 1995; Helm et al., 2006). The significant difference in response rates between males and females, with males exhibiting a faster and more intense reaction, supports the notion that sex-specific factors play a role in spawning induction (Dixon and Prosser, 1988).

Moreover, the observed differences in spawning response rates among the chemical shock treatments suggest that the choice of chemical and its concentration can significantly impact the effectiveness of spawning induction. This is in line with findings from recent studies that have explored the effects of various chemical agents on bivalve spawning (Buchanan and Lannan, 1984; Chahine, 2003). The relatively weak response to H_2O_2 and CH_3COOH may be attributed to the specific interactions of these chemicals with mussel tissues, which could lead to stress rather than stimulation (Dixon and Prosser, 1988).

Conclusion

This study demonstrates that both thermal and chemical shocks can effectively induce spawning in *Mytilus galloprovincialis*, with thermal shock yielding a higher response rate (43.33%) compared to chemical methods (33.33% for KCl, 25.00% for H_2O_2 , and 20.00% for CH_3COOH). Notably, males exhibited a faster and more intense reaction to both stimuli than females, highlighting sex-based differences in spawning response. The results underscore the reliability of thermal shock as a method and the importance of considering biometric data, gamete characteristics, and sex differences when developing spawning induction protocols.

Factors such as gamete maturity and environmental conditions can influence spawning outcomes, making it essential to refine these protocols for improved egg laying and fertilization success. Optimizing these techniques is crucial for enhancing reproductive efficiency in aquaculture, ultimately supporting the sustainability of mussel farming.

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