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Optimizing the reproductive performance of clownfish *Amphiprion percula*: Effects of artificial substrates on spawning, fertilization, and hatchability in captive breeding

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Abstract

The increasing demand for marine ornamental fish, particularly *Amphiprion percula*, has raised concerns about sustainability due to the heavy reliance on wild-caught specimens. Captive breeding programs offer a viable alternative; however, reproductive success varies significantly depending on the artificial substrates used. This study examines the effects of different substrate types on spawning duration, fertilization rate, and egg hatchability in *A. percula* under controlled aquaculture conditions. A completely randomized design was implemented, testing four substrate types—cobek (earthenware, control), PVC pipes, ceramic pieces, and asbestos sheets—with three replicates each. Broodstock were maintained in optimized water quality conditions, with regular monitoring of feeding regimes and spawning behavior. The results demonstrated that substrate type significantly influenced reproductive parameters. The shortest spawning interval was observed in the cobek treatment (7 days), while the longest was recorded in the asbestos treatment (32.67 days). Fertilization rates ranged from 96.67% in the cobek treatment to 93.42% in the PVC pipe treatment. Notably, the hatchability of fertilized eggs remained consistently at 100% across all treatments, suggesting that artificial substrates do not compromise embryonic development. Water quality parameters were maintained within optimal ranges throughout the experiment, ensuring a stable rearing environment. This research contributes to the improvement of captive breeding practices, reducing dependency on wild populations and supporting the sustainable trade of marine ornamental fish. Future studies should explore additional factors influencing breeding success, including environmental cues and broodstock conditioning, to further refine aquaculture methodologies.

Keywords: Clownfish spawning, egg fertilization, hatching rate, substrate effect, water quality

Introduction

The growing demand for marine ornamental fish has driven the expansion of the ornamental fish trade from local markets to international commerce. Indonesia is a major exporter, with seawater ornamental fish exports exceeding 3,354 tons and generating over 33 million USD between 2015 and 2019 (Akmal et al., 2020). Among the most traded species is the clownfish *Amphiprion percula*, a reef-associated species known for its mutualistic relationship with sea anemones (Mariscal, 1972).

Due to its striking coloration and unique ecological traits, *A. percula* has become highly sought after in the aquarium trade. This species exhibits a characteristic bright orange body with three white bands bordered by black outlines, reaching a maximum size of 8–11 cm with notable geographic variation in coloration (Maison & Graham, 2015). Clownfish are protandrous hermaphrodites, meaning individuals begin life as males and can transition to females within a structured social hierarchy (Herrera et al., 2023). Each anemone typically hosts a dominant female, a breeding male, and several subordinate non-breeding males. If the female dies, the breeding male undergoes sex change and assumes the female role, ensuring reproductive continuity (Casas et al., 2022).

Despite their popularity, most clownfish are still harvested from the wild, raising concerns over sustainability and the depletion of natural populations. Their natural spawning cycles are prolonged, further limiting their ability to replenish stocks. To address these challenges, captive breeding programs have been developed. However, traditional breeding practices often use a single substrate type or no substrate at all, which may negatively affect spawning success, fertilization rates, and egg hatchability. The selection of an appropriate egg-laying substrate is a crucial factor in optimizing breeding success and ensuring a stable supply for the ornamental fish trade.

One of the primary challenges in clownfish aquaculture is the inconsistent use of artificial substrates, leading to variability in egg production and larval survival rates. Given the increasing demand for sustainably sourced ornamental fish, it is essential to determine the most effective artificial substrates for enhancing reproductive success. This study aims to assess the effects of different substrate types on spawning duration, fertilization rates, and egg hatchability in *A. percula*, ultimately identifying the most efficient substrate for egg attachment.

Artificial substrates play a crucial role in facilitating reproductive success in captive clownfish populations (Sahusilawane et al., 2023). In their natural habitat, clownfish deposit eggs on hard substrates near their host anemone, ensuring protection and adequate aeration (Gopakumar et al., 2011). Various artificial substrates such as ceramic tiles, PVC pipes, and natural rocks have been utilized in aquaculture, yielding variable results (Job, 2011; Kroll et al., 2014). Additionally, water quality parameters, including temperature, salinity, and dissolved oxygen levels, significantly influence reproductive performance in clownfish, necessitating careful regulation in captive breeding systems (Mariu et al., 2023).

This study will compare the effects of different artificial substrates on spawning duration, fertilization rates, and egg hatchability in *A. percula*, identifying the optimal substrate for broodstock reproduction in captivity. By elucidating the role of substrate selection in enhancing reproductive efficiency, aquaculture practitioners can refine breeding protocols, improve larval survival rates, and reduce dependence on wild-caught specimens. The findings will contribute to sustainable aquaculture practices, ensuring a consistent supply of clownfish while promoting the conservation of wild populations. Furthermore, the results may have broader implications for breeding other marine ornamental species with similar reproductive behaviours, supporting the long-term sustainability of the ornamental fish industry.

Methods

Experimental design

This study investigates the impact of different artificial substrates on *Amphipion percula* reproductive performance in terms of spawning duration, fertilization rate, and hatchability of eggs. A completely randomized design (CRD) was used with four substrate treatments in three replicates each. Treatment A served as the control, consisting of cobek (earthenware) and anemone as the spawning substrate. Treatment B had PVC pipes without anemone, and Treatment C was with ceramic pieces without anemone. Treatment D used asbestos sheets without anemone. Substrate selection was based on its availability, structural properties, and previous use in marine aquaculture studies.

Treatment procedure

The experimental design followed a sequential pattern of activities: preparation of the container, treatment of the substrate, selection and acclimatization of broodstock, feeding regime, and water quality maintenance for optimal spawning conditions. Preparation of the container and supply of water.

In this experiment, twelve glass aquariums measuring 40 × 40 × 60 cm in length were used. Before the experiment, all aquaria were treated with a chlorine solution at 200 ppm and left for 24 hours to ensure microbial decontamination. Then, they were scrubbed with a sponge, washed repeatedly with freshwater, and left to dry to remove chlorine residues. Each aquarium was filled with 48 liters of seawater, previously acclimated to a salinity range of 31-33 ppt. Aeration was provided with air pumps and diffusers throughout, ensuring that dissolved oxygen remained above 5 mg/L. Water temperature and pH were monitored daily using a digital thermometer and a calibrated pH meter, respectively, to keep the spawning conditions stable.

Substrates preparation

Each type of substrate was prepared according to the standard aquaculture procedure. The cobek plates, PVC pipes (10 cm height × 11 cm diameter), asbestos sheets (15 × 15 cm), and pieces of ceramic (15 × 15 × 10 cm) were pre-treated to remove unwanted contaminants. All substrates were soaked in seawater for three days to leach out toxic substances. Subsequently, they were scrubbed and washed appropriately before being sun-dried and then placed into the respective aquariums. Substrates were placed in the aquariums in such a way that they would provide optimum contact surface for the adhesion of the eggs and at the same time allow for easy observation. ´

Broadstock preparation

Twelve pairs of mature *A. percula* were chosen based on size, colour and active swimming-the characteristics that manifest their reproductive competence. The fish underwent acclimatization for one week before the experiment proper. Each aquarium was stocked with one male and one female, thus maintaining a 1:1 sex ratio. The transfer was done in the morning, using a soft mesh net and a water-filled container to reduce handling stress. The broodstock were acclimatized for signs of adaptation, such as feeding response and social interactions, before spawning observations were initiated.

Feeding regime

A strict feeding regime was adopted to improve broodstock reproductive performance. The fish were fed three times daily with a mix of commercial pellets and live feed. Feeding included pellets at 08.00 a.m., frozen blood worms at 10.00 a.m., and again with pellets at 14.00 p.m. Blood worms were first soaked in freshwater for 15 minutes to remove impurities and to improve digestibility. The amount fed was adjusted with respect to consumption by the fish to prevent a buildup of wastes and maintain good water quality.

Water quality maintenance

Water quality parameters, including dissolved oxygen, temperature, pH, and salinity, were continuously monitored. The readings were taken every day at 10.00 a.m. using calibrated meters for accurate measurement. Partial water exchange was done twice a day, replacing 20% of the total water volume each time to remove accumulated waste and maintain optimum rearing conditions. Besides, ammonia and nitrite levels were checked weekly using colorimetric test kits to avoid toxic buildups.

Observation parameters

Several key parameters were measured to assess reproductive success and environmental stability including spawning duration, fertilization rate, hatching rate, and water quality.

Spawning duration

The duration of spawning in the fish for each treatment. The observations ranged from the first day that the broodstock were placed within the spawning environment to the day on which spawning was detected. Spawning events would have been evident by the presence of newly laid eggs on the substrates. Number of days taken before spawning thus was recorded to measure the effect of the substrate type on the timing of reproduction.

Fertilization rate

Fertilization was considered successful through the count of viable fertilized eggs in comparison to the overall number of laid eggs. Among the eggs obtained, the clearly visible ones signified fertilization, whereas unfertilized ones were opaque in appearance. FR = (NFE/TNE) x 100 %, where FR is the fertilization rate (%), NFE is the number of fertilized eggs, and TNE is the total number of eggs produced per spawning event (Larasati et al., 2017). Percentage was calculated for each treatment in order to establish differences in reproductive efficiency.

Hatching rate

The hatching rate was obtained by count of the number of eggs that successfully hatched out relative to the fertilized eggs. This was done using the formula: HR = (Total number of fertilized eggs / Number of hatched eggs) × 100 (Ryan et al., 2023).

Hatching success was used as an indication of the viability of embryonic development and suitability of environmental conditions for egg incubation (Yonarta et al., 2024). Observations were made daily and the number of hatched larvae counted to ensure accuracy in data collection.

Water quality

Water quality was monitored during the entire course of the experiment to assess its effect on the spawning of the broodstock and viability of the eggs. In all tanks, temperature, salinity, pH, and dissolved oxygen were recorded. Other parameters, including ammonia and nitrite concentrations, were measured periodically to detect any deviations that might affect the reproductive results. Stability in water conditions was paramount to the success of the breeding trials.

Data analysis

All collected data were systematically analyzed to identify trends and treatment effects. Quantitative data, including spawning duration, fertilization rate, and hatching rate, were compiled into graphs using Microsoft Excel. Descriptive statistical analysis was performed to summarize water quality variations. One-way analysis of variance (ANOVA) was applied where applicable to determine significant differences among treatments, with post-hoc tests conducted for pairwise comparisons. Statistical analyses were performed using SPSS software to ensure robust and reliable interpretation of results.

Results

Spawning duration

The results indicate that different artificial substrates significantly influenced the spawning duration of *Amphiprion percula*. The spawning duration for each treatment is presented in Figure 1. The shortest spawning duration was observed in Treatment A (control), where spawning occurred within an average of 7 days (168 hours). In contrast, the longest spawning duration was recorded in Treatment D (asbestos substrate), with an average spawning time of 32.67 days (784 hours).

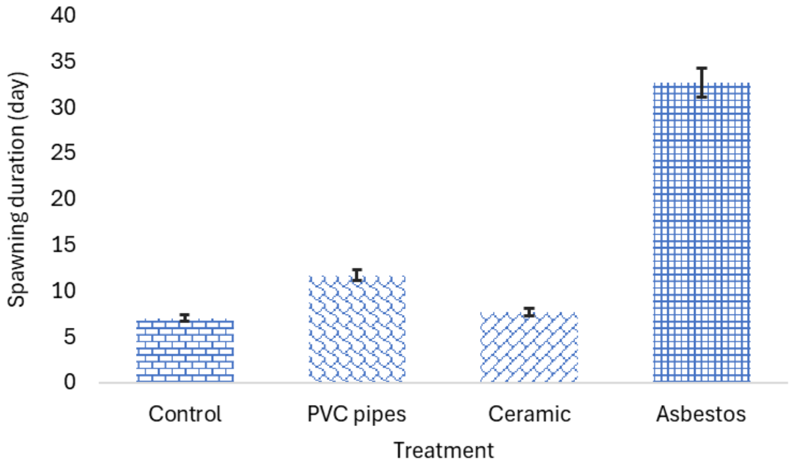

Figure 1. Clownfish spawning duration.

Statistical analysis using ANOVA revealed a highly significant effect of artificial substrates on spawning duration, with F-value = 30.39, exceeding both the 0.05 significance threshold (F-table = 4.07) and the 0.01 threshold (F-table = 7.59). Further analysis using the Least Significant Difference (LSD) test showed that Treatments A and C were not significantly different, whereas Treatment A differed significantly from Treatment B. Moreover, Treatments A, C, and B exhibited significantly shorter spawning durations compared to Treatment D.

Fertilization rate

The fertilization rate varied significantly among different substrate treatments. The highest fertilization rate was observed in Treatment A (control), with an average of 96.67% fertilized eggs (Figure 2). The lowest fertilization rate occurred in Treatment B (PVC pipe substrate), averaging 93.42%. ANOVA results confirmed that artificial substrates had a significant effect on fertilization rate, with an F-value of 12.23, surpassing the critical values at both 0.05 (F-table = 4.07) and 0.01 (F-table = 7.59). LSD analysis indicated no significant difference between Treatments D and B, while significant differences were detected between Treatments D and A, as well as between Treatments A and C. Treatments D, B, and A were significantly different from Treatment C, highlighting substrate-dependent variations in fertilization success.

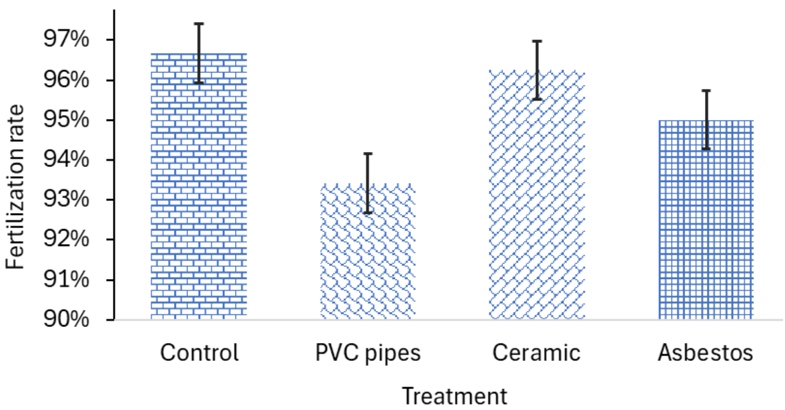

Figure 2. Clownfish fertilization rate.

Hatching rate

The hatching rate remained consistently high across all treatments, with each substrate yielding a 100% hatchability rate. The total number of hatched larvae varied according to the number of fertilized eggs in each treatment. Treatment A (control) resulted in 1673 hatched larvae, Treatment B (PVC pipe) produced 1193 hatched larvae, Treatment C (ceramic) yielded 1709 hatched larvae, and Treatment D (asbestos) produced 585 hatched larvae. These findings indicate that while substrate type influenced fertilization rates and spawning duration, it did not compromise the overall hatching success of fertilized eggs.

Water parameter

Throughout the experiment, water quality parameters remained within the optimal range for *A. percula* reproduction. The recorded temperature ranged from 27.8 to 30.1 °C, salinity levels were maintained between 30 and 33 ppt, and pH values ranged from 7.89 to 8.89. Dissolved oxygen (DO) levels were maintained between 4.7 and 6.32 ppm. These stable water quality conditions ensured a suitable environment for spawning, fertilization, and larval development.

Discussion

This study revealed significant variations in spawning duration, fertilization rate, and hatchability of *Amphiprion percula* eggs across different artificial substrates. The shortest spawning time was observed in Treatment A (control), where the broodstock spawned within an average of seven days. This rapid spawning response could be attributed to the familiarity of the broodstock with the substrate, which closely mimics natural rocky surfaces commonly used by *A. percula* in the wild. Previous research has shown that commercial breeders often utilize earthen flower pots or ceramic surfaces for clownfish spawning, a practice that aligns with the findings of this study (Allen, 1997). The clay-based cobek substrate, used in the control treatment, not only provided a familiar surface but may also have released an earthy odor that served as a chemical cue, stimulating reproductive activity.

The longest spawning duration was recorded in Treatment D, where the asbestos substrate

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resulted in a delay of 32.7 days before spawning occurred. The composition of asbestos, which includes cement, waste paper, and silica minerals, creates a coarse and fibrous texture that may have been unsuitable for spawning. While a rough surface is generally preferred for egg attachment, excessive coarseness may have hindered proper adhesion, leading to hesitancy in spawning behaviour. Additionally, asbestos fibres, which are extremely fine and airborne in certain conditions (Gibbs & Hwang, 1980), could have created a suboptimal environment, potentially causing stress or irritation to the broodstock. These findings suggest that substrate composition and surface texture play a crucial role in influencing the spawning behaviour of *A. percula*.

Fertilization success was highest in Treatment A, with a fertilization rate of 96.67%, and the lowest was recorded in Treatment B (PVC pipes), where the fertilization rate was 93.42%. The lower fertilization rate in the PVC treatment may have resulted from insufficient egg adhesion, leading to increased exposure to external disturbances such as water currents and aeration. Studies have shown that substrate selection significantly affects fertilization success, as it determines the stability and positioning of eggs during sperm penetration (Muslim et al., 2012). The fertilization process in teleost fish involves complex biochemical interactions between sperm and eggs, with successful fertilization requiring the integration of the male haploid nucleus into the egg cytoplasm (Lahnsteiner et al., 2001; Petersen & Mazzoldi, 2010; Ghosh et al., 2012; Roux et al., 2019). Once fertilization occurs, a cortical reaction is triggered, leading to the formation of a perivitelline space that protects the developing embryo (Berois et al., 2011; Ohta et al., 1990). Any disruption in these processes, including inadequate substrate support, may negatively affect fertilization success.

Despite variations in fertilization rates, all fertilized eggs successfully hatched, with a 100% hatchability rate across treatments. The highest number of hatched larvae was recorded in Treatment C (ceramic), while the lowest was in Treatment D (asbestos). These findings suggest that artificial substrates provided a sufficiently stable environment for embryonic development, ensuring that hatching success remained unaffected. Proper handling of eggs during the transfer to hatching containers minimized potential physical damage, further contributing to high hatch rates. Moreover, optimal water quality conditions, particularly the maintenance of dissolved oxygen levels, played a vital role in embryonic development. The presence of aeration stones near the substrates ensured adequate oxygenation, while parental fanning behavior, which enhances oxygen delivery to embryos, likely improved hatching success (Goldberg et al., 2020).

Hatching was consistently observed between 05:00 and 09:00 a.m, coinciding with a temperature range of 26–28 °C. This pattern aligns with the natural reproductive cycle of clownfish, where females actively assist in hatching by fanning their ventral fins to release larvae from the substrate before transferring them into the water column. Such synchronization between hatching behaviour and environmental conditions further emphasizes the importance of controlled rearing conditions in aquaculture settings.

Throughout the study, water quality parameters were maintained within optimal ranges for clownfish breeding and development. Broodstock tanks were maintained at temperatures of 28.0–30.1 °C, with a pH of 8.01–8.22, dissolved oxygen (DO) levels of 4.7–5.95 ppm, and salinity of 31–33 ppt. Similarly, spawning and hatchery containers had temperature ranges of 27.0–29.7 °C, pH values of 8.01–8.89, DO levels of 4.20–6.10 ppm, and salinity levels of 31–33 ppt. These values fall within the recommended seawater quality standards (KNLH, 2004) and are consistent with previous studies indicating that optimal conditions for clownfish culture include temperatures of 27–30 °C and a pH range of 7.0–8.0 (Santikawati et al., 2023). Routine siphoning was conducted twice daily to remove metabolic waste and excess feed, preventing the accumulation of harmful toxins, in accordance with best aquaculture practices (Summerfelt, 1999).

The findings of this study highlight the critical role of substrate selection, water quality management, and environmental conditions in optimizing the reproductive success of *A. percula* in captivity. Future research should focus on long-term reproductive performance under varying substrate and environmental conditions, as well as investigate additional factors such as broodstock conditioning and environmental cues that may further enhance spawning efficiency. These insights will contribute to the development of more effective captive breeding programs, ultimately reducing reliance on wild-caught populations and supporting the sustainability of the marine ornamental fish trade.

Conclusions

This research shows that the selection of substratum significantly influenced spawning result of *A. percula*, whereas the fastest spawning was Treatment A (cobek and anemone) within 7 days because it is similar to natural spawning surface, while the longest spawning time fell to Treatment D-asbestos with the highest value 32.7 days because it was made of rough texture and undesirable properties. The fertilization rates ranged from 96.67% in Treatment A to 93.42% in Treatment B, as mediated by substrate stability and water currents. However, the HR was 100% for all fertilized eggs, indicating that optimal water quality and aeration supported embryo development.

All water quality parameters remained within the optimum range and, therefore, within the suitable levels for spawning and hatching. Regular siphoning and aeration were carried out to maintain water quality and oxygen availability. Overall, substrates resembling natural spawning surfaces enhance reproductive efficiency in *A. percula*. Future research should explore long-term reproductive outcomes across different substrates to improve aquaculture practices.

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Authorship contribution

Iftahuddin Iftahuddin: Conceptualization, methodology, investigation, sample processing and analysis, visualization, original draft preparation, writing - review and editing. **Muliani Muliani:** Methodology, original draft preparation, writing - review and editing, supervision. **Zulfikar Zulfikar:** Conceptualization, methodology, review and editing, supervision.

Data availability

Datasets generated during and/or analysed throughout the present study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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