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### Behavior responses and survival of snakehead fish (*Channa striata*) broodstock during acclimatization

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#### Abstract

Post-transport acclimatization is a critical phase in broodstock management, particularly for stress sensitive species such as snakehead fish (*Channa striata*). This study evaluated behavioral responses, feeding activity, and survival of snakehead broodstock during the first 24 hours of acclimatization following transport. The study employed a descriptive exploratory design using nine acclimatization units, with behavioral exploration conducted at two time points immediately after stocking (T0) and 24 hours post-stocking (T24). Behavior responses recorded included erratic swimming, hitting against walls, jumping, gasping, and rubbing, which were quantified during a 10 minute exploration period. Feeding response and survival rate were assessed at T24, while water quality parameters were monitored throughout the acclimatization period.

The results showed that all behaviors responses were prominently expressed at T0, indicating acute stress responses following transport and handling. After 24 hours of acclimatization, a general reduction in the frequency of erratic swimming, hitting against walls, jumping, and rubbing was observed, suggesting partial behavioral recovery and adaptation to the new environment. In contrast, gasping behavior persisted with relatively minor changes, indicating that respiratory adjustment may require a longer recovery period. Feeding responses emerged in several acclimatization units at T24. Survival rates during the first 24 hours ranged from 80% to 100%, with an overall survival rate of approximately 92%. Water quality remained within tolerable ranges for *Channa striata* and was not considered a primary factor of behavioral variation or mortality. These results indicate that the first 24 hours post-transport represent a critical phase characterized by behavioral responses. Behavioral indicators, combined with feeding response and survival, provide a practical and non-invasive approach to assessing acclimatization success and fish welfare during the early post-transport period in snakehead broodstock.

**Keywords:** acclimatization, behavior responses, fish welfare, *Channa striata*, stress behavior

#### INTRODUCTION

Broodstock management requires special handling, particularly during the post-transport period. Post-transport acclimatization represents the most vulnerable phase in broodstock management, during which fish experience abrupt environmental changes that may trigger stress responses (Singh and Saxena, 2020). Transport related stress has been reported to disrupt physiological stability, suppress feeding activity, and reduce survival rates in cultured fish. During transport and the subsequent acclimatization period, fish are

exposed to changes in temperature, water quality, stocking density, and holding conditions. These changes often induce acute stress responses that directly affect behavior, physiological activity, and survival (Qiang, et al., 2018; Refaey & Li, 2018; Q. Wang et al., 2023).

Snakehead fish (*Channa striata*) is a freshwater species of high economic value that is widely utilized in aquaculture. However, this species is known for its aggressive behavior and high sensitivity to environmental fluctuations, therefore particularly vulnerable to stress during handling and early adaptation to rearing conditions. Failure of acclimatization is commonly indicated by the emergence of abnormal behaviors (Vanderzwalmen et al., 2021), reduced feeding activity (Kleiber et al., 2023), and increased mortality during the early phase of acclimatization. These conditions pose a major challenge in maintaining fish quality and survival during the transition from the original environment to a controlled aquaculture system.

Abnormal behaviors such as erratic swimming, hitting against walls, jumping, gasping, and rubbing have been recognized as reliable indicators of acute stress and impaired welfare in teleost fish (Handa, 2013; Sharma, 2015; Mandic et al., 2022). In addition to behavioral responses, feeding activity and early survival are considered integrative parameters reflecting the ability of fish to recover from stress and re-establish physiological homeostasis (Alvarado et al., 2025). Several studies have demonstrated that fish capable of resuming normal swimming behavior and feeding shortly after stocking generally exhibit higher survival rates and better long term performance in aquaculture systems (White et al., 2017).

Although knowledge of stress physiology and fish welfare assessment continues to develop, studies specifically focusing on the behavior responses of snakehead broodstock remain limited. Most previous research on *Channa striata* has focused on growth performance and reproductive biology. Therefore, this study aimed to evaluate behavior responses and survival dynamics of snakehead broodstock (*Channa striata*) during acclimatization period. The results of this study are expected to provide practical information for broodstock behavior after transportation to support the development of more effective and welfare-oriented acclimatization protocols in snakehead aquaculture. This study provides one of the first behavior-based descriptions of the early post-transport acclimatization phase in snakehead broodstock, offering practical welfare indicators for aquaculture management

## **MATERIALS AND METHODS**

### **Location**

The study was conducted at the Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia. The snakehead broodstock (*Channa striata*) used in this study were obtained from local fishers in Central Java, Indonesia, with an approximate transportation travel time of 2 hours to the laboratory. A total of 135 individuals were used across nine acclimatization units, with each unit containing a batch of fish in a single tank. Fish were transported to the laboratory under standard closed transport with oxygen. Each bag of fish were immediately placed into tanks and allowed to adjust the bag's temperature. The fish were released into the water and the behavioral responses were observed.

### **Exploration study**

The study employed a descriptive exploration design using repeated acclimatization trials. The study used nine acclimatization trials, each represented by a single tank containing a batch of snakehead fish with individual body weights of 250–300 g. Behavioral observations and survival assessments were conducted at the group level. Behavioral observations were conducted at the tank level, and the acclimatization unit (tank) was considered the unit of analysis to ensure independence of observations among replicates. Observations focused on early stress responses and survival during the first 24 hours after stocking. Behavioral and biological parameters were recorded at two observation times: immediately after stocking (T0) and 24 hours after acclimatization (T24). Observation at 24 hours after stocking was considered a critical period during post-transport acclimatization, as reported by Paixão et al., (2024).

Behavioral exploration were conducted at the group level. Each acclimatization trial consisted of a batch of fish housed in a single tank, and behavioral events were recorded as total frequencies per tank during a 10-minute observation period as reported by (Wilson, et al., 2024). Stress-related behaviors recorded included (1) erratic swimming (Sharma, 2015), (2) hitting against wall (Handa, 2013), (3) jumping (Sharma, 2015), (4) gasping (Handa, 2013; Mandic et al., 2022) dan (5) rubbing (Rose et al., 2014). Each behavior was recorded as the frequency of discrete observable events within a fixed 10-minute observation period, without assigning ordinal scores or weighting to behavioral categories. Feeding response was evaluated by offering feed 24 hours after stocking and recording whether fish actively consumed the feed.

### **Survival Rate**

Mortality was recorded continuously during the first 24 hours. Survival rate was determined as the percentage of surviving fish relative to the initial number stocked.

### **Water Quality**

Water temperature was measured using a digital thermometer, pH using a calibrated portable pH meter (Eutech Instrument), and dissolved oxygen (DO) using a portable DO meter (Lutron-5510). Instruments were calibrated prior to measurement by following the guidelines of the instrument.

#### Data Analysis

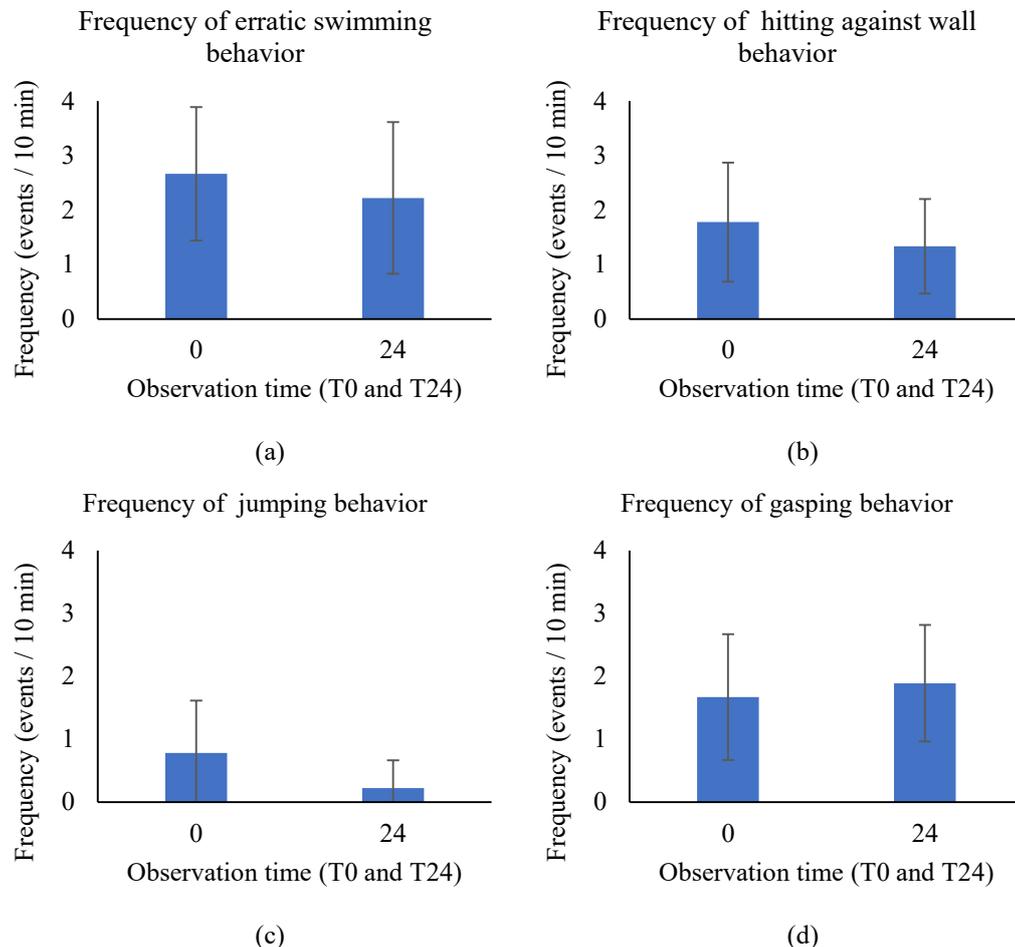
Data were analyzed descriptively. Stress-related behaviors were analyzed based on the frequency of behavioral events observed in each acclimatization unit at two observation times, immediately after stocking (T0) and 24 hours after acclimatization (T24), and were presented in tables and figures. Feeding response was analyzed qualitatively based on direct observation of feeding-related behavior at 24 hours after stocking. Mortality was recorded during the first 24 hours, and survival rate was calculated as the percentage of surviving fish relative to the initial number of fish stocked in each acclimatization unit. All analyses were conducted at the acclimatization unit (tank) level as the unit of analysis.

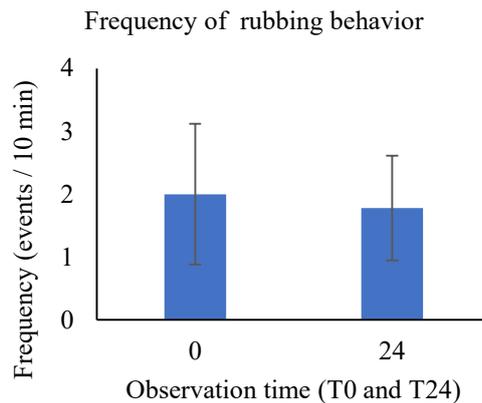
### RESULTS AND DISCUSSION

#### Results

##### Stress-related behavioral responses during acclimatization

Stress-related behaviors were observed in all acclimatization tanks immediately after stocking (T0). Behavioral indicators recorded included erratic swimming, hitting against walls, jumping, gasping, and rubbing. These behaviors reflected acute stress responses following transport and handling. After 24 hours of acclimatization (T24), a general reduction in the frequency of several stress-related behaviors was observed across acclimatization tanks. Erratic swimming showed a clear decreasing trend at T24 compared to T0 (Fig.1.a), indicating partial recovery from acute stress. Similar patterns were observed for hitting against the tank walls and jumping behavior (Fig.1.b,c), which occurred less frequently after 24 hours of acclimatization. In contrast, gasping behavior remained relatively stable or showed slight variation between T0 and T24 (Fig.1.d), suggesting that respiratory adjustment during acclimatization may persist longer than other behavioral responses. Rubbing behavior also decreased after 24 hours (Fig.1.e), although it was still observed in several tanks. Overall, the behavioral observations indicated that acute stress responses were most pronounced immediately after stocking and tended to diminish during the first 24 hours of acclimatization.





(e)

Figure 1. Frequency of stress-related behaviors observed immediately after stocking (T0) and after 24 hours of acclimatization (T24). (a) erratic swimming, (b) hitting against wall, (c) jumping, (d) gasping, and (e) rubbing. Values represent level observations recorded per acclimatization tank during a 10-minute observation period.

#### Feeding response during acclimatization

No feeding-related behavior was observed immediately after stocking (T0). At 24 hours after acclimatization (T24), feeding responses began to emerge in several acclimatization tanks. Feeding response was indicated by active approach toward feed and ingestion behavior observed. However, feeding activity was not uniform across all tanks, as some tanks showed clear feeding behavior (Fig.2.b) while others exhibited limited or no response to feed (Fig.2.a). This variability suggests differences in the rate of behavioral recovery among acclimatization tanks during the early post-transport period.



Figure 2. Feeding responses observed immediately after 24 hours of acclimatization (T24). (a) no response to feed, (b) clear feeding behavior shown by the red circle.

#### Survival rate

Mortality occurred during the first 24 hours after stocking. Survival rates varied among acclimatization tanks (A1–A9), ranging from 80% to 100% (Fig.3). Several tanks exhibited complete survival with no observed mortality, while others experienced limited mortality during acclimatization. Resulting in an overall survival rate of approximately 92%. These results indicate that mortality was confined to the early acclimatization phase and varied among tanks.

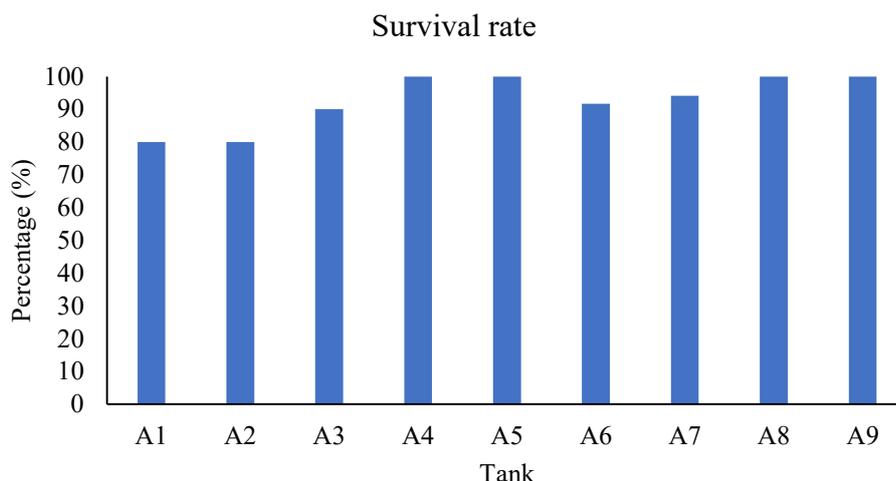


Figure 3. Survival rate of snakehead fish (*Channa striata*) broodstock in each acclimatization unit (A1–A9) during the first 24 hours after stocking. Values represent the percentage of surviving fish relative to the initial number stocked in each tank, each bar represents a single tank-level observation.

### Water Quality

Water quality parameters were relatively stable throughout the acclimatization period and did not show extreme fluctuations as summarized in Table 1. Water temperature, pH, and dissolved oxygen were within ranges that are considered tolerable for *Channa striata*.

Table 1. Water quality parameters during the acclimatization of snakehead fish (*Channa striata*) broodstock over the first 24 hours after stocking.

No.	Parameters	Specifications	Reference
1	pH	7.1 ± 0.11	6-7 (Bambang.S. et al., 2023)
2	DO (mg/L)	3.1 ± 0.21	3-6 mg/l (Bambang.S. et al., 2023)
3	Temp (°C)	29.3 ± 0.40	27-33 °C (Do, Huong et al., 2021).

### Discussion

The results of this study indicate that the first 24 hours after transport represent the most critical phase during the acclimatization of snakehead broodstock, as this period is characterized by a combination of behavioral responses, feeding response behavior, and survival rate. The use of behavioral indicators is consistent with the development of the fish welfare concept, which emphasizes non-invasive observations as a rapid and practical approach to assess fish condition under field conditions (Noble et al., 2020; Barreto et al., 2021). The frequency of behavioral responses observed at T0 such as erratic swimming, hitting against walls, jumping, gasping, and rubbing. It can be identified as indicators of behavior stress or physiological disturbance. Erratic swimming and hitting against walls are classified as signs of stress and neurological disturbance as reported by Vanderzwalmen et al., (2021), that observed similar responses in various cultured fish species during the transport phase. The reduction in erratic swimming behavior at T24 indicates a more effective stress adaptation following stocking. Erratic swimming is recognized as a behavioral indicator of stress, defined as a sign of stress after transportation (Vanderzwalmen et al., 2021). This behavior response is adaptive and successful adaptation is marked by the ability of fish to reduce abnormal behavioral responses after a certain period, as reported by White et al., (2017) that recovery from erratic swimming behavior began within the first three hours.

The erratic swimming behavior was supported by the behavioral response of hitting against walls, which also showed a decreasing trend after acclimatization. Hitting against wall behavior observed after stocking indicates a behavioral response to stress in the new environment. This is consistent with unacclimatized fish showed abnormal swimming behavior and avoidance responses. The reduction in hitting against wall behavior indicates behavioral adjustments (Noble et al., 2020; Paixão et al., 2024). Noble et al., (2020) reported that intense and repetitive abnormal behavior including hitting against wall is an indicator of

declining welfare. However, a decrease in this behavior indicates improved welfare. Jumping behavior in fish is an avoidance response or panic reaction to sudden disturbances. Disturbances suddenly make fish move away from the stressor (Vetter et al., 2017). Jumping behavior was observed at a higher frequency after stocking (T0). These are consistent with the report by Singh and Saxena, (2020) that jumping behavior in snakehead fish is reported under confined conditions, environmental changes suddenly, or attempts to escape from stressors. The reduction in jumping behavior after 24 hours (T24) indicates the ability of fish to stabilize stress responses and adapt to new environmental conditions. Wang et al., (2015) also reported that fish exhibit gradual behavioral recovery after stress exposure under stable aquatic conditions.

Gasping behavior is a classic indicator of a fish's response to hypoxic conditions. Gasping reflects increased tissue oxygen demand that cannot be reached through gill respiration, thus triggering behavioral responses such as the utilization of the surface oxygen layer (Reemeyer & Chapman, 2024). Although dissolved oxygen levels were relatively low, snakehead fish are known to tolerate hypoxic conditions due to accessory air-breathing adaptations, suggesting that observed gasping behavior was more closely related to acute transport stress than water quality alone. Rubbing behavior in snakehead fish indicates a response to mechanical injury likely incurred during capture, handling, and transport. scratches to the skin and fins can trigger a local inflammatory response, increase sensory sensitivity, and cause irritation or itching, prompting the fish to rub its body on hard surfaces in an attempt to relieve discomfort. Sharma (2015) reported that fish exposed to environmental stressors showed increased mucus secretion, pigmentation changes, avoidance behavior and abnormal movements. This increased mucus production is related to irritation and correlated with the emergence of rubbing behavior as a response to the skin. The decrease in the frequency of rubbing behavior indicates a gradual recovery process in the integument. This is in line with the report of Paixão et al., (2024) which showed that the post-transport stress response is most intense in the initial phase and decreases during acclimatization. Therefore, the reduction in rubbing behavior can be interpreted as a sign of physical recovery and a decrease in irritation.

Analysis of feeding response at T24 showed that some acclimatization units exhibited changes toward normal activity and feeding behavior. This condition represents a key indicator of the transitional phase toward behavioral stabilization in cultured fish (Alvarado et al., 2025). The emergence of feeding response in parallel with a decrease in stress-related behaviors suggests that fish were transitioning from an "acute stress" state toward an early behavioral adaptation phase. The recovery of normal activity and feeding interest is considered one of the earliest signs that fish are undergoing adaptive recovery (Kleiber et al., 2023). However, the presence of feeding response and near-normal activity does not necessarily indicate complete recovery, as many fish still exhibited stress-related behaviors. Fish that survived and showed feeding response may still experience physiological strain, including disturbances in immune function, metabolism, and osmoregulatory processes (Assan et al., 2021). Feeding behavior is highly sensitive to stress and often altered under stressful conditions; therefore, feeding response should be interpreted as a confounding variable alongside other indicators rather than as a single definitive measure of recovery (Barreto et al., 2021).

The survival rate during the 24 hours after stocking reflects the ability of snakehead fish to cope with acute stress caused by transport and environmental change. In this study, survival rates ranged from 80% to 100%, indicating variability in adaptive capacity during the early acclimatization phase. Mortality was confined to the early post-stocking period, which is widely recognized as the most critical phase in the acclimatization process of cultured fish (Gåsnes, et al., 2021). Post-transport stress is known to trigger serious physiological disturbances, including osmoregulatory imbalance, increased metabolic demand, and impaired respiratory function. When the physiological compensatory capacity of fish is exceeded, these disturbances may lead to early mortality. Previous studies have reported that transport stress contributes substantially to increased mortality during the initial rearing phase, particularly in species that are sensitive to environmental changes (Qiang, et al., 2018; Refaey & Li, 2018; Q. Wang et al., 2023).

Water quality during the acclimatization of snakehead broodstock was relatively within the tolerable range of this species. Temperatures was  $29.3 \pm 0.40$  °C is close to the temperature observed in the maintenance of snakehead fish species which can survive in the range of 27-33 °C (Do, Huong et al., 2021). This indicates that environmental maintenance has supported metabolic activity during the acclimatization phase. The measured pH value showed a value of  $7.1 \pm 0.11$ , this indicates suitable media conditions for the *Channa* with a pH value of around 6-7 within the normal range for snakehead fish (Sihananto et al., 2023). Low dissolved oxygen (DO) levels of around  $3.1 \pm 0.21$  mg/L can be tolerated by snakehead fish. This is due to their biological habitat, as they have adaptive respiration to low oxygen levels and a wide temperature range (Ansyari, 2021). This study was limited to the first 24 hours of acclimatization and therefore reflects early behavioral adjustment rather than complete recovery. Longer observation periods may provide additional insights into the stabilization of physiological and welfare status.

## Conclusion

The first 24 hours after transport represent a critical acclimatization phase for snakehead broodstock, characterized by pronounced stress-related behavioral responses. Abnormal behaviors such as erratic swimming, hitting against walls, jumping, gasping, and rubbing were most evident immediately after stocking and generally decreased after 24 hours, indicating early behavioral adaptation to the new environment. The emergence of feeding responses and relatively high survival rates further suggest partial recovery from acute stress, although variability among acclimatization units was observed. Overall, behavioral indicators combined with feeding response and survival rate provide a practical and non-invasive approach to assess acclimatization success and welfare status of *Channa striata* broodstock during the early post-transport period.

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