

Original Article

Optimization of safe transport conditions for hatchery-reared golden rabbitfish (*Siganus guttatus*) juveniles: Effects of loading density and transport duration on survival and water quality

Michael B. Andam*

Institute of Aquatic and Applied Sciences, Davao del Norte State College, New Visayas, Panabo City, Davao del Norte, Philippines.

Abstract: The golden rabbitfish (*Siganus guttatus*) is increasingly recognized as a promising aquaculture species in the Indo-Pacific region due to its rapid growth, tolerance to salinity fluctuations, high market demand, and consumer preference. However, one critical bottleneck in its culture is the safe and efficient transport of juveniles from hatcheries to grow-out facilities. Grow-out farms are often located far from hatcheries, making the transport of rabbitfish juveniles to grow-out rearing facilities a critical process. Establishing optimal transport conditions of this species for grow-out culture is essential to minimize stress and enhance survival. This study evaluated the effects of three loading densities (300, 400, and 500 fish·bag⁻¹, corresponding to 13.53, 18.06, and 22.59 g·L⁻¹) and two transport durations (10 and 15 h) on the survival and water quality of *S. guttatus* juveniles. Both loading density and travel duration significantly influenced the survival and water quality of rabbitfish juveniles. Dissolved oxygen (DO), pH, and total ammonia nitrogen (TAN) were critical factors that significantly affected survival, with positive correlations for DO and pH and a negative correlation for TAN. The optimal loading densities identified were 400 fish·bag⁻¹ for 10-h and 300 fish·bag⁻¹ for 15-h transport, respectively. These results provide valuable insights for optimizing transport protocols to reduce stress and unnecessary mortalities of *S. guttatus* juveniles during transfer from nursery facilities to grow-out production sites.

Article history:

Received 27 January 2026

Accepted 18 April 2026

Available online 25 June 2026

Keywords:

Handling stress

Packing density

Rabbitfish

Transportation

Introduction

The golden rabbitfish (*Siganus guttatus*) is a commercially important euryhaline fish species widely distributed across the Indo-Pacific region (Duray, 1998; Ayson et al., 2014; Simora et al., 2015). This species inhabits diverse habitats, including estuarine waters, coastal and mangrove areas, seagrasses, and inshore coral reefs (Hara et al., 1986; Ayson et al., 2014; Caballero et al., 2022). Unlike other diurnal rabbitfish, *S. guttatus* is documented to be nocturnal (Ayson et al., 2014; Caballero et al., 2022). In recent years, the promising aquaculture potential of *S. guttatus* has elicited special interest among fish culturists due to its relatively large size, faster growth rate, hardiness, ability to withstand overcrowding, and wide range of tolerance to salinity fluctuations (Hara et al., 1986; Ayson et al., 2014; Caballero et al., 2022). In addition, this low-trophic-

level, euryhaline teleost is renowned for its low dietary protein requirement (35%), exceptional meat quality and nutritional value, and high market value and consumer preference (Parazo, 1990; Quintio and Castor-Sa-an, 2008; Li et al., 2021). Like other rabbitfish, *S. guttatus* is a suitable mariculture species due to its herbivorous-to-omnivorous feeding habits, which allow it to be fed filamentous algae and artificially formulated diets (Andam et al., 2016; Caballero et al., 2022). This highly esteemed food fish can be reared alone at high densities (Andam et al., 2016; Syah et al., 2020) or in polyculture with milkfish in cages, owing to its efficiency in controlling algal growth from net surfaces through grazing (Andam et al., 2016; Caballero et al., 2022). For this reason, polyculture of rabbitfish with milkfish is moving towards a sustainable culture of this species (Andam et al., 2016). Moreover, this species is well accepted

*Correspondence: Michael B. Andam
E-mail: michael.andam@dnc.edu.ph

by consumers and fetches a relatively higher market price than milkfish or tilapia, resulting in increased profit margins (Ayson et al., 2014; Andam et al., 2016). Thus, due to its desirable attributes, *S. guttatus* is a good alternative species to milkfish for grow-out culture (Ayson et al., 2014; Caballero et al., 2022).

Science-based nursery technology of *S. guttatus* has already been established in brackishwater ponds (Andam et al., 2016; Caballero et al., 2022). However, the nursery production of *S. guttatus* juveniles has remained an integral component of grow-out operations. Hatchery and nursery operations for this species are still conducted in land-based facilities, even when grow-out culture is carried out in pond- or sea-based conditions. In most cases, land-based hatcheries or nurseries are situated several miles from the grow-out culture sites (cages, ponds, net pens, etc.) for several famed fish species. As a result, fish post-larvae or juveniles often need to be transported from hatcheries to grow-out farms over long distances, a process that can take several hours to several days (Singh et al., 2004; Stieglitz et al., 2012; Failaman et al., 2022; Li et al., 2023). In such segmented and multifaceted culture operations, packing and transporting fish fry from the collecting grounds or hatcheries to the farm site is a crucial activity in any fish operation (Ayson et al., 1990; Stieglitz et al., 2012; Failaman et al., 2022).

Routine fish culture operations usually involve handling and transporting fish as the final stage in the production cycle (Harmon, 2009). Transporting live fish constitutes a significant portion of the total operational costs incurred by aquaculture enterprises (Lim et al., 2003; Cupp et al., 2017). As a result, large quantities of fish must be transported as efficiently as possible with minimal loss via land, sea, and air freight (Harmon, 2009). However, road transport can be stressful for live fish, as they are confined to limited space and subjected to successive transport-related stressors, including pre-transport fasting and handling, vehicle loading, container transport, and post-transport unloading (Harmon, 2009; King, 2009; Sampaio and Freire, 2016). Fish welfare during live transport is influenced by several factors, including

dissolved oxygen levels, loading density, water quality, and transport duration (Harmon, 2009).

Depending on the species, size, and transport distance/duration, various methods are used to transport young fish. Rabbitfish juveniles are commonly packed in oxygenated plastic bags for transport from land-based hatcheries and nurseries to sea-based grow-out cages. Transporting rabbitfish juveniles from land-based nurseries to grow-out sites is typically employed when the fish reach 45-50 days post-hatching (DPH) at about 2 cm (Ayson et al., 1990, 2014). Transport loading density is a crucial factor to consider when transporting juveniles from the hatchery to any grow-out site (Failaman et al., 2022; Aya et al., 2024; Yang et al., 2024), which is associated with the quantity of nitrogenous waste generated during extended transport (Lim et al., 2003). Increasing loading density can induce considerable physiological stress and disrupt metabolic processes in fish due to deteriorating water quality, which, over time, may result in mortality, particularly during long-hour transport (≥ 8 h) (Stieglitz et al., 2012; Failaman et al., 2022; Li et al., 2023). Conversely, decreasing loading density increases survival rates and significantly raises fuel and freight costs, making transport one of the most expensive parts of the production process. The success of intensive aquaculture operations largely depends on developing strategies to minimize production losses during transport and maximize fish survival in confined spaces (Garcia and Toledo, 1988; Lim et al., 2003; Harmon, 2009; Cupp et al., 2017). Hence, there is a need to establish optimal transport protocols to sustain *S. guttatus* in sea-based grow-out cage culture.

At present, the transportation of good-quality seedstock has become increasingly more important, driven by the gradual expansion of aquaculture. Although transport protocols at various loading densities have already been reported for several species (Stieglitz et al., 2012; Nazari et al., 2015; Cupp et al., 2017; Failaman et al., 2022; Aya et al., 2024), there is limited scientific documentation on the effects of loading densities and prolonged travel duration on the survival and water quality of *S. guttatus* juveniles.

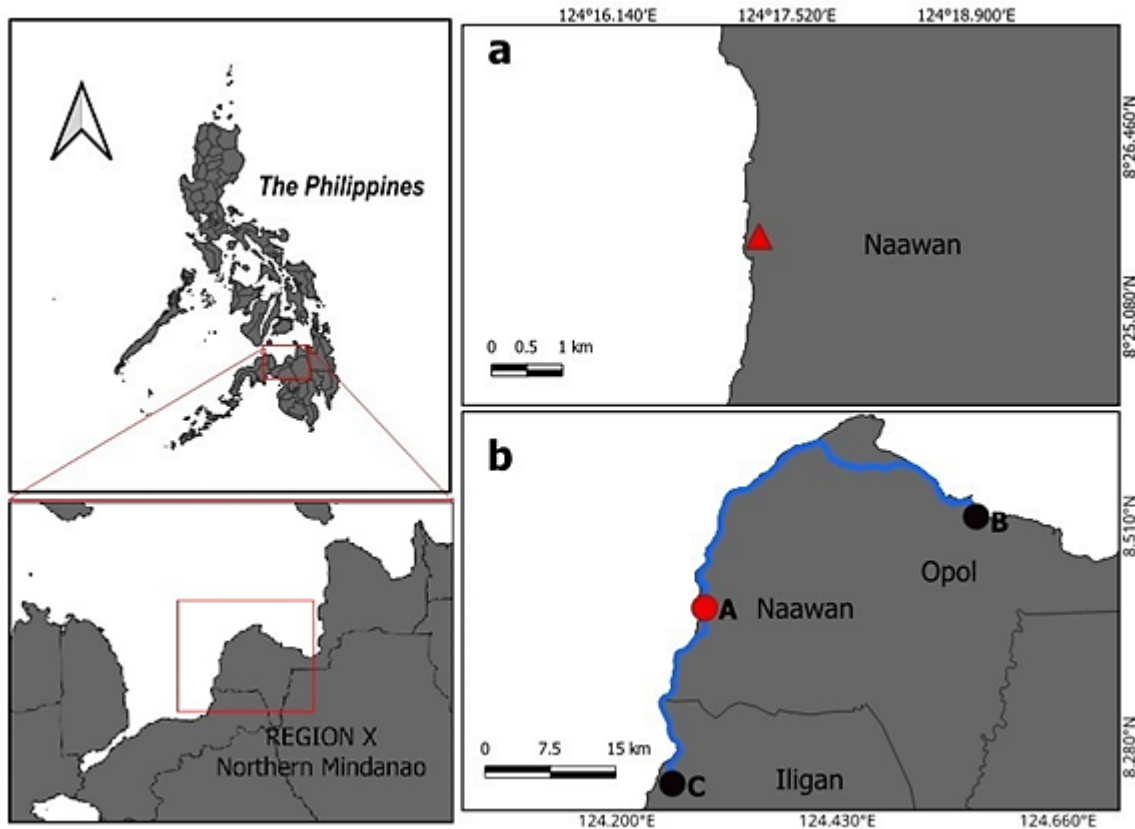


Figure 1. Geographical location and transport route of the *Siganus guttatus* juveniles. (a) Sigamid hatchery of MSU at Naawan, located in Naawan, Misamis Oriental, Philippines, marked by a triangle. (b) Transport route from MSU at Naawan, Misamis Oriental, represented by blue lines.

The only most available transport protocols for *S. guttatus* juveniles are based on conservative loading density of 100 fish \cdot L $^{-1}$ for 8-h transport, 300 fish \cdot L $^{-1}$ for 1-2 h, and 200 fish \cdot L $^{-1}$ for 4 h, respectively (Ayson et al., 1990, 2014). Given the increasing demand for rabbitfish juveniles for grow-out culture, it is essential to determine the optimal loading density to ensure efficient transport while maximizing fish survival. Optimizing juvenile transport density over extended periods (≥ 8 h) to ensure an efficient and sustainable seedstock supply is crucial to the success of mass production of rabbitfish in grow-out systems. So far, no published information has been reported on the effects of high loading density and extended transport duration for *S. guttatus* juveniles. Hence, this study was conducted to establish optimal transport conditions for *S. guttatus* juveniles from hatchery to grow-out production facilities, to minimize stress and ensure optimal fish survival during prolonged closed-system transport in polyethylene bags filled with oxygen.

Materials and Methods

Experimental animal and maintenance: The hatchery-reared *S. guttatus* juveniles (0.18 ± 0.04 g body weight and 2.28 ± 0.16 cm total length) were obtained from the Sigamid Hatchery of Mindanao State University at Naawan (MSU at Naawan)-Hatchery Operation and Services Section, in Naawan, Misamis Oriental, Philippines (Fig. 1). Before the simulated transport experiment, nursery rearing of *S. guttatus* was done from 24 DPH to 50 DPH. The fish were stocked at 0.5 fish \cdot L $^{-1}$ and maintained at 32-35 ppt salinity throughout the nursery rearing period. The fish were provided with continuous aeration to keep dissolved oxygen (DO) levels above 5 ppm. The microalgae (*Chlorella* sp.) were added to and maintained in the tank at $2-3 \times 10^5$ cells \cdot ml $^{-1}$ from 24 to 37 DPH. The rabbitfish were fed with *Brachionus plicatilis* at 5-10 individual \cdot ml $^{-1}$ from 24 DPH to 37 DPH. The fish were also fed with newly hatched *Artemia* nauplii (0.2-0.4 individual \cdot ml $^{-1}$) thrice a day starting at 24 DPH onwards. Weaning of rabbitfish

Table 1. Proximate composition of artificial diets (% dry matter).

| Proximate analysis (% dry matter) | Shrimp feeds (<i>P. monodon</i>) ¹ | Fry mash ² |
|---|---|-----------------------|
| Crude protein | 50 | 40 |
| Crude lipid | 8 | 7 |
| Crude fiber | 3 | 5 |
| Crude ash | 9 | 12 |
| NFE ³ | 30 | 36 |
| Gross energy (KJ·g ⁻¹) ⁴ | 20.14 | 18.38 |

¹BNH Aquaculture Corporation. ²Tatech Malaga Feeds, Santeh Feeds Corporation, Philippines. ³Nitrogen-free extract, computed by difference. ⁴Computed using gross calorific values of 17, 24, and 38 KJ·g⁻¹ for carbohydrate, protein, and lipid, respectively (Jobling, 1993).

Table 2. Summary of conditions used in the simulated transport trial of hatchery-reared golden rabbitfish *Siganus guttatus* juveniles.

| | Treatment 1 | Treatment 2 | Treatment 3 |
|----------------------------------|-------------|-------------|-------------|
| Number of fish·bag ⁻¹ | 300 | 400 | 500 |
| Density (g·L ⁻¹) | 13.53 | 18.06 | 22.59 |
| Transport duration (h) | 10, 15 | 10, 15 | 10, 15 |
| Temperature (°C) | 22-23 | 22-23 | 22-23 |
| Number of replicates (n) | 3 | 3 | 3 |

post-larvae from *Artemia* to an artificial diet was completed once the fish had fully metamorphosed into the juvenile stage. The artificial diet (feed for *P. monodon*) was provided to the fish at 1-2 g·m⁻³·day⁻¹, three times daily, from 24 DPH onwards. In addition, fry mash was provided to the fish from 45 DPH onwards, three times daily (Table 1). To maintain water quality and prevent protozoan infestation in the nursery tank, water management involved draining and siphoning at least 50% of the tank volume, followed by a morning flow-through. The water quality parameters were regularly monitored and maintained within an optimal range conducive to the growth and survival of the rabbitfish (salinity:32-35 ppt, temperature 27-30°C, DO:>5 ppm, pH:7.5-8).

Fish handling, packing, and simulated transport protocol: The rabbitfish juveniles were starved for at least 24 h before packing for the simulated transport experiment to empty their gut and reduce the metabolic waste in the transport bags. Before transport, the fish were harvested from the nursery tank using a fine-meshed seine, acclimated in the 500-L-capacity fiberglass tank at 22-23°C, and transferred into the 12-L-capacity pail, partially filled with the same tank water. Three loading densities (300, 400, and 500 fish·bag⁻¹ corresponding to 13.53, 18.06, and 22.59 g·L⁻¹, respectively) were tested for transport

duration of 10 and 15 h (Table 2), given the growing demand for rabbitfish juveniles among fish growers throughout the Philippines. The fish were scooped and packed into closed, double-lined polyethylene bags (25.4 cm W x 55.88 cm L) with 4 L of cooled, preconditioned seawater (22-23°C; 34-35 ppt). Each replicate transport bag was inflated with medical-grade oxygen until the air space had the same volume as the transport water (1:1; water-to-gas ratio) and sealed tightly with rubber bands. Three replicate oxygenated transport bags containing the rabbitfish juveniles were randomly assigned in the Styrofoam box (dimensions: 55.88 cm L x 38.1 cm W x 43.18 cm H) per transport loading density treatment (Fig. 2). The rabbitfish juveniles were transported late in the afternoon (1633 h) using a multi-cab from MSU-Naawan to Opol, Misamis Oriental, and Vice Versa, then to Iligan City to MSU-Naawan in Naawan, Misamis Oriental, Philippines (Fig. 1). The designated path, spanning roughly 155 km, was repeatedly covered throughout the transport period. Scheduled stops were incorporated to facilitate short breaks of 15-30 min. These pauses were systematically included in the transport protocol to maintain consistency and ensure the reliability of the experimental conditions. The fish were transported for up to 15 h, as this is the estimated travel time from MSU Naawan, Misamis Oriental, to Pangasinan in the Northern Philippines,



Figure 2. The experimental animals were acclimated in the 500-L capacity circular fiberglass tank with a water temperature of 22-23°C (a, b), then transferred into the 12-L capacity pail before packing in the double-lined polyethylene transport bag filled with 4-L of pre-cooled seawater (c, d). The rabbitfish juveniles were packed in transport bags at various loading densities, inflated with oxygen, sealed tightly with rubber bands, and then stored in a Styrofoam box for thermal insulation (e, f).

where rabbitfish culture is widely practiced. Transport duration began when the transport bags containing the experimental animals were completely sealed and stored in the Styrofoam box, and ended after 15 h, the prescribed maximum duration of the study.

Water quality parameters: The initial concentrations of the water quality parameters in the transport water were determined before adding the experimental fish to the transport bags. After transport for each time and loading density per triplicate, the same parameters were measured. Once each bag was opened, dead fish were removed, and then the live ones were conditioned in 1-m³ tanks. DO and pH levels were measured using the Winkler method

(Grasshoff, 1981) and a digital pH meter, respectively. In addition, total ammonia nitrogen (TAN) was determined following the standard method for the examination of water and wastewater (APHA, 1998). **Survival rate determination:** The survival rates of *S. guttatus* juveniles after each simulated transport duration at different loading densities were monitored to assess the animals' responses within their respective transport bags. At the end of the experiment, the influence of different transport loading densities and durations on survival was quantified based on the formula of Wang et al. (2020) as follows: survival (%) = (final number of rabbitfish juveniles survived / initial number of rabbitfish juveniles) x 100.

Table 3. Factorial analysis on the survival and water quality parameters of rabbitfish juveniles confined in closed transport bags at different loading densities (LD) and transport durations (TD). Values (means \pm SE) annotated with different superscripts within the same column are significantly different ($P < 0.05$).

| Treatment | Survival (%) | DO (ppm) | pH | TAN (ppm) |
|-----------------------------------|--------------------------------|------------------------------|------------------------------|------------------------------|
| LD (fish·bag⁻¹) | | | | |
| 300 | 99.61 \pm 0.17 ^a | 5.53 \pm 0.20 ^a | 6.87 \pm 0.07 ^a | 1.60 \pm 0.59 ^a |
| 400 | 83.38 \pm 10.96 ^b | 5.47 \pm 0.14 ^b | 6.64 \pm 0.25 ^b | 1.61 \pm 0.58 ^a |
| 500 | 36.97 \pm 24.70 ^c | 3.70 \pm 1.46 ^c | 5.95 \pm 0.61 ^c | 1.72 \pm 0.68 ^b |
| TD (h) | | | | |
| 10 | 85.26 \pm 2.05 ^a | 5.50 \pm 0.17 ^a | 6.79 \pm 0.12 ^a | 1.03 \pm 0.01 ^a |
| 15 | 61.38 \pm 2.05 ^b | 4.30 \pm 1.03 ^b | 6.17 \pm 0.44 ^b | 2.26 \pm 0.07 ^b |
| LD effect (P value) | 0.000 | 0.000 | 0.000 | 0.000 |
| TD effect (P value) | 0.000 | 0.000 | 0.000 | 0.000 |
| LD x TD (P value) | 0.000 | 0.000 | 0.000 | 0.002 |
| Acceptable level | | >5.0 ¹ | 6.5-9.0 ¹ | <1.0 ² |

¹Water quality requirement for the propagation and growth of fish and other aquatic resources (DENR-DAO, 2016); Boyd (1990); El-Sayed (2006). ²Caballero et al. (2022).

Table 4. Comparison of the mean survival and water quality parameters of rabbitfish juveniles confined in closed transport bags at different loading densities and transport durations. Values (means \pm SE) annotated with different superscripts within the same column are significantly different ($P < 0.05$).

| Duration (h) | Density (fish·bag ⁻¹) | Survival (%) | DO (ppm) | pH | TAN (ppm) |
|-------------------------|-----------------------------------|-------------------------------|------------------------------|------------------------------|---------------------------------|
| 10 | 300 | 99.78 \pm 0.11 ^a | 5.72 \pm 0.03 ^a | 6.94 \pm 0.03 ^a | 1.014 \pm 0.004 ^a |
| | 400 | 94.33 \pm 1.74 ^a | 5.61 \pm 0.05 ^a | 6.88 \pm 0.03 ^a | 1.030 \pm 0.003 ^{ab} |
| | 500 | 61.67 \pm 2.73 ^b | 5.16 \pm 0.05 ^b | 6.56 \pm 0.05 ^b | 1.041 \pm 0.006 ^b |
| P-value | | 0.00001 | 0.0002 | 0.001 | 0.018 |
| 15 | 300 | 99.44 \pm 0.22 ^a | 5.33 \pm 0.03 ^a | 6.80 \pm 0.04 ^a | 2.192 \pm 0.007 ^a |
| | 400 | 72.42 \pm 7.86 ^b | 3.39 \pm 0.04 ^b | 6.39 \pm 0.04 ^b | 2.194 \pm 0.005 ^a |
| | 500 | 12.27 \pm 1.90 ^c | 2.24 \pm 0.04 ^c | 5.33 \pm 0.04 ^c | 2.405 \pm 0.057 ^b |
| P-value | | 0.00003 | 0.0000 | 0.0000 | 0.006 |
| Acceptable level | | | >5.0 ¹ | 6.5-9.0 ¹ | <1.0 ² |

¹Water quality requirement for the propagation and growth of fish and other aquatic resources (DENR-DAO, 2016); Boyd (1990); El-Sayed (2006). ²Caballero et al. (2022).

Statistical analysis: All data were first tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) before analysis. The effects of loading density and transport duration on the survival and water quality parameters of rabbitfish juveniles confined in closed transport bags were analyzed using two-way analysis of variance (ANOVA). When significant interactions were detected, mean values were further examined using one-way ANOVA, followed by Tukey's HSD post hoc test, to determine differences among treatments ($P < 0.05$). Pearson correlation analysis was also conducted to assess the relationships between post-transport water-quality parameters and survival rates. ANOVA and Tukey's HSD tests were performed using IBM SPSS Statistics Version 20 for Windows, while correlation analysis was carried out with PAST Version 5.2.1. All values

were presented as means \pm standard error (SE).

Results

Survival of *S. guttatus* juveniles across loading densities and travel durations: In this study, the survival rate of *S. guttatus* juveniles was significantly influenced by both the loading density and duration of transport. In addition, the interaction between the loading density and duration of transport significantly influenced the survival of *S. guttatus* juveniles (Table 3). Generally, the survival of *S. guttatus* juveniles significantly decreased with increasing transport loading density and travel duration (Tables 3-4). *Siganus guttatus* juveniles confined at 300 fish·bag⁻¹ significantly obtained the highest survival (99%) after 10 to 15 h of simulated transport. However, the survival of rabbitfish juveniles packed from a

transport density of 300 fish.bag⁻¹ was not significantly different from that of the fish confined in 400 fish.bag⁻¹ after 10 h simulated transport duration ($P>0.05$; Table 4). The lowest survival was significantly exhibited by rabbitfish juveniles at 500 fish.bag⁻¹ after 10 to 15 h of confinement ($P<0.05$). Increasing the number of fish per transport bag from 400 to 500 fish.bag⁻¹ led to a significant decrease in survival rates, which dropped from 61.67 to 12.27% after 10 to 15 h of confinement in the transport bags (Table 4).

Water quality conditions across loading densities and travel durations: At the end of the experiment, the results showed that the various loading densities ($P=0.000$) and transport durations ($P=0.000$) significantly influenced the water quality (DO, pH, and TAN) of rabbitfish juveniles (Table 3). In addition, the interactive effects of both loading density and transport duration were significantly observed on the water quality of rabbitfish juveniles ($P=0.000$; Table 3). The water quality parameters across different loading densities and transport durations are presented in Tables 3 and 4. The transport loading densities had a significant influence on DO, pH, and TAN across treatment groups. As loading density increases, DO and pH levels decline, whereas TAN tends to increase with longer travel durations. The influence of transport loading densities on DO was significant after the 10-h transport test. 10 h after packing, a significant decline in DO concentration relative to the initial value was observed in all treatments. Transport densities of 500 fish.bag⁻¹ exhibited significantly lower DO levels than those with a density of 300 and 400 fish.bag⁻¹. Meanwhile, DO concentrations in treatments with transport densities of 300 and 400 fish.bag⁻¹ remained similar throughout this period. However, DO levels were significantly decreased after 15 h of simulated transport ($P<0.05$).

Similarly, a significant decline in water pH levels across all transport densities was observed after 10 h of confinement. The treatment with a transport density of 300 fish.bag⁻¹ recorded the highest pH value of 6.94 ± 0.03 , while the lowest pH of 6.56 ± 0.05 was

noted at a density of 500 fish.bag⁻¹. The pH reduction became more gradual for transport densities of 300, 400, and 500 fish.bag⁻¹ up to the 15th hour. At this point, the transport density of 300 fish.bag⁻¹ significantly exhibited the highest pH of 6.80 ± 0.04 , whereas the 500 fish.bag⁻¹ density had the lowest pH of 5.33 ± 0.04 . Conversely, TAN concentration increased with loading densities and travel durations (Table 4). At the 10th hour of confinement, TAN in 500 fish.bag⁻¹ was significantly higher than that of 300 fish.bag⁻¹. However, TAN in treatments at 400 and 500 fish.bag⁻¹ did not differ significantly during this period. TAN concentrations showed a similar trend across all treatment groups up to the 15th hour of transport.

The relationship between transport water quality parameters (DO, pH, and TAN) and survival rates of *S. guttatus* juveniles at various loading densities over 10 h and 15-h durations is presented in Figure 3. A strong negative correlation was observed between survival rates and loading density ($r = -0.82$), whereas survival rates showed a significant, moderate negative correlation with TAN ($r = -0.47$). These findings suggest that increased loading density and elevated TAN levels tend to reduce survival rates. Furthermore, survival rates exhibited a significant, strong positive correlation with DO ($r = 0.86$) and pH ($r = 0.95$), indicating that higher DO levels and stable pH conditions may enhance survival during extended transport. Additionally, TAN exhibited a very strong positive correlation with transport duration ($r = 0.99$), while it showed negative correlations with both DO ($r = -0.77$) and pH ($r = -0.65$). These results suggest that prolonged transport time, along with associated fluctuations in TAN, DO, and pH, can negatively affect survival. Therefore, these factors must be carefully regulated to improve survival outcomes. These correlations are statistically significant ($P<0.05$), underscoring the importance of managing water quality parameters and loading density during extended transport periods to maximize the survival of *S. guttatus* juveniles. Identifying optimal transport conditions can enhance the reliability of juvenile transport in aquaculture operations, ultimately

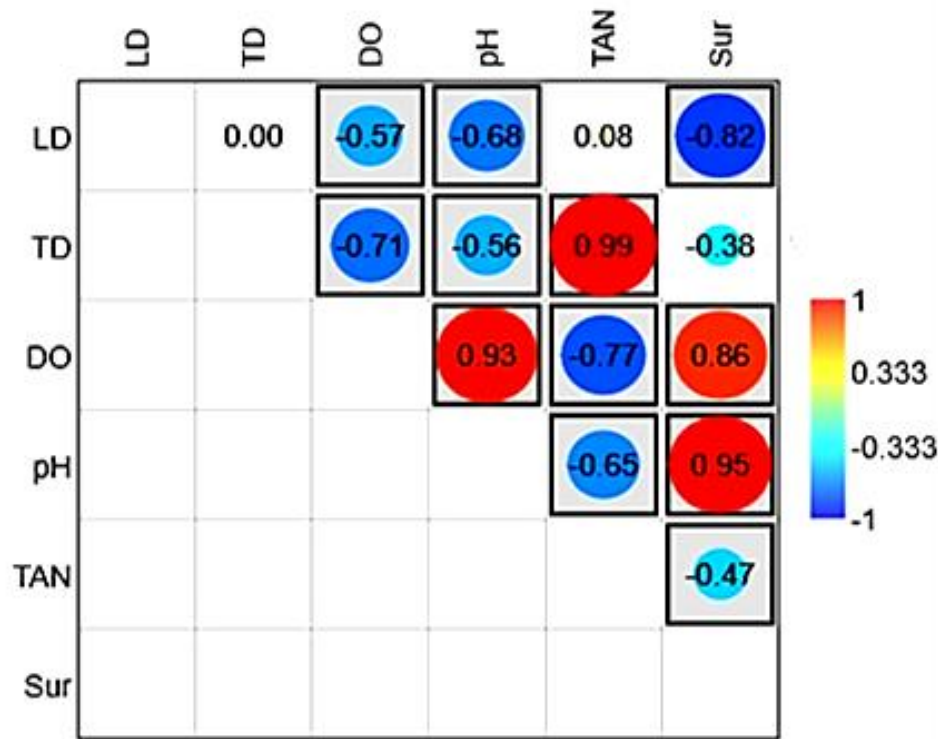


Figure 3. Pearson correlation matrix between water quality parameters and survival of *Siganus guttatus* juveniles at various loading densities and travel durations. Darker circles indicate stronger correlations (i.e., the color intensity ranging from -1 to 1), with red representing positive correlations and blue signifying negative correlations. Boxed values signify a significant correlation ($P < 0.05$). Legend: LD: Loading Density, TD: Travel Duration, DO: Dissolved Oxygen, pH, TAN: Total Ammonia Nitrogen, Sur: Survival Rate.

contributing to more efficient and sustainable management practices for this species.

Discussions

The survival of *S. guttatus* juveniles at various loading densities in closed transport bags has not been previously documented for a long-distance transport duration (15 h). The results of the present study indicated that transport loading density significantly affects the survival of rabbitfish juveniles, regardless of transport duration, with the sharpest decline occurring at the maximum tested density of 500 fish·bag⁻¹. The effect became more pronounced with increasing transport duration, as reflected by a drop in survival rates from 61.67±2.73 (10 h) to 12.27±1.90 (15 h). These results underscore the species' sensitivity to crowding stress, suggesting the importance of considering loading density when transporting rabbitfish juveniles for extended periods. Generally, increasing loading densities and transport duration have resulted in a significant reduction in

survival in the rabbitfish juveniles. A similar finding was reported by Ayson et al. (1990), who documented that increasing the loading density from 100 to 200 fish·L⁻¹ reduced fish survival after 4 and 8 h of transport. Confinement of *S. guttatus* juveniles in transport bags for 10 h at a density of 300 fish·bag⁻¹ resulted in significantly higher survival (99.78±0.11) compared to those fish packed in 500 fish·bag⁻¹ (61.67±2.73). The optimal loading density for high survival after 10 h of simulated transport is 400 fish·bag⁻¹, corresponding to 18.06 g·L⁻¹. However, rabbitfish juveniles confined to 400 fish·bag⁻¹ showed a similar survival trend to those packed at 300 fish·bag⁻¹. These results suggest that rabbitfish juveniles can be transported up to 400 fish·bag⁻¹ for 10-h travel duration from the usual recommendation of a packing density of 100 fish·L⁻¹ for 8-h transport, 300 fish·L⁻¹ for 1-2 h at 28°C and 32 ppt transport water; and 200 fish·L⁻¹ for 4-h transport at 20°C and 20 ppt in a 2.1 cm total length of *S. guttatus* (47 days old) (Ayson et al., 1990; Ayson et al., 2014).

Similarly, confinement of *S. guttatus* juveniles in transport bags for 15 h at a density of 300 fish·bag⁻¹ resulted in significantly higher survival (99.44±0.22) compared to those fish packed in 400 and 500 fish·bag⁻¹ (72.42±7.86 and 12.27±1.90, respectively). The optimum loading density that yields high survival after 15 h of simulated transport is 300 fish·bag⁻¹, corresponding to 13.53 g.L⁻¹. For juvenile Cobia, the optimal transport density during a 24-h live transport is 10-20 g.L⁻¹ (Stieglitz et al., 2012). Similarly, an optimal density of 60 g.L⁻¹ was identified for dusky grouper *Epinephelus marginatus* in an 8-h simulated transport (Pereira-Cardona et al., 2017). In addition, 39.09 g.L⁻¹ was found to be the optimum transport density for juvenile silver therapon *Leiopotherapon plumbeus* up to 12-h simulated transport (Aya et al., 2024). Collectively, these findings suggest that the optimal transport densities may vary across fish species depending on factors such as travel duration. While information on the transport of rabbitfish juveniles remains limited, the current study indicates that the optimal transport biomass is notably higher than that reported for *S. guttatus* juveniles transported over 1-4 h.

Transporting post-larvae or juveniles from hatcheries to grow-out farms is a critical stage in aquaculture. While high stocking densities during transport can lower production costs, excessively high densities deteriorate water quality and compromise the physiological and biochemical functions of the fish (Liu et al., 2022; Yang et al., 2024). Consequently, stocking density is a key factor influencing both fish physiology and water quality, with parameters such as temperature, DO, pH, and ammonia identified as essential indicators that require close monitoring (Luz and Favero, 2024). In closed transport systems, water quality can be a significant stressor, potentially limiting fish health and survival (Ross and Ross, 1999; Emata, 2000). The availability of DO, along with the accumulation of ammonia and carbon dioxide (CO₂), has been recognized as the primary limiting factor (Erikson et al., 1997; Grøttum et al., 1997; Gomes et al., 1999). The decline in survival rates at higher densities and prolonged transport duration is likely

due to accumulated physiological stress and physicochemical stressors, characterized by increased oxygen consumption and rapid buildup of toxic metabolic byproducts, such as ammonia and CO₂ (leading to pH reduction) (Liu et al., 2022; Yang et al., 2024). These variations in water quality affect fish physiological responses during transport, leading to heightened stress and increased mortality (Lim et al., 2003).

The findings of this study indicate that, among the water parameters quantified, DO levels were the primary driver of mortality, limiting transport density and duration. The survival of *S. guttatus* juveniles was significantly affected by low DO concentrations in transport bags, resulting from high loading densities and extended travel durations. As loading density and travel time increased, survival rates declined. This decrease in survival was accompanied by a corresponding drop in DO levels within the transport bags. DO levels after 10 h of transport decreased significantly relative to the initial level and were further reduced after 15 h of simulated transport, suggesting that the observed changes in DO levels were regressive. However, a downward trend in DO values after 15 h of transport can be due to increased fish respiration (Lim et al., 2003; Manliclic et al., 2018). These findings indicate that in a closed system, DO levels progressively decline, while metabolic by-products continuously build up throughout the transport period. An oxygen deficiency may have occurred in the transport bags, with DO concentrations ranging from 2.24 to 3.39 ppm. Under crowded conditions, fish exhibit increased physical activity, which causes rapid DO consumption and further limits DO availability in the transport bags (Lim et al., 2003; Manliclic et al., 2018). *Siganus guttatus* shows signs of distress, such as restlessness and gasping at the water surface, when DO levels drop to 2.24 ppm, indicating severe oxygen depletion (Carumbana and Luchavez, 1979). Prolonged exposure to such conditions often proves fatal. When transported at high loading densities, fish experience mechanical abrasion from frequent contact with one another, which is a significant source of stress (Ross and Ross,

1999). Additionally, frequent collisions among fish in overcrowded environments may lead to physical injuries, further contributing to high mortality rates. DO levels below 3 ppm induce stress in most aquatic organisms, while concentrations <2 ppm are insufficient to sustain fish life. For optimal growth and activity, DO levels should be within the 5-6 ppm range. In the case of *S. guttatus* at transport densities of 300 and 400 fish·bag⁻¹, DO concentrations ranged from 5.61 to 5.72 ppm, exceeding the minimum threshold of 5 ppm required for the conducive growth and propagation of aquatic animals (Boyd, 1990; El-Sayed, 2006; DENR-DAO, 2016; Caballero et al., 2022).

Similarly, a significant decrease in water pH was observed during the longest transport durations at higher loading densities, leading to increased seawater acidity. The significant decline in water pH observed following 10 h of simulated transport in fish confined at higher loading densities can be attributed to the elevated CO₂ concentration from fish respiration, which is recognized as a stressor (Purcell et al., 2006; Parodi et al., 2014; Sutphin and Hueth, 2015; Failaman et al., 2022). The surge in water acidity may lead to gill dysfunction in some marine species (Munday et al., 2009; Martyshev, 2020). However, at 10 and 15 h of transport, pH levels were significantly higher at 300 fish·bag⁻¹ than those of fish confined at 500 fish·bag⁻¹, indicating that less CO₂ was produced in this treatment group (Failaman et al., 2022). These findings suggest that *S. guttatus*, is physiologically constrained in response to environmental variations, especially during extended transport durations.

Metabolic ammonia can accumulate within a closed transport container system and has been identified as a limiting factor in the transport of high-density biomass finfish (Nazari et al., 2015; Sutphin and Hueth, 2015). This study showed that transporting rabbitfish juveniles at high loading densities resulted in higher TAN concentrations. This result suggests that in a closed system, TAN values tend to increase, and that this metabolic by-product accumulates throughout the entire duration of travel. TAN concentrations increased after 10 h of transport

relative to initial levels and further increased after 15 h, suggesting that the observed changes were cumulative. Generally, transport water TAN concentrations increased with increasing loading density and travel duration, and were higher after 15 h of transport than at 10 h. Previous studies documented similar trends of increasing TAN concentrations with prolonged transport duration (Smutná et al., 2002; Cupp et al., 2017; Espinoza-Ramos et al., 2022; Aya et al., 2024). The observed increase in TAN concentration at various loading densities over time is likely due to the accelerated accumulation of metabolic by-products in the transport bag, thus degrading the transport water quality (Randall and Tsui, 2002; Franklin and Edward, 2019; Fang et al., 2023; Aya et al., 2024).

A major factor contributing to fish mortality in transport bags is the accumulation of toxic ammonia (Watson et al., 2010; Barbieri and Bondioli, 2015). An increase in TAN concentrations impairs blood oxygen transport, inducing hypoxia and fish mortality (Zhang et al., 2014). The transport water at the highest loading density appeared slightly more turbid compared to the other density levels. The safe level of TAN concentration for rabbitfish culture in ponds is set at <1.0 ppm to ensure that fish thrive (Caballero et al., 2022). Unionized ammonia concentrations above 1.5 ppm pose a threat to *Penaeus vannamei*, triggering increased excretion, elevated blood pH, and gill damage, ultimately hindering their ability to absorb oxygen effectively (Rapiz et al., 2025). A previous study found that early juveniles of *L. plumbeus* could withstand TAN concentrations of up to 1 ppm (Aya et al., 2021), exceeding the generally accepted range of 0-0.5 ppm (Boyd, 1990; El-Sayed, 2006). The TAN concentrations obtained in the study exceeded the recommended thresholds for rabbitfish juveniles to thrive. Moreover, the highest TAN concentrations at the maximum loading density coincided with the lowest recorded pH of 5.33±0.04 in the transport water, as documented in previous studies (Crosby et al., 2011; Espinoza-Ramos et al., 2022). This suggests that the drop in pH values may be attributed to the increased excretion of nitrogenous compounds, with a

significant portion of TAN present as NH_4^+ , along with CO_2 produced as metabolic and respiratory by-products in fish (Randall and Wright, 1989; Watson et al., 2010; Thorarensen and Farrell, 2011). Consequently, an increase in waste metabolite concentrations is likely linked to both the loading density and the transport duration for this species (Lim et al., 2003).

Conclusion

This study aimed to investigate how different transport loading densities and travel durations affect the survival and water quality during the simulated transport of *S. guttatus* juveniles. This study is the first to explore the effects of high loading densities on the survival of juvenile rabbitfish over an extended 15-h period under simulated transport conditions. The survival rates of *S. guttatus* juveniles were significantly influenced by both loading density and transport duration. Key water quality parameters, including DO, pH, and TAN, were critical factors affecting the survival of *S. guttatus* juveniles. These parameters varied with loading density and transport duration, with lower DO and pH and higher TAN observed at a density of 500 fish.bag⁻¹. The optimal loading densities were determined to be 400 fish.bag⁻¹ (18.06 g.L⁻¹) for 10-h and 300 fish.bag⁻¹ (13.53 g.L⁻¹) for 15-h transport duration. These results provide valuable insights for efficiently transporting *S. guttatus* juveniles from nursery facilities to grow-out production sites. The findings of this study will provide valuable information to help nursery operators effectively transport juveniles to grow-out production farms while ensuring high post-transport survival rates. However, further studies are required to confirm these results in actual fish transport conditions across both wet and dry seasons.

Acknowledgments

The author would like to express his sincere appreciation to the Department of Science and Technology (DOST)–Science Education Institute (SEI)–Accelerated Science and Technology Human Resource Development Program (ASTHRDP) for

their financial support. Deepest gratitude is also extended to the technical staff of the MSU at Naawan Research and Extension Division in Naawan, Misamis Oriental, Philippines, for their invaluable assistance, including the provision of materials, personnel support, and technical guidance throughout this study. The author further acknowledges the technical assistance and invaluable contribution of Dr. Jocelyn N. Gorospe, which enabled the completion of this study. This research was conducted as part of the author's course requirements for the MS in Aquaculture program. The author is also deeply grateful to Mr. Rustan C. Eballe and Mr. Frank E. Rodrigora for their help in counting and packing the rabbitfish juveniles.

Ethics statement: Ethical review and approval were not required for this study involving fish, as it adheres to internationally accepted ethical standards, local legislation, and institutional requirements for the humane handling and treatment of aquatic animals in research. All experimental procedures were conducted in accordance with the Philippine National Standard (PNS) on the Code of Good Aquaculture Practices (GAqP) (PNS/BAFS 135:2014; BAFS, 2020). Rearing, handling, and animal welfare protocols were strictly followed in accordance with the ethical standards set forth in the Philippine Republic Act Number 8485, otherwise known as the Animal Welfare Act of 1998. Moreover, the study adhered to standard protocols to safeguard animals from unnecessary harm or distress (American Psychological Association, 1986).

Funding information: This study was supported and funded by the Department of Science and Technology (DOST)–Science Education Institute (SEI)–Accelerated Science and Technology Human Resource Development Program (ASTHRDP).

References

- American Psychological Association. (1986). Guidelines for ethical conduct in the care and use of animals. *Journal of the Experimental Analysis of Behavior*, 45(2): 127.
- Andam M.B., Lumasag G.J., Gorospe J.N., Gorospe J.G.

- (2016). Pond based juvenile production of siganids: growth performance of 30-day post hatch *Siganus guttatus* (Bloch, 1787) at three stocking densities. *Journal of Environment and Aquatic Resources*, 4: 75-93.
- APHA (American Public Health Association). (1998). Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, New York, USA.
- Aya F.A., Gutierrez R.C., Garcia L.M. (2024). Simulated transport of silver therapon (*Leiopotherapon plumbeus*) fingerlings at different durations and loading densities: Effects on survival and water quality. *The Philippine Agricultural Scientist*, 107(2): 8.
- Aya F.A., Nillasca V.S.N., Garcia L.M.B. (2021). Improved survival and growth of silver therapon *Leiopotherapon plumbeus* early juveniles through co-feeding with *Artemia* and commercial feeds. *Journal of Applied Ichthyology*, 37(6): 925-931.
- Ayson F.G., Parazo M.M., Reyes Jr, D.M. (1990). Survival of young rabbitfish (*Siganus guttatus* Bloch) under simulated transport conditions. *Journal of Applied Ichthyology*, 6(3): 161-166.
- Ayson F.G., Reyes O.S., de Jesus-Ayson E.G.T. (2014). Seed production of rabbitfish *Siganus guttatus*. Aquaculture Department, Southeast Asian Fisheries Development Center.
- BAFS (Bureau of Agriculture and Fisheries Standards). (2020). Philippine national standards code of good aquaculture practices (PNS/BAFS 135:2014). Bureau of Agriculture and Fisheries Standards, Quezon City, Philippines.
- Barbieri E., Bondioli A.C.V. (2015). Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. *Aquaculture Research*, 46(3): 565-571.
- Boyd C.E. (1990). *Water quality in ponds for aquaculture*. Birmingham, Alabama, Auburn University Press. 482 p.
- Caballero P.A., Coniza E.B., Dayrit R. (2022). Nursery and grow-out culture of rabbitfish *Siganus guttatus* in brackishwater ponds. Aquaculture Department, Southeast Asian Fisheries Development Center.
- Carumbana E.E., Luchavez J.A. (1979). Oxygen consumption and short term effects of reduction in salinity and dissolved oxygen concentration on *Siganus canaliculatus*, *S. spinus* and *S. guttatus* reared under laboratory conditions. *Silliman Journal*, 26: 172-185.
- Crosby T.C., Hill J.E., Martinez C.V., Watson C.A., Yanong R.P. (2006). On-farm transport of ornamental fish: FA-119/FA119, 11/2006. EDIS, 2006(33).
- Cupp A.R., Fredricks K.T., Porcher S.T., Smerud J.R., Hartleb C.F., Gaikowski M.P. (2017). Survival and behavioural responses of cool and warm water fish sedated with AQUI-S® 20E (10% eugenol) at high loading densities. *Aquaculture Research*, 48(4): 1576-1589.
- Cupp A.R., Schreier T.M., Schleis S.M. (2017). Live transport of Yellow Perch and Nile Tilapia in AQUI-S 20E (10% eugenol) at high loading densities. *North American Journal of Aquaculture*, 79(2): 176-182.
- DENR-DAO. (2016). Administrative Order No. 08, Series of 2016: Water Quality Guidelines and General effluents Standards 2016. Department of Environment and Natural Resources, Visayas Avenue, Diliman, Quezon City, Philippines. 25 p.
- Duray M.N. (1998). *Biology and culture of siganids*. Aquaculture Department, Southeast Asian Fisheries Development Center.
- El-Sayed A.F.M. (2006). *Tilapia culture*. CABI publishing CABI International Willingford. Oxfordshire, UK, 24(3): 276-278.
- Emata A.C. (2000). Live Transport of pond-reared milkfish *Chanos chanos* Broodstock. *Journal of the World Aquaculture Society*, 31(2): 279-282.
- Erikson U., Sigholt T., Seland A. (1997). Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture*, 149(3-4): 243-252.
- Espinoza-Ramos L.A., Pepe-Victoriano R., Huanacuni J.I., Nande M. (2022). Effect of transportation time and stocking density on seawater quality and survival of *Anisotremus scapularis* (Perciformes: Haemulidae). *Journal of the World Aquaculture Society*, 53(5): 1042-1050.
- Failaman A.N., Traifalgar R.F.M., Corre Jr, V.L. (2022). Survival of nursery-reared juvenile milkfish, *Chanos chanos*, at different transport density and temperature. *Journal of Applied Aquaculture*, 34(4): 938-952.
- Fang D., Mei J., Xie J., Qiu W. (2023). The effects of transport stress (temperature and vibration) on blood biochemical parameters, oxidative stress, and gill histomorphology of pearl gentian groupers. *Fishes*, 8(4): 218.
- Franklin D.A., Edward L. (2019). Ammonia toxicity and adaptive response in marine fishes. *Indian Journal of Geo-Marine Sciences (IJMS)*, 48(3): 273-279.

- Garcia L.M.B., Toledo J.D. (1988). Critical factors influencing survival and hatching of milkfish (*Chanos chanos* Forsskal) eggs during simulated transport. *Aquaculture*, 72(1-2): 85-93.
- Gomes L.C., Golombieski J.I., Chippari-Gomes A.R., Baldisserotto B. (1999). Effect of salt in the water for transport on survival and on Na⁺ and K⁺ body levels of silver catfish, *Rhamdia quelen*, fingerlings. *Journal of Applied Aquaculture*, 9(4): 1-9.
- Grasshoff K. (1981). *Marine Electrochemistry*. M. Whitfield, D. Jagner (Eds.). John Wiley and Sons. pp: 327-420.
- Grøttum J.A., Staurnes M., Sigholt T. (1997). Effect of oxygenation, aeration and pH control on water quality and survival of turbot, *Scophthalmus maximus* (L.), kept at high densities during transport. *Aquaculture Research*, 28(2): 159-164.
- Hara S., Kohno H., Taki Y. (1986). Spawning behavior and early life history of the rabbitfish, *Siganus guttatus*, in the laboratory. *Aquaculture*, 59(3-4): 273-285.
- Harmon T.S. (2009). Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture*, 1(1): 58-66.
- Jobling M. (1993). Bioenergetics: feed intake and energy partitioning. In: J.C. Rankin, F.B. Jensen (Eds.) *Fish Ecophysiology*. Chapman and Hall Fish and Fisheries Series, vol 9. Springer, Dordrecht.
- King H.R. (2009). Fish transport in the aquaculture sector: An overview of the road transport of Atlantic salmon in Tasmania. *Journal of Veterinary Behavior*, 4(4): 163-168.
- Li J., Guo Y., Zhao X., Zhou S., Ma, Z., Yu G., ... Wang X. (2023). The effects of vibration frequency on oxidative stress, digestive enzymes and ATPases of Crimson Snapper (*Lutjanus erythropterus*) fry during transport. *Fishes*, 8(12): 603.
- Li L., Wang C., Olsen R.H., Li X., Meng H., Xu L., Shi L. (2021). Characterization of a Streptococcus species isolated from *Siganus guttatus* in South China. *Aquaculture*, 545: 737163.
- Lim L.C., Dhert P., Sorgeloos P. (2003). Recent developments and improvements in ornamental fish packaging systems for air transport. *Aquaculture Research*, 34(11): 923-935.
- Liu H., Fu Z., Yu G., Ma Z., Fu Z. (2022). Effect of transport density on greater amberjack (*Seriola dumerili*) stress, metabolism, antioxidant capacity and immunity. *Frontiers in Marine Science*, 9: 931816.
- Luz R.K., Favero G.C. (2024). Use of salt, anesthetics, and stocking density in transport of live fish: A review. *Fishes*, 9(7): 286.
- Manliclic A.D.C., Corpuz M.N.C., Vera Cruz E.M. (2018). Optimum conditioning period before packing, salt-treated water, and blue background color improved the survival of Nile tilapia (*Oreochromis niloticus* L.) fingerlings during transport. *Philippine Agricultural Scientist*, 101(1): 76-83.
- Martyshev F.G. (2020). Transport of live fish. In: *Pond fisheries*. London, CRC Press, UK. pp: 419-430.
- Munday P.L., Donelson J.M., Dixson D.L., Endo G.G. (2009). Effects of ocean acidification on the early life history of a tropical marine fish. *Proceedings of the Royal Society B: Biological Sciences*, 276(1671): 3275-3283.
- Nazari T., Yavari V., Salati A.P., Movahedinia A. (2015). Effect of density on some physiological responses to transportation stress in *Mesopotamichthys sharpeyi* (Günther 1874) fingerlings. *International Journal of Aquatic Biology*, 3(5): 331-338.
- Parazo M.M. (1990). Effect of dietary protein and energy level on growth, protein utilization and carcass composition of rabbitfish, *Siganus guttatus*. *Aquaculture*, 86(1): 41-49.
- Parodi T.V., Cunha M.A., Becker A.G., Zeppenfeld C.C., Martins D.I., Koakoski G., ... Baldisserotto B. (2014). Anesthetic activity of the essential oil of *Aloysia triphylla* and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, *Rhamdia quelen*. *Fish Physiology and Biochemistry*, 40(2): 323-334.
- Pereira-Cardona P.M., Lisboa V., Barbas L.A.L., Robaldo R.B. (2017). Transport of juvenile dusky grouper *Epinephelus marginatus* under different packing densities: metabolic and haematological responses. *Aquaculture Research*, 48(10): 5356-5362.
- Purcell S.W., Blockmans B.F., Agudo N.N. (2006). Transportation methods for restocking of juvenile sea cucumber, *Holothuria scabra*. *Aquaculture*, 251(2-4): 238-244.
- Quinitio G.F., Sa'an C. (2008). Development of the gastrointestinal tract and associated organs of the rabbitfish *Siganus guttatus* (Bloch) larvae. *UPV Journal of Natural Science*, 13: 133-148.
- Randall D.J., Tsui T.K.N. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin*, 45(1-12): 17-23.

- Randall D.J., Wright P.A. (1989). The interaction between carbon dioxide and ammonia excretion and water pH in fish. *Canadian Journal of Zoology*, 67(12): 2936-2942.
- Rapiz F.G.B., Verzosa R.C., Nemenzo-Calica P., Clapano M.B. (2025). Physico-chemical analysis of water in *Litopenaeus vannamei* ponds in Dahican, City of Mati, Davao Oriental, the Philippines. *Philippine Journal of Science*, 154(1): 135-147.
- Ross L.G., Ross B. (1999). Anaesthetic and sedative techniques for aquatic animals. John Wiley and Sons. 159 p.
- Sampaio F.D., Freire C.A. (2016). An overview of stress physiology of fish transport: Changes in water quality as a function of transport duration. *Fish and Fisheries*, 17(4): 1055-1072.
- Simora R.M.C., Traifalgar R.F.M., Legario F.S. (2015). Characterization of extracellular enzymes from culturable autochthonous gut bacteria in rabbitfish (*Siganus guttatus*). *ELBA Bioflux*, 7(1): 67-76.
- Singh R.K., Vartak V.R., Balange A.K., Ghughuskar M.M. (2004). Water quality management during transportation of fry of Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). *Aquaculture*, 235(1-4): 297-302.
- Smutna M., Vorlova L., Svobodova Z. (2002). Pathobiochemistry of ammonia in the internal environment of fish. *Acta Veterinaria Brno*, 71(2): 169-181.
- Stieglitz J.D., Benetti D.D., Serafy J.E. (2012). Optimizing transport of live juvenile cobia (*Rachycentron canadum*): effects of salinity and shipping biomass. *Aquaculture*, 364: 293-297.
- Sutphin Z.A., Hueth C.D. (2015). Effects of loading density during transport on physiological stress and survival of Sacramento-San Joaquin Delta fishes. *California Fish and Game*, 101(2): 108-30.
- Syah R., Tampangallo B.R., Undu M.C., Asaad A.I.J., Laining A. (2020). Rabbitfish (*Siganus guttatus*) culture in floating net cage with different stocking densities. *IOP Conference Series: Earth and Environmental Science*, 564(1): 012022.
- Thorarensen H., Farrell A.P. (2011). The biological requirements for post-smolt Atlantic salmon in closed-containment systems. *Aquaculture*, 312(1-4): 1-14.
- Wang W., Zhang Y., Liu Y., Adányi N., Zhang X. (2020). Effects of waterless live transportation on survivability, physiological responses and flesh quality in Chinese farmed sturgeon (*Acipenser schrenckii*). *Aquaculture*, 518: 734834.
- Watson C., Kilgore K.H., Martinez C. (2010). Shipping fish in boxes. *South Regional Aquaculture Center*. 3903: 1-9.
- Yang Y., Narayan E., Rey Planellas S., Phillips C.J., Zheng L., Xu B., ... Descovich K. (2024). Effects of stocking density during simulated transport on physiology and behavior of largemouth bass (*Micropterus salmoides*). *Journal of the World Aquaculture Society*, 55(2): e13054.
- Zhang Y., Wang W., Yan L., Glamuzina B., Zhang X. (2019). Development and evaluation of an intelligent traceability system for waterless live fish transportation. *Food control*, 95: 283-297.