# Juvenile mangrove red snapper (*Lutjanus argentimaculatus*) is euryhaline but utilizes feed better in seawater than in brackish water

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**ABSTRACT**: The mangrove red snapper (*Lutjanus argentimaculatus*) inhabits seawater and brackish water environments, but the optimal salinity for rearing the species in aquaculture systems has never been assessed. Here, triplicate groups of juvenile *L. argentimaculatus* (9.16–9.17 g body weight) were reared in various salinities (0, 7.5, 15, 22.5, and 30‰) for eight weeks; and growth, feed utilization, digestive enzyme activities, muscle quality, and whole-body composition were investigated at the end of trial. Food rejection and mortality gradually increased over the first three weeks among fish reared in 0 and 7.5‰ treatments; while the growth performances of the other three remaining treatments were similar, with a specific growth rates of 1.78–2.08% body weight/day, $p > 0.05$. However, feed conversion ratio of the fish reared in 30‰ treatment, which was 4.12 g feed/g gain, was significantly ($p < 0.05$) lower than those of the fish reared in 15 and 22.5‰ treatments, which were 5.31 and 5.16 g feed/g gain, respectively. In addition, it was observed that digestive enzyme activities, muscle quality, and whole-body composition were not affected by the different levels of water salinity. These findings confirm the euryhaline characteristics of juvenile mangrove red snapper, and the observed feed conversion ratios support rearing this species in a salinity of 30‰.

**KEYWORDS**: feed utilization, *Lutjanus* sp., mangrove jack, muscle quality, whole-body composition

## INTRODUCTION

The mangrove red snapper (*Lutjanus argentimaculatus*) is an economically important edible fish found in the waters of Asian countries, which include Thailand, Malaysia, Singapore, and the Philippines; and Australia [1]. The species is also naturally distributed in the Atlantic on the coastal shelf from Massachusetts to Florida and around the Gulf of Mexico [2, 3]. In areas where the species is available, market demand and prices are high; and in Southeast Asia, aquaculture production of the fish is the highest [4].

The mangrove red snapper is a small fish classified as carnivorous. Adults of the species spawn at deep offshore reefs [5, 6], and the newly hatched larvae spend several weeks in the plankton before settling in brackish coastal waterways, around mangrove forests, or estuaries [7, 8]. A number of reports have confirmed the presence of larvae and juveniles of this fish in estuaries, coastal areas, and freshwater [6, 9–12]. Although, the breadth of range is a characteristic of a euryhaline species [13], the tolerance of mangrove red snapper to hyper- and hypo-salinity could vary with its development.

Newly hatched mangrove red snapper larvae have been reported to tolerate abrupt salinity changes from 32‰ to 8, 16, 24, and 40‰ [13]. A growth trial conducted by Chi and True [9] investigated tolerances of 10, 17, and 25‰.

However, short-term observations of fish of various sizes and ages have been made without reference to feed utilization or existing records, and have not matched the long-term observations from aquaculture production. Therefore, the present study aimed to determine the optimal salinity for rearing juvenile mangrove red snapper. A growth trial covering freshwater, brackish, and seawater salinities was formulated with reference to available documents. To match local conditions, a maximum salinity of 30‰, the approximate salinity of seawater in Thailand, was chosen [14]. The effects of various levels of water salinity were observed on fish performance, including survival, growth, feed utilization, digestive enzyme activities, muscle quality, and whole-body composition. The findings from this study would support Thailand's decision makers on aquaculture production.

## MATERIALS AND METHODS

### Animal protocols

This project was conducted at Phang-Nga Coastal Fisheries Research and Development Center, under the regulation of the Department of Fisheries. All the animal protocols conformed to the "Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes", National Research Council, Thailand (Application No. U1-06514-2560).

### Fish trial

Juvenile mangrove red snapper was provided by Phang-Nga Coastal Fisheries Research and Development Center, Phang-Nga, Thailand. Before starting the eight-week growth trial, the fish were acclimatized for two weeks in a 500 l cylindrical plastic tank (1.8 m diameter × 0.8 m height). The acclimatized fish were screened and individuals of uniform size ($n = 150$, 9.16–9.17 g body weight) were selected and randomly distributed into five alternative treatments. Each treatment was conducted in triplicate aquaria (49 cm wide × 50 cm long × 38 cm high, containing 70 l of seawater) containing ten fish each. The fish were reared in diluted natural seawater adjusted to treatments of 0, 7.5, 15, 22.5, and 30‰ salinity levels. The water for each treatment was prepared by serially diluting the natural seawater and, then, dechlorinating the diluted seawater by aeration with air pumps.

Twice daily, at 09.00 h and 15.00 h, the fish were fed *ad libitum* a floating diet for marine carnivorous fish (Charoen Pokphand PCL., Bangkok, Thailand). The feed comprised > 42% crude protein, > 6% crude lipid, < 4% crude fiber, and < 12% moisture. Hand feeding was used throughout the experiment. Uneaten feed was collected 30 min after feeding, oven-dried at 60 °C for 48 h, and weighed. After deducting the weight of dried uneaten feed, the weight of consumed feed was used to calculate feed conversion ratios (FCR). Eighty percent of the water in every aquarium was changed twice daily, and the water quality parameter ranges during the trial were pH, 7.03–8.32; temperature, 25–28 °C; and dissolved oxygen, 5.40–8.61 mg/l.

Mortality was recorded daily. At the end of the trial, all the fish were fasted for 24 h and, then, sacrificed by chilling in ice. The body weight (BW) and total length of all the fish were recorded. The BW and the total length values were used to calculate weight gain (WG), condition factor (CF), and specific growth rate (SGR). The viscera were carefully removed; stomach, intestine, and liver were identified and weighed; and the values were used for the calculation of stomasomatic index (SSI), intestosomatic index (ISI), and hepatosomatic index (HSI). Stomachs and intestines of three fish from each tank were used in the analysis of digestive enzyme activities. Epaxial white muscle ($n = 3$ per tank) from below the dorsal fin was used for muscle quality analysis. The remaining fish ($n = 4$) from each tank were used for the whole-body composition study.

Survival, growth performance and feed conversion parameters were calculated as follows:

$$\text{Survival (\%)} = 100 \times \frac{\text{final fish number}}{\text{initial fish number}},$$
$$\text{WG (g)} = \text{final BW (g)} - \text{initial BW (g)},$$
$$\text{CF} = 100 \times \frac{\text{BW (g)}}{\text{total length (cm)}^3},$$
$$\text{SGR (\% BW/day)} = 100 \times \frac{\ln W_t - \ln W_0}{t - t_0},$$

where $W_t$ = mean BW (g) at day t, $W_0$ = mean BW (g) at day $t_0$,

$$\text{FCR (g feed/g gain)} = \frac{\text{dry feed consumed (g)}}{\text{wet WG (g)}},$$
$$\text{SSI (\%)} = 100 \times \frac{\text{wet weight of stomach (g)}}{\text{BW (g)}},$$
$$\text{ISI (\%)} = 100 \times \frac{\text{wet weight of intestine (g)}}{\text{BW (g)}},$$
$$\text{and} \quad \text{HSI (\%)} = 100 \times \frac{\text{wet weight of liver (g)}}{\text{BW (g)}}.$$

### Digestive enzyme activity

Stomach and intestine samples were weighed and immediately homogenized in cold 0.2 M $Na_2HPO_4$-$NaH_2PO_4$ buffer (pH 8) at a 1:3 (w/v) ratio in a tissue homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenate was centrifuged at $15,000 \times g$ for 30 min at 4 °C. Supernatants were collected and kept at $-20$ °C. Following the method described by Lowry et al [15], protein concentrations in the crude extracts were assayed using bovine serum albumin (BSA) as the standard.

Pepsin activity was determined at pH 2 at 40 °C using a hemoglobin substrate according to the method of Worthington [16]. One enzyme unit was defined based on an increase of 1.0 in absorbance at 280 nm. Trypsin (pH 9 at 50 °C) and chymotrypsin (pH 7 at 50 °C) activities were assayed using *N*-benzoyl-*L*-Arg-*p*-nitroanilide and *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide as the respective substrates, following the protocols described by Rungruangsak-Torrisen et al [17]. In both assays, liberated *p*-nitroanilide was determined at 410 nm. Lipase activity was determined at pH 7.5 at 30 °C using *p*-nitrophenyl palmitate as the substrate, according to the protocol described by Winkler and Stuckmann [18]. Liberated *p*-nitrophenol was spectrophotometrically quantified at 410 nm against the linear range of the standard. Alpha-amylase activity was assayed at pH 9 at 45 °C using a soluble starch substrate [19]. Liberated maltose was quantified by spectrophotometry at 540 nm. One unit of trypsin, chymotrypsin, lipase, and amylase

was defined as the amount catalyzing the conversion of 1 μmol of substrate per minute under the specified condition.

### Muscle quality

#### *Protein synthesis capacity*

Each frozen muscle sample (approximately 50 mg) was weighed and sonicated in 1 ml of TRIzol® reagent (Invitrogen, Carlsbad CA, USA). The sonicated muscle was mixed with 200 μl of chloroform and centrifuged at $5,000 \times g$ for 10 min at 4 °C to separate RNA (upper aqueous phase) and protein (lower organic phase) layers. To precipitate RNA and protein, aliquots of approximately 200 μl of each layer were mixed with 1 ml of isopropanol, washed with 90% ethanol, and then dried in a hot-air oven at 55 °C for 20 min. The dried samples of RNA and protein were dissolved in 1 ml of 0.1 M sodium acetate (pH 5) and 1 ml of 1% sodium dodecyl sulfate (SDS) before spectrophotometrically measuring absorbances at 260 and 280 nm, respectively. The quantification of RNA and protein was based on their extinction coefficients of $E_{260} = 40$ μg RNA/ml and $E_{280} = 2.1$ mg protein/ml, after comparing to standards of yeast RNA dissolved in 0.1 M sodium acetate buffer (pH 5) and BSA dissolved in 1% SDS, respectively [20].

#### *Actin and myosin determination*

Based on protein denaturation temperature, a differential scanning calorimeter (DSC7, Perkin Elmer, Waltham, Massachusetts, USA) was used to determine denaturation enthalpy ($\Delta$H) of myosin and actin [21]. Approximately 10 mg of white muscle were placed in an aluminum pan sealed with an aluminum lid; and the pan was placed in a calorimeter and heated from 20 to 120 °C at a rate of 5 °C/min in 20 ml/min of $N_2$. Peaks of individual proteins were identified according to Schubring [22]; and onset ($T_o$), peak ($T_p$), and conclusion ($T_c$) temperatures were labelled from thermograms. Native myosin and actin contents were determined from the amount of energy used to completely denature each protein within a specific temperature range, and the values were automatically converted from the areas under peaks in the thermograms.

### Whole-body composition

Moisture, crude protein, crude lipid, and crude ash from the whole-body of reared fish were determined according to the methods described by the AOAC [23]. Gravimetric analysis of moisture was determined using oven-dried sample (WOF-155; Wisd Laboratory Instruments, Wertheim, Germany) at 105 °C for 24 h. The Kjeldahl analyzer (Kjeltec™ 8100; Foss, Höganäs, Sweden) and Soxhlet extraction unit (Soxtec™ 8000; Foss, Suzhou, China) were used for crude protein and

crude lipid analyses, respectively. Crude ash was gravimetrically determined using sample burned at 600 °C for 2 h in a muffle furnace (E30-HT; Thai Furnaces Engineering, Lampang, Thailand). All values were expressed as % of wet weight.

### Statistical analysis

This study followed a completely randomized design with triplicate observations and ten fish in each group. The data were analyzed using the Statistics Package for the Social Science (SPSS) Version 17 (SPSS Inc., Chicago, USA). Data were expressed as the tank means ± standard error of means (SEM). Differences between means were evaluated by one-way analysis of variance followed by Duncan's multiple range test ($p < 0.05$) as a *post hoc* test.

## RESULTS

### Survival, growth, and feed utilization

All fish in 0 and 7.5‰ treatments slowly died within the first three weeks of the trial, while survival in the other three treatments was 96.7 to 100% at the end of trial ($p > 0.05$, Table 1). Among the 15, 22.5, and 30‰ treatments, growth performances (final weight, WG, total length, CF, and SGR) and organ indexes (SSI, ISI, and HSI) did not differ ($p > 0.05$). Superior feed utilization characteristics, indicated by lower FCRs, were observed in fish reared in 30‰ salinity compared with the 15 and 22.5‰ ($p < 0.05$).

### Digestive enzyme specific activities

There were no differences in specific activities of pepsin, trypsin, chymotrypsin, lipase, and amylase among the fish reared in 15, 22.5, and 30‰ treatments ($p > 0.05$, Table 2).

### Protein synthesis capacity in white muscle

RNA concentrations, protein concentrations, and RNA/protein ratios (μg/mg) in fish white muscle did not differ between the 15, 22.5, and 30‰ treatments ($p > 0.05$, Table 3).

### Myosin and actin in white muscle

The major muscle proteins in mangrove red snapper were first characterized using DSC. The $T_o$, $T_p$, and $T_c$ of muscle myosin were in the ranges (min–max) of 42.4–46.6 °C, 48.9–51.9 °C, and 52.9–58.4 °C, respectively. The respective ranges for actin were 65.7–67.7 °C, 70.3–71.9 °C, and 72.9–74.4 °C (Fig. 1). Based on enthalpy values, the amounts of native form myosin and actin in white muscle were not significantly different across the 15, 22.5, and 30‰ treatments ($p > 0.05$, Table 3).

**Table 1** Survival, growth performance, and feed utilization parameters of juvenile red snapper (*L. argentimaculatus*) reared in different water salinities for eight weeks.

| Parameter | Salinity level (‰) | | | | | $p$-value |
|---|---|---|---|---|---|---|
| | 0 | 7.5 | 15 | 22.5 | 30 | |
| Survival (%) | $0 \pm 0^b$ | $0 \pm 0^b$ | $100 \pm 0^a$ | $100 \pm 0^a$ | $96.7 \pm 3.3^a$ | < 0.001 |
| Final weight (g) | – | – | $25.8 \pm 1.3$ | $26.6 \pm 1.0$ | $30.7 \pm 1.5$ | 0.066 |
| Total length (cm) | – | – | $10.5 \pm 0.1$ | $10.4 \pm 0.1$ | $10.9 \pm 0.1$ | 0.235 |
| WG (g) | – | – | $24.8 \pm 1.3$ | $25.7 \pm 1.0$ | $29.8 \pm 1.5$ | 0.066 |
| CF | – | – | $2.23 \pm 0.02$ | $2.35 \pm 0.02$ | $2.38 \pm 0.07$ | 0.105 |
| SGR (%BW/day) | – | – | $1.78 \pm 0.08$ | $1.84 \pm 0.06$ | $2.08 \pm 0.08$ | 0.066 |
| FCR (g feed/g gain) | – | – | $5.31 \pm 0.23^a$ | $5.16 \pm 0.18^a$ | $4.12 \pm 0.08^b$ | 0.006 |
| SSI (%) | – | – | $1.36 \pm 0.21$ | $1.76 \pm 0.03$ | $2.25 \pm 0.09$ | 0.103 |
| ISI (%) | – | – | $1.11 \pm 0.06$ | $1.05 \pm 0.04$ | $1.14 \pm 0.08$ | 0.920 |
| HSI (%) | – | – | $0.81 \pm 0.06$ | $0.76 \pm 0.05$ | $0.60 \pm 0.04$ | 0.619 |

WG, weight gain; CF, condition factor; SGR, specific growth rate; BW, body weight; FCR, feed conversion ratio; SSI, stomasomatic index; ISI, intestosomatic index; and HSI, hepatosomatic index. Data are expressed as tank means $\pm$ SEM ($n = 3$). Significant differences are indicated by different superscripts ($p < 0.05$). Means were tested by one-way ANOVA using Duncan's multiple range test for *post hoc* analysis.

**Table 2** Specific activities of main digestive enzymes in juvenile red snapper (*L. argentimaculatus*) reared in different water salinities for eight weeks.

| Digestive enzyme | Salinity level (‰) | | | $p$-value |
|---|---|---|---|---|
| | 15 | 22.5 | 30 | |
| Pepsin (U/mg protein) | $3.22 \pm 0.68$ | $3.35 \pm 0.30$ | $3.07 \pm 0.29$ | 0.549 |
| Trypsin (mU/mg protein) | $279 \pm 18$ | $275 \pm 15$ | $294 \pm 8$ | 0.738 |
| Chymotrypsin (mU/mg protein) | $139 \pm 6$ | $180 \pm 9$ | $173 \pm 5$ | 0.314 |
| Lipase (mU/mg protein) | $32.3 \pm 0.2$ | $25.0 \pm 2.1$ | $28.3 \pm 5.3$ | 0.336 |
| Amylase (U/mg protein) | $17.3 \pm 2.2$ | $15.6 \pm 2.8$ | $14.1 \pm 1.8$ | 0.300 |

Data are expressed as tank means $\pm$ SEM ($n = 3$). Means were tested by one-way ANOVA using Duncan's multiple range test for *post hoc* analysis.

## Whole-body composition

There were no differences in moisture, crude protein, crude lipid, and crude ash across the 15, 22.5, and 30‰ treatments ($p > 0.05$, Table 4).

## DISCUSSION

While there were no differences in survival, growth performance, and organ indexes between the 15, 22.5, and 30‰ treatments, the FCR was significantly improved in the 30‰ treatment. This finding indicated that the 30‰ salinity was more suitable for juvenile mangrove red snapper than the 22.5‰, or the 15‰. This observation was in partial agreement with the findings of Chen et al [24], who reported a high survival rate of mangrove red snapper fingerlings reared for five days at 30 or 35 °C, within a salinity range of 10 to 40‰. Since *L. argentimaculatus* is a euryhaline

**Table 3** Qualities of white muscle of juvenile red snapper (*L. argentimaculatus*) reared in different water salinities for eight weeks.

| Parameter | Salinity level (‰) | | | $p$-value |
|---|---|---|---|---|
| | 15 | 22.5 | 30 | |
| *Protein synthesis capacity* | | | | |
| RNA (µg/g) | $1{,}687 \pm 33$ | $1{,}554 \pm 182$ | $1{,}443 \pm 192$ | 0.772 |
| Protein (mg/g) | $172 \pm 19$ | $187 \pm 8$ | $209 \pm 8$ | 0.197 |
| RNA/protein ratio (µg/mg) | $9.81 \pm 0.27$ | $8.01 \pm 1.22$ | $6.73 \pm 1.42$ | 0.216 |
| *Myosin and actin enthalpy* | | | | |
| Myosin (J/g) | $1.08 \pm 0.13$ | $1.19 \pm 0.10$ | $1.09 \pm 0.13$ | 0.764 |
| Actin (J/g) | $0.25 \pm 0.02$ | $0.25 \pm 0.02$ | $0.26 \pm 0.02$ | 0.905 |
| Myosin + actin (J/g) | $1.33 \pm 0.14$ | $1.44 \pm 0.11$ | $1.35 \pm 0.13$ | 0.811 |
| Actin/myosin ratio | $0.24 \pm 0.02$ | $0.22 \pm 0.02$ | $0.26 \pm 0.03$ | 0.493 |

Data are expressed as tank means $\pm$ SEM ($n = 3$). Means were tested by one-way ANOVA using Duncan's multiple range test for *post hoc* analysis.
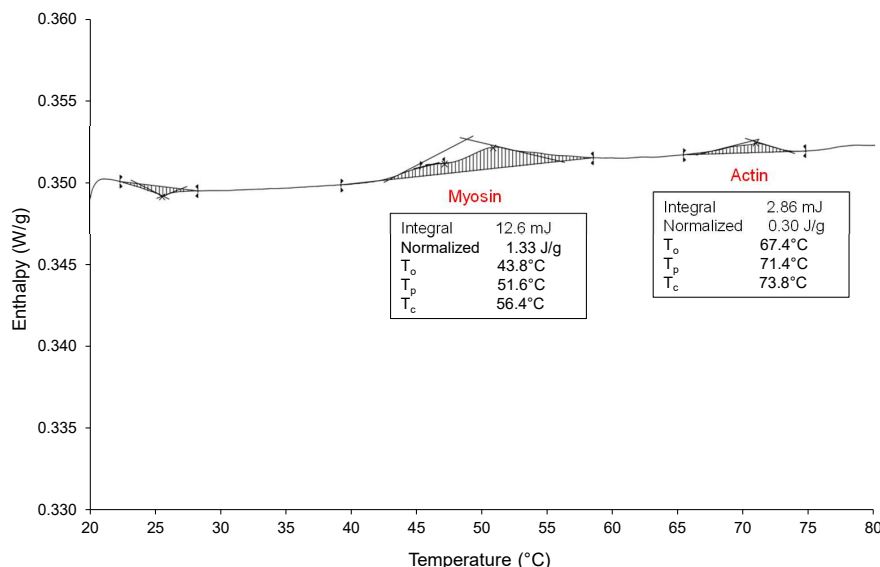
**Fig. 1** Thermogram of myosin and actin in white muscle of juvenile mangrove red snapper (*L. argentimaculatus*). All treatments showed common thermal characteristics, with only one sample from the fish reared in 30‰ used as representative. $T_o$ = onset temperature, $T_p$ = peak temperature, $T_c$ = conclusion temperature.

**Table 4** Whole-body compositions (% of fresh weight) of juvenile red snapper (*L. argentimaculatus*) reared in different water salinities for eight weeks.

| Component | Salinity level (‰) | | | *p*-value |
|---|---|---|---|---|
| | 15 | 22.5 | 30 | |
| Moisture | 72.0 ± 0.5 | 70.2 ± 1.7 | 71.8 ± 1.1 | 0.529 |
| Crude protein | 18.0 ± 0.4 | 19.0 ± 1.0 | 18.1 ± 0.8 | 0.647 |
| Crude lipid | 2.10 ± 0.23 | 2.54 ± 0.41 | 2.20 ± 0.30 | 0.442 |
| Crude ash | 5.45 ± 0.54 | 5.59 ± 0.50 | 5.39 ± 0.54 | 0.965 |

Data are expressed as tank means ± SEM ($n = 3$). Means were tested by one-way ANOVA using Duncan's multiple range test for *post hoc* analysis.

fish, larvae and juveniles of the species can be found in freshwater areas, as well as in estuaries and coastal areas. Meanwhile, adults spawn in deeper seas on offshore reefs [5, 6, 10–12]. However, our long-term trial showed the unsuitability of rearing this species in freshwater (0‰) and brackish water at 7.5‰. It is possible that the fish can physically acclimatize their osmoregulation over a short period of time, such as during foraging, migration, or spawning. Nonetheless, the ability to adapt to changes in salinity for a short duration is of no benefit in aquaculture where fish must have time to grow reaching the market size.

Chi and True [9] reported a survival rate of ∼40 to 60% after rearing juvenile mangrove red snapper (∼0.3 g initial body weight) in salinities of 10, 17, and 25‰ for four weeks. In the present study, fish (9.16–9.17 g initial body weight) reared in salinities of 0‰

and 7.5‰ failed to survive the first three weeks due to food rejection, whereas fish reared in salinities of 15, 22.5, and 30‰ exhibited relatively high survival rates (96.7 to 100%) at the end of the eight-week trial. It is possible that the initial size of the fish and the duration of rearing, as well as the condition, may affect the survival of juveniles. In general, younger fish of many teleost species tolerate a wider range of salinity than adults [25, 26]. In embryos and yolk-sac larvae, osmotic pressure is regulated through the skin, probably with the aid of ion pumps, such as chloride cells [27]. This assumption is supported by the observed tolerance to abrupt salinity changes of newly hatched larvae (day 0) of mangrove red snapper compared with day 7, day 14, and day 21 larvae [13].

Information regarding the growth performance of juvenile mangrove red snapper reared in cultured conditions is still lacking. The growth performance of juvenile fish in the present study, in terms of SGR, was within the range observed in other euryhaline carnivorous fish [14, 28, 29]. Corroborating with our results, SGR values ranging from 1.45–1.60% BW/day were reported in mangrove red snapper with an initial weight of 13.4 g after receiving a practical diet with 45.4% crude protein for 17 weeks [30]. However, the SGR of fish in this study (1.78 to 2.08%/day) was inferior to the value reported by Chi and True [9] of ∼4.33%/day. The difference in SGR might be due to the difference in feeds used in the present study, which was floating pellets for older juveniles, and the combination of minced fresh fish and *Artemia* nauplii for younger juveniles in the aforemention report.

Since the correct feeding regime for this species is still a matter of conjecture, over-feeding was applied in the present study since it inhibited aggressive behavior in the juveniles. Consequently, FCRs were relatively high. Nonetheless, the fish reared in a salinity of 30‰ exhibited superior feed utilization relatively to the fish reared in 15‰ and 22.5‰. However, the desirable feed utilization in the preferred treatment was not sufficient to improve SGR during the eight-week study period. Prolongation of the rearing duration might clarify the effect of salinity in improving SGR. In general, marine teleost fish regulate blood osmotic pressure to maintain the body-fluid salinity at around 10–15‰ and keep the salt concentration below the seawater's level [31, 32]. Hence, the energy cost of osmoregulation under isosaline and isosmotic conditions would be lower than under hypersaline and hyperosmotic conditions. Moreover, fish reared in suboptimal salinity might ingest more food to gain sufficient energy for ionic regulation, as well as for growth compensation [33].

Water salinity could alter pH, ion concentrations and composition; therefore, the physicochemical condition of the gut would be modified, and the zymogen activation could be affected [34–36]. In addition, fish adjusted their drinking rates to control digestive enzyme activities [36]. Therefore, it was possible to track the utilization of feed through the activity of digestive enzymes. Based on our observations, different salinity levels of 15, 22.5, and 30‰ had no effects on specific activities of proteolytic (pepsin, trypsin, and chymotrypsin), amylolytic (amylase), and lipolytic (lipase) enzymes. The results suggested that the enzyme activities of the studied juvenile red snapper were highly flexible to make ingested food accessible. Interestingly, our findings were in line with the insignificant effects of salinity on pepsin activity reported in hybrid grouper (*Epinephelus coioides × E. lanceolatus*) reared in salinities of 10, 15, 20, and 30‰ [14] and the unchanged amylase activity reported in yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*) reared in 6, 12, 24, 35, and 48‰ salinities [37]. In general, digestive enzyme activities are significantly correlated with feed utilization [38, 39]. However, enzymes involved in nutrient utilization include not only digestive enzymes that break down macromolecules, but also enzymes such as brush-border and metabolic enzymes. Further investigations into these enzymes might reveal the connection between water salinity and feed conversion.

RNA (protein synthesis capacity), protein, and the RNA/protein ratio (protein turnover rate) in white muscle have been used as a sensitive indicator during growth phases; whereas amounts of myosin and actin, the major myofibrillar proteins, directly reflect the quality of fish muscle [21, 22, 29]. For proximate composition, the values are associated with many aspects of fish biology and ecology such as appetite, growth, feed utilization, survival, and reproduction [40]. In the present study, no negative effects of salinity were observed in the analyses of muscle quality and whole-body composition. These findings indicated that fish maintain muscle quality and body composition when reared in different salinities.

In conclusion, based on our observations in the present growth trial, juvenile mangrove snapper exhibited euryhaline traits. They tolerated freshwater (0‰) and slightly brackish (7.5‰) conditions for a short time. Although the fish briefly tolerated these conditions, they increasingly rejected food; and 100% mortality occurred at week 3. Therefore, the fish should not be reared for a long-term under these conditions in an aquaculture system. In the salinity treatments of 15, 22.5, and 30‰, all fish grew at similar rates, but the 30‰ treatment fish exhibited a superior feed utilization. The unchanged parameters over a wide range of salinity strongly supported the plasticity of these fish to compensate growth, digestive functionality, muscle quality, and whole-body composition. Our findings suggested that juvenile mangrove red snapper should be reared in a salinity of 30‰ to maximize feed conversion. Further studies on diet quality and feeding practice might benefit the rearing of mangrove red snapper (*L. argentimaculatus*) in less saline conditions.

## REFERENCES

1. Emata AC, Borlongan IG (2003) A practical broodstock diet for the mangrove red snapper, *Lutjanus argentimaculatus*. *Aquaculture* **225**, 83–88.
2. Hoese HD, Moore RH (1977) *Fishes of the Gulf of Mexico: Texas, Louisiana, and Adjacent Water*, Texas A&M University Press, College Station, TX, USA.
3. Phelps RP, Papanikos N, Bourque BD, Bueno FT, Hastey RP, Maus DL, Ferry A, Davis DA (2009) Spawning of red snapper (*Lutjanus campechanus*) in response to hormonal induction or environmental control in a hatchery setting. *Rev Fish Sci* **17**, 149–155.
4. Muyot FB, Magistrado ML, Muyot MC, Mutia MTM (2021) Growth performance of the mangrove red snapper (*Lutjanus argentimaculatus*) in freshwater pond comparing two stocking densities and three feed types. *Philippine J Fish* **28**, 1–17.
5. Day JH, Blaber SJM, Wallace JH (1981) Estuarine fishes. In: Day JH (ed) *Estuarine Ecology with Particular Reference to Southern Africa*, Balkema, Rotterdam, The Netherlands, pp 197–222.
6. Doi M, Singhagraiwan T (1993) *Biology and Culture of the Red Snapper, Lutjanus argentimaculatus*, Eastern Marine Fisheries Development Center, Department of Fisheries, Rayong, Thailand.
7. Russell DJ, McDougall AJ, Fletcher AS, Ovenden JR, Street R (2003) *Biology, Management and Genetic Stock*

*Structure of Mangrove Jack (Lutjanus argentimaculatus) in Australia*, Department of Primary Industries and the Fisheries Research Development Corporation, Queensland, Australia.

8. Zagars M, Ikejima K, Arai N, Mitamura H, Ichikawa K, Yokota T, Tongnunui P (2012) Migration patterns of juvenile *Lutjanus argentimaculatus* in a mangrove estuary in Trang province, Thailand, as revealed by ultrasonic telemetry. *Environ Biol Fishes* **94**, 377–388.

9. Chi VV, True JD (2018) Effects of habitat structure and salinity on growth and survival of juvenile mangrove red snapper *Lutjanus argentimaculatus* (Forsskal, 1775). *Songklanakarin J Sci Technol* **40**, 1240–1247.

10. Ebner BC, Morgan DL (2013) Using remote video to estimate freshwater fish species richness. *J Fish Biol* **82**, 1592–1612.

11. Lake JS (1971) *Freshwater Fishes and Rivers of Australia*, Thomas Nelson, Sydney, Australia.

12. Russell DJ, McDougall AJ (2005) Movement and juvenile recruitment of mangrove jack, *Lutjanus argentimaculatus* (Forsskål), in northern Australia. *Mar Freshwater Res* **56**, 465–475.

13. Estudillo CB, Duray MN, Marasigan ET, Emata AC (2000) Salinity tolerance of larvae of the mangrove red snapper *Lutjanus argentimaculatus* during ontogeny. *Aquaculture* **190**, 155–167.

14. Sutthinon P, Thongprajukaew K, Saekhow S, Ketmanee R (2015) Juvenile hybrid grouper (*Epinephelus coioides × E. lanceolatus*) are euryhaline and can grow in a wide range of salinities. *Aquac Int* **23**, 671–682.

15. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**, 265–275.

16. Worthington V (1993) *Worthington Enzyme Manual: Enzymes and Related Biochemicals*, Worthington Chemical, NJ, USA.

17. Rungruangsak-Torrissen K, Moss R, Andresen LH, Berg A, Waagbø R (2006) Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). *Fish Physiol Biochem* **32**, 7–23.

18. Winkler UK, Stuckmann M (1979) Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J Bacteriol* **138**, 663–670.

19. Areekijseree M, Engkagul A, Kovitvadhi U, Thongpan A, Mingmuang M, Pakkong P, Rungruangsak-Torrissen K (2004) Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson 1900. *Aquaculture* **234**, 575–587.

20. Sunde J, Taranger GL, Rungruangsak-Torrissen K (2001) Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (*Salmo salar* L.). *Fish Physiol Biochem* **25**, 335–345.

21. Matos E, Silva TS, Tiago T, Aureliano M, Dinis MT, Dias J (2011) Effect of harvesting stress and storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): A differential scanning calorimetry study. *Food Chem* **126**, 270–276.

22. Schubring R (2009) Differential scanning calorimetry. In: Rehbein H, Oehlenschlager J (eds) *Fishery Products – Quality, Safety and Authenticity*, Wiley-Blackwell, England, pp 173–213.

23. AOAC (2005) *Official Methods of Analysis of AOAC International*, 18th edn, Association of Official Analytical Chemists, Arlington, VA, USA.

24. Chen SL, Shyu CZ, Lue MY (1990) Tolerance of red snapper, *Lutjanus argentimaculatus*, fingerlings to temperature, salinity and dissolved oxygen. *Bull Taiwan Fish Res Inst* **49**, 37–42.

25. Alderdice DF (1988) Osmotic and ionic regulation in teleost eggs and larvae. In: Hoar WS, Randall DJŽ (eds) *Fish Physiology*, Vol **11**, Academic Press, San Diego, USA, pp 163–251.

26. Bone Q, Marshall NB, Blaxter JHS (1995) *Biology of Fishes*, 2nd edn, Chapman & Hall, London, UK.

27. Ayson FG, Kaneko T, Hasegawa S, Hirano T (1994) Development of mitochondrion-rich cells in the yolk-sac membrane of embryos and larvae of tilapia, *Oreochromis mosambicus*, in freshwater and seawater. *J Exp Zool* **270**, 129–135.

28. Galkanda Arachchige HSC, Davis RP, Nazeer S, Ibarra Castro L, Davis DA (2021) Effect of salinity on growth, survival, and serum osmolality of red snapper, *Lutjanus campechanus*. *Fish Physiol Biochem* **47**, 1687–1696.

29. Kaewtapee C, Thongprajukaew K, Jittanoon T, Nuntapong N, Preedaphol K, Saekhow S (2022) Mixed feeding schedule switching between high and low protein diets for Asian seabass (*Lates calcarifer*). *Anim Feed Sci Technol* **284**, 115204.

30. Catacutan MR, Pagador GE, Doyola-Solis E, Teshima S, Ishikawa M (2011) Growth and feed efficiency in mangrove red snapper, (*Lutjanus argentimaculatus* Forsskal 1775) fed practical diets supplemented with *L*-ascorbyl-2-monophosphate-Mg. *Isr J Aquac Bamidgeh* **63**, 1–7.

31. Brett JR, Groves TDD (1979) Physiological energetics. In: Hoar WS, Randall DJ, Brett JR (eds) *Fish Physiology, Bioenergetics and Growth*, Vol **8**, Academic Press, New York, USA, pp 279–352.

32. Jobling M (1994) *Fish Bioenergetics*, Chapman & Hall, London, UK.

33. Wootton RJ (1995) *Ecology of Teleost Fishes*, Biological Sciences, Chapman & Hall, London, UK.

34. Boeuf G, Payan P (2001) How should salinity influence fish growth? *Comp Biochem Physiol* **130**, 411–423.

35. Moutou KA, Panagiotaki P, Mamuris Z (2004) Effects of salinity on digestive activity in the euryhaline sparid *Sparus aurata* L.: a preliminary study. *Aquac Res* **35**, 912–914.

36. Usher ML, Talbot C, Eddy FB (1988) Drinking in Atlantic salmon molts transferred to seawater and the relationship between drinking and feeding. *Aquaculture* **73**, 237–246.

37. Mozanzadeh MT, Safari O, Oosooli R, Mehrjooyan S, Najafabadi MZ, Hoseini SJ, Saghavi H, Monem J (2021) The effect of salinity on growth performance, digestive and antioxidant enzymes, humoral immunity and stress indices in two euryhaline fish species: Yellowfin seabream (*Acanthopagrus latus*) and Asian seabass (*Lates calcarifer*). *Aquaculture* **534**, 736329.

38. Rodjaroen S, Thongprajukaew K, Jaihao P, Saekhow S, Nuntapong N (2020) Mixed feeding schedules switching between dietary crude protein levels for mono-sex male Nile tilapia (*Oreochromis niloticus*). *Aquac Rep* **18**, 100509.

39. Thongprajukaew K, Kovitvadhi U, Kovitvadhi S, Som-
    sueb P, Rungruangsak-Torrissen K (2011) Effects of dif-
    ferent modified diets on growth, digestive enzyme ac-
    tivities and muscle compositions in juvenile Siamese
    fighting fish (*Betta splendens* Regan, 1910). *Aquaculture*
    **322–323**, 1–9.
40. Breck JE (2014) Body composition in fishes: Body size
    matters. *Aquaculture* **433**, 40–49.