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*Short Communication*

# The combined effects of salinity and temperature on the proximate composition and energetic value of spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869)

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**ABSTRACT.** Combined effects of temperature (25, 30 and 35°C) and salinity (15, 25, 35 and 45 g L$^{-1}$), on the body composition and energetic value in the juvenile spotted rose snapper (*Lutjanus guttatus*), were investigated over 90 days. Significant effects of temperature, salinity, and their interaction on body composition and energetic value were analyzed. Low temperatures (25°C) significantly reduced the protein and increase lipid content in the body. In a temperature of 30°C, the highest energetic value was recorded, and in the highest salinity (45 g L$^{-1}$), the energetic value was reduced. The salinity of 15 has the highest growth, protein content, and energetic value. It was found that the optimal temperature-salinity combination for the best relationship of growth (3.8% d$^{-1}$), protein (21.9%), and lipid (4.9%) content, and energetic value (2.61 kJ g$^{-1}$) was at 30 to 32.0°C and salinity of 35 g L$^{-1}$. The effect of temperature was directly proportional to the increase in protein and inversely with the content of lipids in the body; salinity has its main effect negatively on these parameters in the highest salinity (45 g L$^{-1}$).

**Keywords:** *Lutjanus guttatus*, spotted rose snapper**, s**alinity, temperature, proximal analyses.

The temperature and salinity are the main physical factors affecting marine fish (Huang *et al*., 2015), and the biological effects of these factors are complex (Likongwe *et al*., 1996). The marine fish research has been directed on the effects of temperature-salinity fluctuations on growth, survival, feeding, physiological indices and immune capacities (Likongwe *et al*., 1996; Castillo-Vargasmachuca *et al*., 2013; Huang *et al*., 2015), while there has been little attention to the effect of these factors and their interaction on proximate composition and energy value. The nutritional content of fish is used as an indicator of fish quality (Reinitz, 1983) and has been used for the assessment of fish health, determination of the efficiency nutrient transfer from feed to fish and to make it possible to predictably modify carcass composition (Shearer, 1994). The proximate composition of fish can be affected by endogenous and exogenous factors that operate simultaneously. In general, it has been found that primarily the temperature (Vollenweider *et al*., 2011) and the ration size affect the proximate composition,

and that salinity has little or no effect (Küçükgülmez *et al*., 2010). However, the combined effect of temperature and salinity on the proximate composition has been little studied for marine fishes (Edirisinghe *et al*., 2013). Therefore, the aim of the present work was to investigate the effects of the temperature and salinity on the proximate composition of spotted rose snapper grown in experimental conditions.

Four hundred juvenile *L. guttatus* (52.2 ± 0.3 mm and 2.10 ± 0.03 g; mean ± SE) were obtained from the Center for Food Research and Development A.C. Unit-Mazatlán (Mazatlán, Sinaloa, México). They were transported at 20°C to the Coastal Bioengineering Laboratory, National Engineering School of Fisheries, Autonomous University of Nayarit, in San Blas, Nayarit, México (21°29'52.79"N, 105°12'03.86"W). The fishes were acclimatized in a fiberglass tank (500 L) with continuous closed flux for one week for each combination of salinity and temperature. Salinity was then adjusted daily at a rate not exceeding 2, and the temperature was adjusted at a rate of 3°C d$^{-1}$. During the

acclimatization, feed pellets (NUTRIPEC-Purina: 42% protein 12% lipids and 10% moisture, 2 mm size) were provided *ad libitum* three times a day. Artificial lighting was supplied by four fluorescent daylight strip lights (500 lx measured at the water surface) controlled by a timer set for a daily photoperiod of 12 h. The daylight phase began at 06:30 h, various 1000 L tanks were used to store freshwater (FW), seawater (SW, 35) and to prepare the waters at different salinities. The saline waters used in the experimental tanks were always obtained using SW that was either diluted with FW or made more concentrated with saturated brine. The laboratory temperature was 25ºC. The water tempera-ture was adjusted by means of submersible thermostats (200 watts Sunny®).

Growth, survival, and proximate composition were studied in two-factorial designs, where three temperatures (25, 30, and 35°C) and four salinities (15, 25, 35 and 45 g L$^{-1}$) were tested in triplicate combinations. At the beginning of the experiment, 216 juveniles were randomly distributed into 36 tanks (80 L) at a density of six fish per tank in the same conditions as those of acclimatization. Each tank was equipped with an air diffuser, and the airflow was set at 1.15 L min$^{-1}$ to maintain the oxygen concentration in the water close to saturation. To prevent the fish from jumping out, the tanks were covered by nets. There was an independent recirculation system for each treatment. The fish were handfed slightly in excess (4% of the estimated biomass) with commercial pellets (NUTRIPEC-Purina: 42% protein, 12% lipids, and 10% moisture).

Water temperature (°C), salinity (g L$^{-1}$), dissolved oxygen (mg L$^{-1}$), and pH were recorded daily, and ammonia (mg L$^{-1}$), nitrites (mg L$^{-1}$) and nitrates (mg L$^{-1}$) were recorded fortnightly, accordingly with the technique used by Castillo-Vargasmachuca *et al.* (2013) and Alcalá-Carrillo *et al.* (2016). The juveniles were cultured for 90 days under these conditions. The growth indicators (mean initial weight, mean final weight, food conversion ratio, specific growth rate (SGR), The Fulton´s condition factor (K) and survival, were calculated according to Castillo-Vargasmachuca *et al.* (2013) and Alcalá-Carrillo *et al.* (2016). The proximate composition of *L. guttatus* (flesh only) was estimated at the beginning of the experiment and after 90 days for each of the treatments following standard AOAC (2005) procedures. The energy content was calculated using the energy equivalents of 36.43 and 20.10 kJ g$^{-1}$ Brett (1995) for lipid and protein, respectively.

The homogeneity of variances and the normal distributions of physical, chemical and biometric variables of the experiment were analyzed. Two-way analysis of variance (ANOVA) was used to test the interaction between temperature and salinity. The differences between the means were compared using the Tukey test (Montgomery, 2012) with a confidence interval of 95%. Additionally, specific growth rate, protein, and lipid content results were analyzed by interpreting contour plots (Minitab 16). The statistical analysis of the data was performed with a statistical package (Statistica 5.0, StatSoft 1995).

During the experiment, the temperature and salinity had a maximum variation of 0.5°C and 1.0 g L$^{-1}$, respec-tively. In general, in the experimental system, the pH, ammonia, nitrites, and nitrates showed mean concentrations of 7.5 to 8.0, 0.15 to 0.25 mg L$^{-1}$, 0.10 to 0.15 mg L$^{-1}$ and 0.15 to 0.90 mg L$^{-1}$, respectively.

It was found that at salinities of 15 to 25 g L$^{-1}$ (Table 1), the highest SGR (4.0% to 4.2% d$^{-1}$) was recorded at a temperature of 35°C ($P < 0.05$) and that at a salinity of 45 the lowest SGR (2.6% d$^{-1}$) was recorded at a temperature of 35°C ($P < 0.05$).

For all temperatures, the lowest survivals (20 to 70%) were found at a salinity of 45 ($P < 0.05$) and the highest survivals (93.3% to 96.6%) for all temperatures were found at a salinity of 25 g L$^{-1}$ ($P < 0.05$).

At the beginning of the experiment, a proximate composition of 21.6% protein, 3.5% lipids, 0.9% ash and 74.6% water was recorded. After 90 days, it was found that the higher temperature (35ºC) significantly increased the protein concentration for salinities of 15 to 35 g L$^{-1}$ and had a decreasing effect at a salinity of 45 g L$^{-1}$. The highest concentration of lipid and water were significant ($P < 0.05$) at the lowest temperatures (25°C). The lowest energetic value (2.41 to 2.48 kJ g$^{-1}$) was shown at the lower temperature (25°C). Increasing temperature increased the protein concentration in the spotted rose snapper (Fig. 1a). At 32°C, a negative effect was observed in the higher salinities (36 to 45 g L$^{-1}$). The lipids decreased with increasing temperature (Fig. 1b). At 25 to 30°C, the SGR increments are directly proportional, but at 35°C had the highest SGR (4.2% d$^{-1}$). In relation to the best proportion of protein-lipid-SGR in the proximate composition (Fig. 1), it was found that higher protein (22%) and lower lipids (4.7%) were related with SGR (3.7% d$^{-1}$) at 30 to 32°C and salinity of 35 g L$^{-1}$ (Figs. 1d, 1a). Organisms grown in this temperature-salinity combination had the highest energetic value (2.60-2.61 kJ g$^{-1}$; Table 1). The highest SGR (4.0 to 4.1% day$^{-1}$) was combined with the highest protein content (22%) and the mean concentration of lipids (4.8 to 4.9%; Figs. 1d, 1b), and high level of lipids (5.9%) was combined with low protein (19%) and low SGR (3.1% d$^{-1}$; Figs. 1d, 1c). The Fulton´s condition factor (K) ranged between 0.85 and 1.65 and had no significant relationship with the effect of the temperature-salinity combination.

**Table 1.** Growth, survival and proximate composition parameters of *L. guttatus* at different temperatures and salinities for 90 days in a recirculating system.  In each column, values with the different superscripts (a, b, c, d) are significantly different SGR, specific growth rate, FCR, feed conversion rate.

| Temperature (°C) | Salinity (g L⁻¹) | SGR (% d⁻¹) | FCR | Survival (%) | Protein (%) | Lipid (%) | Ash (%) | Water (%) | Energetic value (kJ g⁻¹) |
|---|---|---|---|---|---|---|---|---|---|
| 25 | 15 | $3.3 \pm 0.0^b$ | $1.7 \pm 0.0^b$ | $90.0 \pm 0.4^{ab}$ | $18.4 \pm 0.1^c$ | $6.1 \pm 1.0^a$ | $0.9 \pm 0.21^b$ | $74.6 \pm 0.52^a$ | $2.48 \pm 0.16^b$ |
| 25 | 25 | $3.0 \pm 0.0^b$ | $1.7 \pm 0.0^b$ | $96.3 \pm 0.5^a$ | $18.1 \pm 0.5^c$ | $5.9 \pm 2.1^a$ | $1.2 \pm 0.16^b$ | $74.8 \pm 0.13^a$ | $2.42 \pm 0.09^b$ |
| 25 | 35 | $2.7 \pm 0.0^c$ | $2.1 \pm 0.0^{bc}$ | $93.6 \pm 0.4^a$ | $17.0 \pm 0.7^c$ | $6.6 \pm 1.6^a$ | $1.0 \pm 0.12^b$ | $75.4 \pm 0.45^a$ | $2.43 \pm 0.10^b$ |
| 25 | 45 | $2.7 \pm 0.0^c$ | $2.1 \pm 0.0^c$ | $70.0 \pm 1.0^c$ | $17.0 \pm 0.1^c$ | $6.6 \pm 5.1^a$ | $1.0 \pm 0.24^b$ | $75.1 \pm 0.60^a$ | $2.41 \pm 0.08^b$ |
| 30 | 15 | $3.8 \pm 0.0^a$ | $1.3 \pm 0.0^a$ | $93.0 \pm 0.4^{ab}$ | $21.9 \pm 1.9^b$ | $5.3 \pm 1.7^{ab}$ | $1.6 \pm 0.14^a$ | $72.1 \pm 0.19^{ab}$ | $2.60 \pm 0.05^a$ |
| 30 | 25 | $3.6 \pm 0.0^{ab}$ | $1.4 \pm 0.0^a$ | $93.3 \pm 0.5^a$ | $21.4 \pm 2.4^b$ | $5.4 \pm 1.8^{ab}$ | $1.7 \pm 0.12^a$ | $71.5 \pm 0.65^b$ | $2.61 \pm 0.07^a$ |
| 30 | 35 | $3.8 \pm 0.0^a$ | $1.5 \pm 0.0^a$ | $86.6 \pm 1.0^b$ | $21.9 \pm 0.1^{ab}$ | $4.9 \pm 2.5^b$ | $1.8 \pm 0.08^a$ | $71.4 \pm 0.14^b$ | $2.61 \pm 0.09^a$ |
| 30 | 45 | $3.2 \pm 0.0^b$ | $1.5 \pm 0.0^a$ | $66.6 \pm 1.7^c$ | $21.2 \pm 0.2^b$ | $4.0 \pm 2.1^b$ | $1.1 \pm 0.02^b$ | $73.7 \pm 0.03^a$ | $2.39 \pm 0.15^b$ |
| 35 | 15 | $4.2 \pm 0.0^a$ | $2.1 \pm 0.0^{bc}$ | $83.3 \pm 1.0^b$ | $22.7 \pm 0.9^a$ | $4.9 \pm 0.2^b$ | $1.0 \pm 0.20^b$ | $71.4 \pm 0.17^b$ | $2.61 \pm 0.13^a$ |
| 35 | 25 | $4.0 \pm 0.0^a$ | $2.2 \pm 0.0^b$ | $92.6 \pm 1.0^a$ | $22.2 \pm 2.9^a$ | $4.4 \pm 1.7^b$ | $1.9 \pm 0.11^a$ | $71.5 \pm 0.19^b$ | $2.55 \pm 0.11^{ab}$ |
| 35 | 35 | $3.5 \pm 0.0^{ab}$ | $2.3 \pm 0.0^c$ | $86.6 \pm 1.0^b$ | $22.1 \pm 0.3^a$ | $4.0 \pm 0.6^b$ | $1.8 \pm 0.35^a$ | $72.2 \pm 0.17^b$ | $2.47 \pm 0.08^b$ |
| 35 | 45 | $2.6 \pm 0.0^c$ | $1.8 \pm 0.0^b$ | $20.0 \pm 0.9^d$ | $21.0 \pm 0.1^b$ | $4.0 \pm 1.1^b$ | $1.7 \pm 0.2^a$ | $73.3 \pm 0.5^{ab}$ | $2.37 \pm 0.14^b$ |
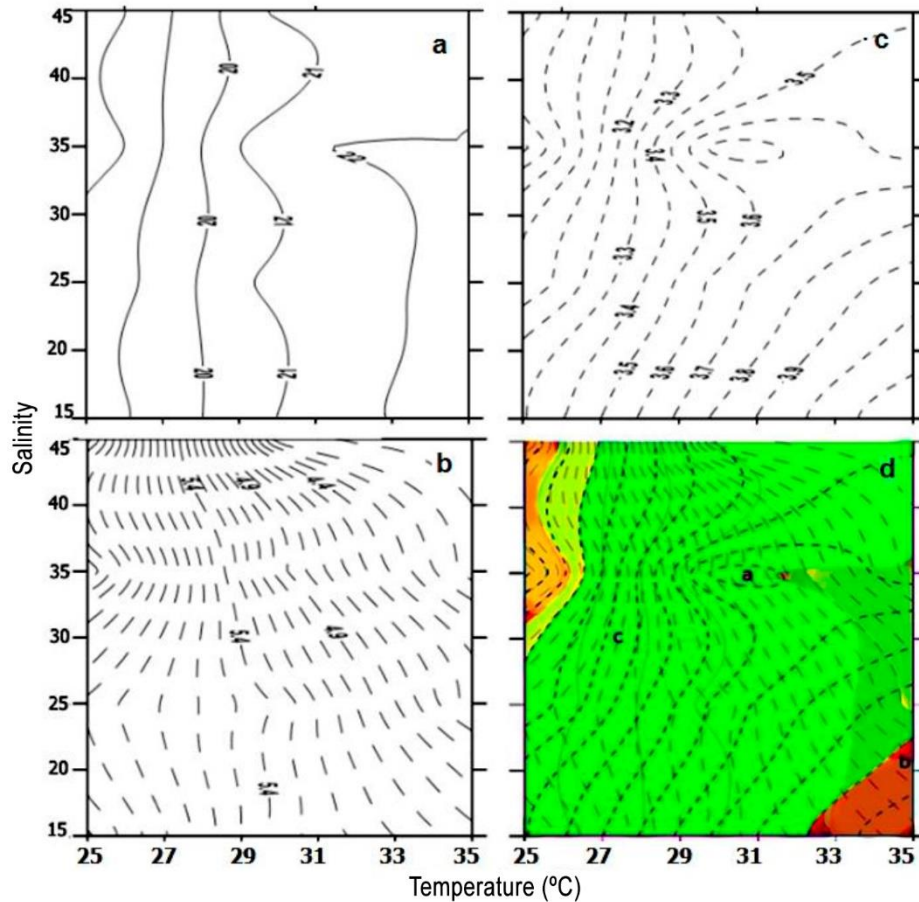


**Figure 1.** Map contour of temperature-salinity effect on protein, lipid, and SGR. a) protein, b) lipid, c) SGR, and d) protein (continuous), lipid (broken) and SGR (dashed).

Temperature is the most important factor affecting the proximate composition of *L. guttatus* with a negative effect on the lipid and water content and a positive effect on the protein content and energetic value. It was found that increasing the temperature increases the SGR in *L. guttatus*, as it has been recorded for other species of fish (Xiao-Jun & Ruyung, 1992). However, salinity had a negative effect on the SGR at

the highest temperatures (35°C) and salinities (45 g L⁻¹) tested. This result is inferred because the opposite result is obtained when the experimental temperature and salinity reach the upper extreme of the tolerated range, as is the case in other species (Hasan & MacIntoch, 1991).

The composition of fish tissues can be determined directly through proximate analyses or estimated indirectly using indices of physiological body condition (Fitzgerald *et al.*, 2002). It has been found that body composition changes with temperature, size, age, diet, ration size and the stage of development (Van-Ham *et al.*, 2003; Ljubojević *et al.*, 2015; Mozsár *et al.*, 2015). The percentages of protein (17.0-22.7%), lipid (4.0-6.7%), ash (0.9-1.9%), water (71.4-75.4%) and energy content (2.37-2.62 kJ g⁻¹) in this study are within the ranges recorded for snappers (Abbas *et al.,* 2005, 2015; Abbas & Siddiqui, 2009).

During the present study, the snapper was characterized by low lipid content (<6.7%) and very high protein content (17.2-22.7%), which is in agreement with the observations of Edirisinghe *et al.* (2013) and Hanna (2001), for sea fish species. The proportionally high protein content in sea fish compared to many other fish species may be an adaptation to low food intake periods. The crude protein content in *L. guttatus* tended to decrease at low temperatures (25°C) and tended to increase at high temperatures (35°C), as has been previously reported for marine fish (Fang *et al.*, 2010). Crude protein was negatively correlated with water content, as has been reported for other coastal-water fish (Pangle & Sutton, 2005). The present results are in agreement with Brown & Murphy (1991), the water content of the juvenile snapper was inversely related to SGR (Table 1). The optimal growth area (Figs. 1d, 1a) shows that snapper can efficiently utilize lipids and protein sparing, which subsequently improve growth.

The increasing lipid content and decreasing protein content in the muscle tissue is a relationship found by the authors in a previous study of *L. guttatus* and carp (Ljubojević *et al.*, 2015). The lipid content of a fish is an indicator of the surplus energy available for future maintenance, growth, and reproduction (Tocher, 2003). However, such a relationship was not found in this study because the snappers were fingerlings and juveniles. The energy content was negatively correlated with the water content for *L. guttatus*, similar to the findings for juvenile lake herring (Pangle & Sutton, 2005).

In this work there was no relationship between the K-factor and the proximate composition, and the relationship between these two parameters can be highly variable among fish species and can be influenced by the time of sampling but independent of gender (Mozsár *et al.*, 2015). Our results also indicate that the retention of protein in the muscle tissue was achieved when the temperature level increased and the salinity level decreased below 35 g L⁻¹. Additionally, the energetic value was higher at 30°C and at salinities of 15 to 35 g L⁻¹. Salinity had its main negative effect on growth and body composition at 45 g L⁻¹ and the combination of the highest temperature (35°C).

## ACKNOWLEDGMENTS

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