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*Short Communication*

# Effects of temperature and salinity on growth and survival of the Pacific red snapper *Lutjanus peru* (Pisces: Lutjanidae) juvenile

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**ABSTRACT.** The present study evaluates the effects of temperature (25 and 30ºC) and salinity (25, 35 and 45 psu) on juvenile growth and survival. All the experiments were carried out under rearing conditions. A total of 270 specimens were used for the experiments. The results showed that more than 86% of the snapper survived at 35 to 45 psu salinity. Significant differences in growth parameters, such as the specific growth rate and weight gain were observed in fish reared at temperatures of 25 and 30°C and salinities of 35 and 45 psu. Increased salinity beyond 45 psu negatively affected growth of the Pacific red snapper used in this trial. The effects of temperature and salinity on growth performance a survival rate indicated that red snapper is an euryhaline species, that may tolerate wide salinity ranges, showing that has a good potential to grow in waters of lower salinity than the sea.

**Keywords:** *Lutjanus peru,* growth, survival, temperature, salinity, aquaculture, Mexico.

# Efectos de la temperatura y salinidad sobre el crecimiento y supervivencia de juveniles de huachinango *Lutjanus peru* (Pisces: Lutjanidae)

**RESUMEN.** El presente trabajo determina los efectos de la temperatura (25 y 30ºC) y salinidad (25, 35, 45 ups) sobre el crecimiento y supervivencia de juveniles. Todos los experimentos se realizaron en laboratorio. Un total de 270 organismos fueron utilizados en los experimentos. Los resultados mostraron que más del 86% de los peces sobreviven a salinidades de 35 y 45 ups. Se encontraron diferencias significativas en la tasa específica de crecimiento y ganancia en peso en los organismos criados a temperaturas de 25 y 30ºC y salinidades de 35 y 45 ups. Aumento de la salinidad más allá de 45 ups afecta el crecimiento del huachinango utilizado en este experimento. La tolerancia a la salinidad bajo 35 ups muestra que la especie tiene el potencial para crecer en aguas de menor salinidad que el mar.

**Palabras clave:** *Lutjanus peru,* crecimiento, supervivencia, temperatura, salinidad, acuicultura, México.

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The adaptability of aquatic organisms to changes in temperature and salinity is influenced by various abiotic and biotic parameters (Chung, 1994a, 1994b). The salinity and temperature acclimation is a key factor in the physiological response of tropical aquatic organisms (Brett, 1979). Though acclimation is easiest with a decrease rather than an in increase in salinity, which is in contrast to thermal adaptation (Chung, 1996, 2001). Nevertheless the relative natural capacity of different species to adjust to fluctuating environmental conditions is reflected in their resistance to sudden changes during experimental acclimatization.

The experiments of tolerance to temperature and salinity changes carried out in fishes have been usually developed through naturally decreasing temperature or well switching freshwater (FW) to brackish

water and seawater (SW) (Sifa *et al.*, 2002; El-Zaeem, 2011). Marine fish culture in brackish water has been common in Southeast Asia and India since the late 40s (Job & Chacko, 1947). Additionally, saltwater fish species have been tested to identify their capability to acclimate to freshwater. The results of these experiences indicated that some marine fish species take three to 12 days to acclimatize from seawater to freshwater: However, the milkfish *Chanos chanos* can tolerate salinity changes in a matter of hours without any ostensible mortality (Ganapati & Alikunhi, 1952). Hence the successful culture of saltwater fish in freshwater depends mainly on the speed with which they are acclimatized to the new environment, without any mortality being caused during the acclimatization process.

In Latin America, snapper species have been assessed and recommended for mariculture activities because they are easy to manipulate, accept artificial feed, and are not aggressive when they are maintained in high densities (Arnold *et al.*, 1978; Tucker & Jory 1991; Boza-Abarca *et al.*, 2008). Ibarra-Castro & Duncan (2007) detailed the commercial importance of snapper species, and other authors have explained the interest of aquaculture and fishing industries in developing snapper culture (Tucker & Jory, 1991; Leu *et al.*, 2003; Dumas *et al.*, 2004; Ogle & Lotz, 2006; Boza-Abarca *et al.*, 2008; Castillo-Vargasmachuca *et al.*, 2007; Boza-Abarca *et al.*, 2011; Castillo-Vargasmachuca *et al.*, 2012; Alvarez-Lajonchere *et al.*, 2012). Growing this species in cage protected sites or open sea (Bergheim, 2012) and ponds on land has been considered as a future possibility. For this reason the goal of the present study was to determine the temperature and salinity tolerance of the Pacific red snapper (*Lutjanus peru*) juvenile under controlled laboratory conditions. This research represents the first comprehensive study focused on the salinity tolerance of this species.

The trial was conducted at the Autonomous University of Nayarit, Mexico, in the National School of Fisheries Engineering, Coastal Bioengineering Laboratory. At the present time in Mexico the Pacific red snapper juveniles are not available from commercial or experimental hatcheries, consequently, for the purposes of this study, juveniles were captured off Platanitos Beach (Nayarit, Mexico), using 24 m long trawling nets with 3.17 cm (1.25 in) mesh; 30 min trawl hauls were conducted at 27 m depth. The collected fish were transported in six 1,000 L plastic tanks with constant aeration and acclimated for up to 120 h. Fish were assessed for pathogens and treated to ensure that they were healthy for the subsequent experiments.

The experiments were carried out under laboratory conditions, where temperature was set at 25ºC. The artificial lighting was supplied by three 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 07:00 h. A 2,000 L tank equipped with an air diffusor was used to house the fish prior to the experiment. The salinity and temperature experiments were developed in 18 cylindrical tanks of 100 L water volume. Each tank was equipped with an air diffusor, and the airflow was set at 1.35 L $min^{-1}$ to maintain an oxygen concentration in the water close to saturation. Nets to prevent the fish from jumping out covered the tanks, with a recirculation system independently for each treatment.

Additionally, various tanks from 500 to 1,000 L were used to store FW and SW (33 to 35 psu) and prepare waters at different salinities. FW was obtained from the local municipal water supply, heated to 27ºC, aerated to eliminate residual chlorine and stored for 24 h before use. SW was provided through direct pumping from the sea and filtered with a textile filter (30 µm) and stored for 24 h. 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. This procedure was adopted to avoid a negative effect on osmoregulation when salt waters are prepared through the simple mixing of freshwater and sea salt (Griffith, 1974).

The water temperature was measured to the nearest 0.1ºC with a calibrated mercury thermometer. The salinity (±0.1 psu) was monitored with an YSI85 oxymeter, which was first calibrated with a standard SW sample (Ocean Scientific International IAPSO Standard Seawater). The pH was measured with a pH meter, and the dissolved oxygen concentration (±0.1 mg $L^{-1}$) was recorded with an YSI85 oxymeter. The total ammonia nitrogen (TAN) concentration was measured with an YSI9000 colorimeter (±0.1 mg $L^{-1}$), and free and total chlorine (±0.2 mg $L^{-1}$) was measured using an Aquamerck kit. A Mettler balance accurate to within 0.01 g was used to weigh the fish.

Groups of *L. peru* juvenile (mean total weight: 68.5 ± 0.9 g) were subjected successively to daily increments of salinity. The increments or decrement was 2 psu $day^{-1}$. The following protocol was the same for each group of fish and for each daily increment in salinity. In the laboratory, each group of 36 fish was housed in a 500 L tank for acclimation and handfed pellets (NUTRIPEC: 42% protein) *ad libitum* three times a day. Acclimation lasted for 10 days, and mortality was evaluated once a day. On the last day of the acclimation period, the fish were not fed, and they

were anesthetized (200 ppm of phenoxy-2-ethanol) and randomly distributed one by one into the three test tanks in each treatment, which were previously filled with 80 L. This random distribution of fish was stopped when each tank contained 15 fish. On the next day at 9:00 am, and at the same time on each of the following days, the fish were handfed slightly in excess (4% of the estimated biomass) of commercial pellets (42% protein, 12% lipids, and 10% moisture), and the temperature (T) of the filters and tanks were measured. At that time, once a week, other physico-chemical parameters were measured either *in situ* for dissolved oxygen (DO), or by taking 100 mL samples to determine pH and total ammonia nitrogen concentration At 9:30 am, half of the water (30 L) in the tanks was siphoned off and any faeces and uneaten food was carefully removed. During this operation, the plastic tubes were taken out of the tanks and replaced. The behavior of the fish was observed, and the mortality (number of fish that died within the previous 24 h) was monitored. The death of a fish was defined when any spontaneous movement stopped and a lack of response to mechanical stimuli was observed. Dead fish were removed and weighed.

The survival and growth rates were studied in triplicate combinations of three salinities (25, 35 and 45 psu) and two temperatures (25 and 30ºC). The locations of the treatments were randomized among tanks. Groups of 15 animals were initially distributed into the 18 tanks that contained normal SW. Salinity was then adjusted at a rate not exceeding 2 psu $day^{-1}$, and temperature was adjusted at a rate of 3ºC $day^{-1}$. Acclimation to all treatments was achieved without problems within 5 days.

Juveniles were grown under these conditions for 60 days. The growth performance indicators were calculated according to Hashim *et al.* (2002), as follow: days of culture; average initial total weight ($W_i$, g); average final total weight ($W_f$, g); Weight gain (g $week^{-1}$), average initial total length ($L_i$, cm); average final total length ($L_f$, cm), specific growth rate (SGR, % $day^{-1}$); feed conversion ratio (FCR) and survival (%). Growth and survival were compared at the beginning and at the end of the trial. The specific growth rate (SGR) was calculated at the end of the experimental period using this formula (Ricker, 1975): SGR = [(ln (final wet body weight) – ln (initial wet body weight)) / time (days)] 100. The total length-total weight relationships were calculated from the allometric equation $W_T = a\ L_T^{\ b}$ (Ricker, 1975), where $W_T$ is total body weight (g), $L_T$ the total length (cm), *a* and *b* are the coefficients of the functional regression between $W_T$ and $L_T$.

The mean and standard deviation of the water quality variables (T, DO, pH, and TAN) were calculated for each test and the entire series of tests. The homogeneity of variances and normal distributions were tested, and the means between the experimental conditions were compared with a two-level factorial design (ANOVA) to test the interaction of salinity and temperature. Differences between means were compared using Tukey´s test with a 95% confidence interval ($P < 0.05$).

There were no differences in the mean water quality variables within treatments throughout the experimental period. Water temperature, salinity and DO were relatively stable and varied by less than 1ºC (25º to 30ºC), 1 psu (25 to 45 psu) and 3.0 mg $L^{-1}$, respectively. The pH ranged from 7.3 to 8.1 and total ammonia nitrogen fluctuated from 0.02 to 0.09 mg $L^{-1}$.

The effect of water temperature (25 and 30ºC) and salinity (25, 35 and 45 psu) on the survival rate of red snapper juveniles is showed in Table 1. Decreasing water salinity had an effect on the survival rate. After 48 h, a low mortality rate was reported in the lowest salinity concentrations (25 psu). The survival of the juveniles maintained at 35 and 45 psu of salinity (83 to 100%) was significantly ($P < 0.05$) higher than the juveniles maintained at 25 psu (75%). After 60 days, the juveniles maintained at 35 psu of salinity suffered 0% mortality. Few differences were found in the survival rates between 25 and 30ºC.

Variance analyses of growth in terms of length and weight showed statistically significant differences ($P < 0.05$) for fish grown at 25 and 30ºC. The values showed a tendency of decreasing growth as salinity decreased (Table 1).

The equation and the allometric factor of the organisms in each treatment are shown in Table 2. If the allometric factor (b) value is 3 or less, the fish will tend to show increased length rather than weight. There was a tendency for a factor ($b > 3$) for organisms grown at 30ºC (Table 2). The effects of temperature and salinity on survival and growth showed a generally wide tolerance of *L. peru* to salinity and temperature changes. Generally, good survival (83 to 100%) was obtained below 30ºC and above 25 psu of salinity. Despite the differences, the conditions of temperature and salinity for good growth were approximately 25 to 30ºC and 25 to 45 psu.

The growth and survival rates achieved in this work may be considered acceptable in comparison with those reported in other similar studies for the same species (Garduño-Dionate *et al.*, 2010; Castillo-Vargasmachuca *et al.*, 2012). The water temperature presented a considerable effect on overall activity,

**Table 1.** Growth, production and survival parameters of *L. peru* at different temperatures and salinities for 60 days, in a recirculating system.

| Temperature (ºC) | 25 | | | 30 | | |
|---|---|---|---|---|---|---|
| Salinity (psu) | 25 | 35 | 45 | 25 | 35 | 45 |
| $L_i$ (cm) | $17.8 \pm 1.2^a$ | $18.2 \pm 0.8^a$ | $17.6 \pm 1.0^a$ | $18.4 \pm 0.5^a$ | $17.5 \pm 1.4^a$ | $18.1 \pm 0.3^a$ |
| $L_f$ (cm) | $25.5 \pm 1.4^a$ | $25.9 \pm 1.0^a$ | $25.8 \pm 0.7^a$ | $26.8 \pm 0.7^a$ | $27.0 \pm 0.9^a$ | $26.6 \pm 0.8^a$ |
| $W_i$ (g) | $67.4 \pm 2.5^a$ | $69.3 \pm 1.9^a$ | $67.5 \pm 2.1^a$ | $68.8 \pm 1.2^a$ | $69.9 \pm 1.7^a$ | $68.4 \pm 1.0^a$ |
| $W_f$ (g) | $143.9 \pm 2.5^c$ | $155.1 \pm 1.8^b$ | $145.4 \pm 2.8^c$ | $158.9 \pm 1.3^b$ | $167.1 \pm 1.9^a$ | $156.9 \pm 1.5^b$ |
| FCR | 2.1 | 1.9 | 2.2 | 1.7 | 1.8 | 2.0 |
| Weight gain (g week$^{-1}$) | $9.6 \pm 0.5^c$ | $10.7 \pm 0.5^{b,c}$ | $9.7 \pm 0.3^c$ | $11.3 \pm 0.2^b$ | $12.2 \pm 0.3^a$ | $11.1 \pm 0.4^b$ |
| SGR (% day$^{-1}$) | $1.26 \pm 0.7^b$ | $1.34 \pm 0.6^b$ | $1.28 \pm 0.5^b$ | $1.40 \pm 0.5^a$ | $1.45 \pm 0.7^a$ | $1.39 \pm 0.3^a$ |
| Survival (%) | $75^c$ | $100^a$ | $100^a$ | $75^c$ | $100^a$ | $86^{.b}$ |

Similar letters show no significant difference between means in each row

**Table 2.** Estimated parameters of the length ($L_T$)–weight ($W_T$) relationships ($W_T = a\ L_T^b$, in g and cm) for *L. peru* cultured at different salinities and temperatures for 60 days. Values for the slope (b) are showed.

| Temperature (ºC) | 25 | 25 | 25 | 30 | 30 | 30 |
|---|---|---|---|---|---|---|
| Salinity (psu) | 25 | 35 | 45 | 25 | 35 | 45 |
| Equation | $W_T=0.1176L_T$ | $W_T=0.0073L_T$ | $W_T=0.0402L_T$ | $W_T=0.0101L_T$ | $W_T=0.0045L_T$ | $W_T=0.0087L_T$ |
| b* | 2.824 | 3.141 | 2.892 | 3.063 | 3.349 | 3.103 |
| df** | 62 | 64 | 64 | 63 | 65 | 62 |
| $R^2$ | 0.689 | 0.710 | 0.721 | 0.801 | 0.750 | 0.790 |

*b: Allometric factor; **df = Degrees of freedom; $R^2$ = Coefficient of determination.

food consumption and growth rate. In fact, the effect was lower in red snapper juveniles grown at 25°C compared to those grown at 30°C.

This agrees with findings for this species in the wild (Santamaría-Miranda *et al.*, 2003). The experimental results indicated that *L. peru* may adjust to gradual temperature increments. When the temperature was maintained at 25 to 30ºC, fish did not suffer any effects such as irreversible harm, losing equilibrium, ceasing respiration, or stop food consumption. This range can assure that fish have normal activity and a high survival rate. Results obtained in the laboratory were similar to field data obtained by Garduño-Dionate *et al.* (2010) and Castillo-Vargasmachuca *et al.* (2012).

Serrano *et al.* (2010) indicated that gray snapper *L. griseus*, in laboratory conditions, preferred intermediate salinities in the range of 9 to 23 psu and in extreme salinities they reduce their activity reflecting compensation of higher osmoregulatory cost. The results of this experiment showed that 75 to 100% of red snapper juveniles, with no prior exposure to salinity, were able to survive direct transfer from 25 to 45 psu of salinity. Serrano *et al.* (2011) obtained similar results with the gray snapper, confirming that juvenile acclimated successfully to hypo and hypersaline environment (0-60 psu) after an adjustment of 96 h, and hence should be considered a euryhaline species such as the Pacific red snapper. The adaptation of fish to different environmental salinities induces changes and activation of ion transport mechanisms. This adaptation is usually accompanied by changes in oxygen consumption, suggesting variations in the energetic demands for osmoregulation. The acclimation process of fish after transfer from SW to FW can be divided into two periods: (i) the critical period, during which changes in osmotic parameters occur rapidly, and (ii) the chronic or regulatory period, during which these parameters reach homeostasis (Zhao *et al.*, 2011). Knowledge of the salinity tolerance of any fish species is important, and the implications of our research are useful for the management and cultivation of Pacific red snapper. Although this fish is currently not cultured, our results suggest that technically could be successfully grown in salinities of 25-35 psu.

Many studies have demonstrated the importance of estuaries and coastal lagoons as breeding areas of demersal fish on the continental shelf of tropical and subtropical areas (Gunter, 1967; Bozeman & Dean,

1980). However, in addition to *L. peru*, there are many species that do not enter estuarine environments during their complete life cycle (Longhurst & Pauly, 1987). In this regard, Blaber & Blaber (1981) suggest the existence of an estuarine effect in the coastal zone of the tropics acting as an important breeding area. This is indicated by the presence of many juvenile of various species. There are no previous quantitative evaluations of Pacific red snapper in estuarine environments, and therefore, no comparisons of abundance indexes could be made; however, according to the seasonality in the reproductive intensity of this species (Cruz-Romero *et al.*, 1991; Reyna-Trujillo, 1993), juvenile abundance can vary with the season. This effect influences salinity tolerance and shows the high potential of growing *L. peru* in water ponds (25 to 35 psu) where shrimp culture activity is realized, utilizing the months (November to March) when ponds are empty. Nevertheless, it is necessary to produce fingerlings under laboratory conditions and do not depend on wild juvenile populations.

In general, the results in this study showed that higher specific growth rate was observed in the 30ºC water experimental group, and the greatest survival ($P < 0.05$) was recorded in the 35 and 45 psu trial groups. There was a tendency for better growth at 35 psu of salinity. Significant differences in growth parameters, such as SGR and weight gain, were observed in fish reared at temperatures of 30ºC and salinities of 35 and 45 psu. Although it seems reasonable to accept that increased salinity beyond 45 psu, could slow the growth of the Pacific red snapper used in this trial. Salinity tolerance under 35 psu showed *L. peru* has the potential to grow in these waters and may be produced in shrimp brackish ponds as a new alternative to increase marine aquaculture.

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