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Research Article

Influence of the temperature on the early larval development of the Pacific red snapper, *Lutjanus peru* (Nichols & Murphy, 1922)

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ABSTRACT. The Pacific red snapper, *Lutjanus peru*, is a commercially important species throughout its distribution range, making it a good alternative for aquaculture; however, there is few information regarding environmental conditions and their influence on early development of this species. Temperature is one of the main factors affecting embryo and larval development in marine fishes. In this paper, the effects of different temperatures upon hatching rate, growth, consumption of yolk sac and oil droplet and the formation of the digestive system and eye pigmentation were evaluated in larvae of this species under experimental conditions. Eggs incubated between 20 and 32°C showed hatching rates higher than 90%. However, larvae maintained at 26°C showed significantly larger notochord length and were the first to complete the pigmentation of the eyes and the formation of the digestive system when still possessing enough reserves in the yolk sac. Therefore, according to the results obtained, it is recommended that the incubation of eggs and larval rearing in Pacific red snapper takes place between 25 and 26°C.

Keywords: *Lutjanus peru*, temperature, larval development, hatching, survival, growth, aquaculture.

Influencia de la temperatura sobre el desarrollo larval temprano del huachinango del Pacífico, *Lutjanus peru* (Nichols & Murphy, 1922)

RESUMEN. El huachinango del Pacífico, *Lutjanus peru*, es una especie de importancia comercial a lo largo de su rango de distribución, lo cual lo hace un buen candidato para la acuicultura. Sin embargo, existe escasa información acerca de las condiciones ambientales y su influencia sobre el desarrollo temprano de esta especie. La temperatura es uno de los principales factores que afectan el desarrollo embrionario y larval en los peces marinos. En este trabajo se evaluaron los efectos de diferentes temperaturas sobre la tasa de eclosión, crecimiento, consumo del saco vitelino y de la gota lipídica, formación del sistema digestivo y pigmentación de los ojos en larvas de esta especie bajo condiciones experimentales. Los huevos incubados entre 20 y 32°C presentaron tasas de eclosión mayores al 90%. Sin embargo, las larvas mantenidas a 26°C presentaron una longitud notocordal significativamente mayor y fueron las primeras en completar la pigmentación de los ojos y la formación del sistema digestivo cuando aún poseían suficientes reservas en el saco vitelino. De acuerdo a los resultados obtenidos se recomienda que la incubación de huevos y la cría larval del huachinango del Pacífico se realice entre 25 y 26°C.

Palabras clave: *Lutjanus peru*, temperatura, desarrollo de larvas, cultivo, supervivencia, crecimiento, acuicultura.

INTRODUCTION

Species belonging to the Lutjanidae family are prized for human consumption and they are of great importance tance to fisheries and aquaculture in coastal areas of Latin America and the Caribbean (Bennetti *et al*., 2001). Particularly, the Pacific red snapper, *Lutjanus peru*, is one of the most desirable species throughout its

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distribution range from Bahía Magdalena in the Baja California Peninsula, Mexico, to the north coast of Peru (Allen, 1985; Díaz-Uribe *et al*., 2004), and in recent years there has been increased interest in developing its cultivation.

There has been progress in the management of this species breeding. For example, successful gonad maturity has been developed in captivity; spawning have been obtained through the application of Human gonadotropic hormone (HGH) and high fertilization and hatching rates (>90% and >85% respectively) have been obtained as well (Píntos-Terán *et al*., 2003; Dumas *et al*., 2004). However, as in most marine fish species, one of the biggest problems they face is the low larval survival.

In this context, temperature management plays a fundamental role in the production of eggs and larvae of good quality, since this is one of the factors which significantly affects growth and development of larvae (Herzing & Winkler, 1986). Temperature has also a direct influence on the fertilization rates and hatching of eggs (Hart & Purser, 1995), embryonic development (Brown *et al*., 2011), larval size at hatching (Hansen & Falk-Petersen, 2001), absorption time of the yolk sac and oil droplet (Pauly & Pullin, 1988), and the efficient use of energy reserves (Heming, 1982).

Therefore, the aim of this study was to evaluate the effect of different temperatures on the hatching rate, notochord length, yolk sac volume, diameter of the oil droplet, growth and survival and development of the digestive system and eye pigmentation in larvae of the Pacific red snapper, *L. peru*, produced under laboratory conditions.

MATERIALS AND METHODS

Broodstock management and egg collection

The eggs and larvae of Pacific red snapper used to conduct this study were obtained in May 2011from a natural spawning of 20 brooders, 10 females (4.32 ± 1.10 kg of body weight and 64.15 ± 3.73 cm of total length) and 10 males (5.61 ± 0.32 kg of body weight and 69.76 ± 1.10 cm of total length). Fish were caught in wild conditions five years earlier and kept in captivity at Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, Mexico. All specimens were stocked in a rectangular 120 m^3 pond with 10% water exchanged daily, under natural photoperiod and temperature (24°08'N, 110°25'W). Two months before spawning, brooders were fed daily to satiation with squid and sardine. Eggs were deposited by gravity into an adjacent container to the pond. At the beginning of the experiment, a sample of eggs (n = 100) was taken. Eggs were photographed with a digital camera (Cool SNAP-Pro color Media Cybernetics®) mounted on an optical microscope (Media Cybernetics®). The images were processed manually with the Image-Pro Plus version 5.0 (Media Cybernetics®) which measured the diameter of the eggs and the oil droplet.

Experimental design

In order to assess the effect of the temperature on the Pacific red snapper larval development, 10 treatments of temperature with three replicates were evaluated: 10 ± 1°C, 13 ± 1°C, 16 ± 1°C, 20 ± 1°C, 23 ± 1°C, 26 ± 1°C, 29 ± 1°C, 32 ± 1°C, 35 ± 1°C and 38 ± 1°C. The experiment was conducted in a closed system of incubation into a laboratory with air-conditioned to 20°C and artificial illumination with 18 h of light and 6 of darkness.

A vessel of 250 L capacity with fresh water at the desired temperature was used for each treatment. In treatments T1 to T3, every two hours the temperature was measured; if temperature rose, crushed ice was added to maintain temperatures of 10, 13 and 16°C. In order to maintain temperatures of treatments T5 to T10, submersibles heaters with thermostat (Thermo Jet 300W, Lomas®) were used to achieve desired temperatures. In the vessel of each treatment, three glass bottles of 2.5 L with seawater at 35 ± 1 filtered to 1 µm and sterilized with UV light and constant aeration where fertilized eggs (n = 200 $bottle^{-1}$) were stocked. During experiment, the larvae were not fed.

Hatch rate

The number of larvae in every bottled was counted to obtain the hatch rate determined by the following relationship:

$$Hatch\ rate(\%) = \left(\frac{hatched\ larvae}{200}\right)100$$

where: 200 is the number of eggs stocked in every bottle.

Notochord length-growth

A sample of larvae (n = 15) was taken from one of the glass bottles of each treatment at different times: at the time of hatching, *i.e.*, 0 h after hatching (HAH), 6 HAH, 18 HAH, 30 HAH, 42 HAH, 66 HAH, 72 HAH, and 114 HAH to measure notochord length. The larvae were photographed with a digital camera (Cool SNAP-Pro color Media Cybernetics®) mounted on an optical microscope (Media Cybernetics®). The images were processed manually with the Image-Pro Plus version 5.0 (Media Cybernetics®). The growth (*GR*) was calculated using the following formula:

$$GR(mm/day) = \frac{Lf - Lh}{t}$$

where: *Lf* is the final notochord length of larvae (mm); *Lh* is the notochord length of larvae (mm) at hatching.

Consumption of the yolk sac and oil droplet

In order to determine the consumption of the yolk sac and oil droplet, samples of larvae were taken and photographed at the same time as described above. The length and width of the yolk sac and oil droplet were measured in each treatment. The following formula was used to calculate the yolk sac volume (*YSV*) (Blaxter & Hempal, 1966):

$$YSV\ (mm^3) = \frac{\pi}{6L\ W^2}$$

where *L*: yolk sac length (mm); *W*: yolk sac width (mm).

To calculate the oil droplet volume (*ODV*), the formula described by Williams *et al*. (2004) was used, as follows:

$$ODV(mm^3) = \frac{4}{3}\pi r^3$$

where *r* is the oil droplet radius (mm).

Larval survival, digestive system development, and eye pigmentation

Two replicates were allocated per treatment to assess the effect of temperature on larval survival. These replicates were sampled until 96 HAH, to count the number of live and dead larvae to determine survival:

$$Survival(\%) = \frac{LV}{LV + LD}\ 100$$

where *LV*: number of live larvae; *LD*: number of dead larvae. Photographs were taken to determine the volume of the yolk sac and oil droplet, aimed to assess the development of digestive system, and eye pigmentation at each experimental temperature.

Statistical analysis

Results are expressed as mean ± standard deviation. The normality of data was tested by the Kolmogorov-Smirnov method. The treatment means were compared by analysis of variance (ANOVA) ($P < 0.05$). The Tukey multiple comparison tests were used to evaluate the significance of the differences observed between treatments, when the null hypothesis was rejected. All statistics were performed with Statistica for Windows, version 6.0 and graphs were built with SigmaPlot for Windows, version 11.0.

RESULTS

The diameter of the eggs sampled at the beginning of the experiment was 809.6 ± 2.7 µm, and the oil drop diameter was 133.2 ± 0.8 µm.

Effect of temperature on the hatch rate (%)

Eggs incubated at 10 and 38°C failed to hatch. Eggs held from 20 to 32°C showed hatching rates higher than 90%, significantly higher ($P < 0.05$) than those observed in the treatments of 13, 16 and 35°C. However, in the latter treatment (36°C), the eggs reached hatching, but the larvae died shortly after. Therefore, for egg incubation in *L. peru*, we can distinguish three types of temperatures: a) Lethal: temperatures where no hatching was observed (10 and 38°C), b) Critical: temperatures where hatching rates were lower than 50% (13, 16, and 35°C), and c) Comfort: temperatures where the hatching rate was above 90% (20, 23, 26, 29, and 32°C) (Table 1). The relationship between temperature and hatching rate was adjusted to a parabolic equation ($R^2 = 0.92$), from this, it was predicted that the maximum hatching percentage occurred at 24.8°C (Fig. 1).

Effect of temperature on the notochord length, yolk sac volume and the oil droplet at hatching

At hatching, the larvae at 23°C showed a notochord length of 2.03 ± 0.02 mm, being significantly larger ($P < 0.05$); however, the larvae maintained at 26°C had the highest yolk sac volume (0.13 ± 0.02 mm^3). No significant differences were observed in the volume of the oil droplet in all treatments (Table 2).

Effect of temperature on the increase in the notochord length

Increase in notochord length of the larvae was directly related to incubation temperature. The notochord length observed in larvae at 26°C was 2.91 ± 0.01 mm, which was significantly higher ($P < 0.05$) than those recorded in the other treatments at 120 HAH (Fig. 2). From 18 HAH, the notochord length at 20, 23, 26 and 29°C, showed no significant variations until the end of the experiment, whereas larvae maintained at 32°C, showed no significant increases at 6 HAH. The latter treatment showed the significantly smaller notochord length ($P < 0.05$) (Fig. 2).

Effect of temperature on the consumption of yolk sac volume and oil droplet

The yolk sac consumption was adjusted to an exponential decay curve in all treatments. The lower yolk sac volume was observed at 72 HAH in larva at 20 ($R^2 = 0.98$), 23 ($R^2 = 0.99$) and 26°C ($R^2 = 0.95$), deple-

Table 1. Hatch rates (%) in eggs of Pacific red snapper, *L. peru*, at different experimental temperatures (°C). Different letters shows significant differences among treatments (ANOVA, Tukey's test $P < 0.05$).

Temperature (°C)	Hatching rates (%)
10	0
13	10.2 ± 4.1^{d}
16	46.3 ± 4.8^{b}
20	92.5 ± 3.9^{a}
23	95.7 ± 4.2^{a}
26	92.3 ± 3.7^{a}
29	91.8 ± 4.5^{a}
32	90.1 ± 4.1^{a}
35	30.5 ± 4.4^{c}
38	0

ting around 90 HAH, while larvae at 29 ($R^2 = 0.99$) and 32°C ($R^2 = 0.99$) depleted the yolk sac at 42 HAH (Fig.3).

Oil droplet consumption was adjusted to a sigmoid function. At 114 HAH, the lower oil droplet volumes in larvae at 20 ($R^2 = 0.95$), 23 ($R^2 = 0.99$) and 26°C ($R^2 = 0.97$) were recorded, whereas larvae at 29 ($R^2 = 0.99$) and 32°C ($R^2 = 0.95$) had already exhausted the reserves (Fig. 4).

Effect of temperature on larval survival rate (%) and survival time (HAH)

No significant difference was observed in the survival of larvae at the different treatments on the fourth day of the experiment (96 HAH). However, in the treatments of 23 and 26°C the larvae were still alive at 114 and 162 HAH, respectively, followed by treatments of 20, 29 and 32°C with 72 HAH each other (Table 3).

Effect of temperature on the digestive system development and eye pigmentation

Results about the influence of the incubation temperature on the digestive system development and eye pigmentation are summarized in Table 4. Larvae at 26°C completed the pigmentation of the eyes and digestive system formation at 42 HAH, when they still had enough yolk sac reserves, followed by the larva at 23°C, completing these processes at 66 HAH, just when they exhausted their yolk reserves. On the other hand, larvae at 29 and 32°C began the digestive system development and pigmentation of the eyes when they had exhausted their reserves.

DISCUSSION

A natural spawning from breeders of the Pacific red snapper kept in captivity was obtained, the females being

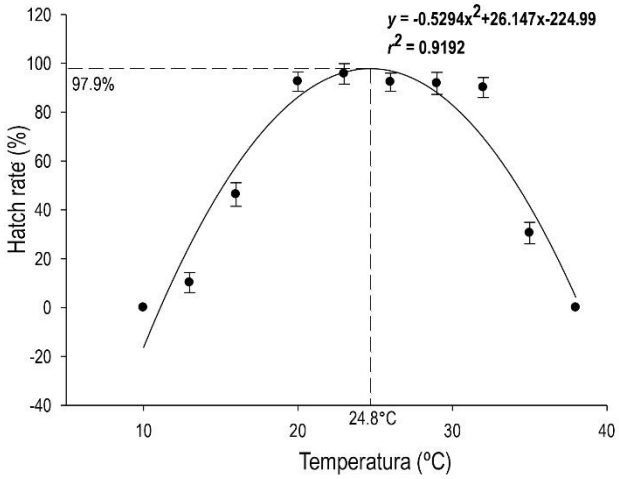

Figure 1. Hatch rate (%) in eggs of Pacific red snapper, *L. peru*, at different temperatures. Spotted line showing the optimal hatching temperature (°C) and maximal hatch rate (%) obtained. A second order polynomial regression defined the relationship between hatching rate (%) with temperature (°C).

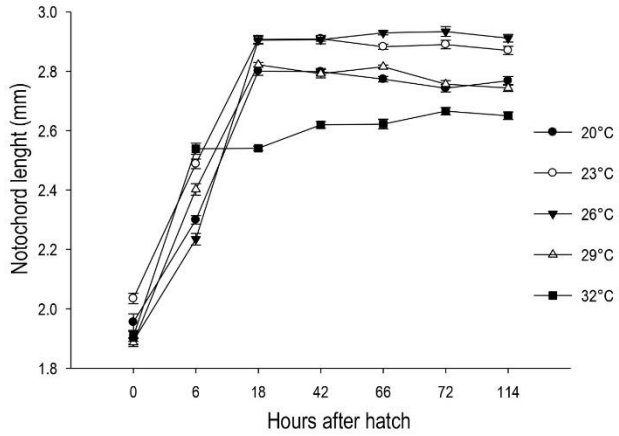

Figure 2. Notochord length (mm) in larvae of Pacific red snapper, *L. peru*, at different temperatures.

64 cm of total length with an average eggs diameter of 810 µm. This represents a major advance, since success spawning had only been obtained by human chorionic gonadotropin (HCG) applied to breeders about 40 cm of size with doses of 1500 IU kg^{-1}, gathering an average egg diameter of about 930 µm (Pintos-Terán *et al.*, 2003; Dumas *et al.*, 2004; Zabala-Leal *et al.*, 2009) which is significantly higher than those obtained in this study.

Contrary to the above, Papanikos *et al.* (2003, 2008) found that eggs obtained naturally were larger than those obtained by hormonal applications, in the northern red snapper, *Lutjanus campechanus*. However, the differences between the average eggs diameters re-

Table 2. Notochod length (mm), yolk sac (mm^3) and oil droplet volume (mm^3) in larvae of Pacific red snapper, *L. peru*, at hatch (0 hours after hatch). Different letters shows significant differences among treatments (ANOVA, Tukey's test $P < 0.05$).

Temperature (°C)	Notochord lenght (mm)	Yolk sac volume (mm^3)	Oil droplet volume (mm^3)
20	1.95 ± 0.03 b	0.09 ± 0.01 b	1.38E-03 ± 7.00E-05 a
23	2.03 ± 0.02 a	0.1 ± 0.01 b	1.36E-03 ± 1.00E-04 a
26	1.89 ± 0.01 c	0.13 ± 0.02 a	1.46E-03 ± 4.00E-05 a
29	1.89 ± 0.01 c	0.11 ± 0.02 a,b	1.45E-03 ± 7.00E-05 a
32	1.91 ± 0.01 c	0.11 ± 0.02 a,b	1.42E-03 ± 5.00E-05 a

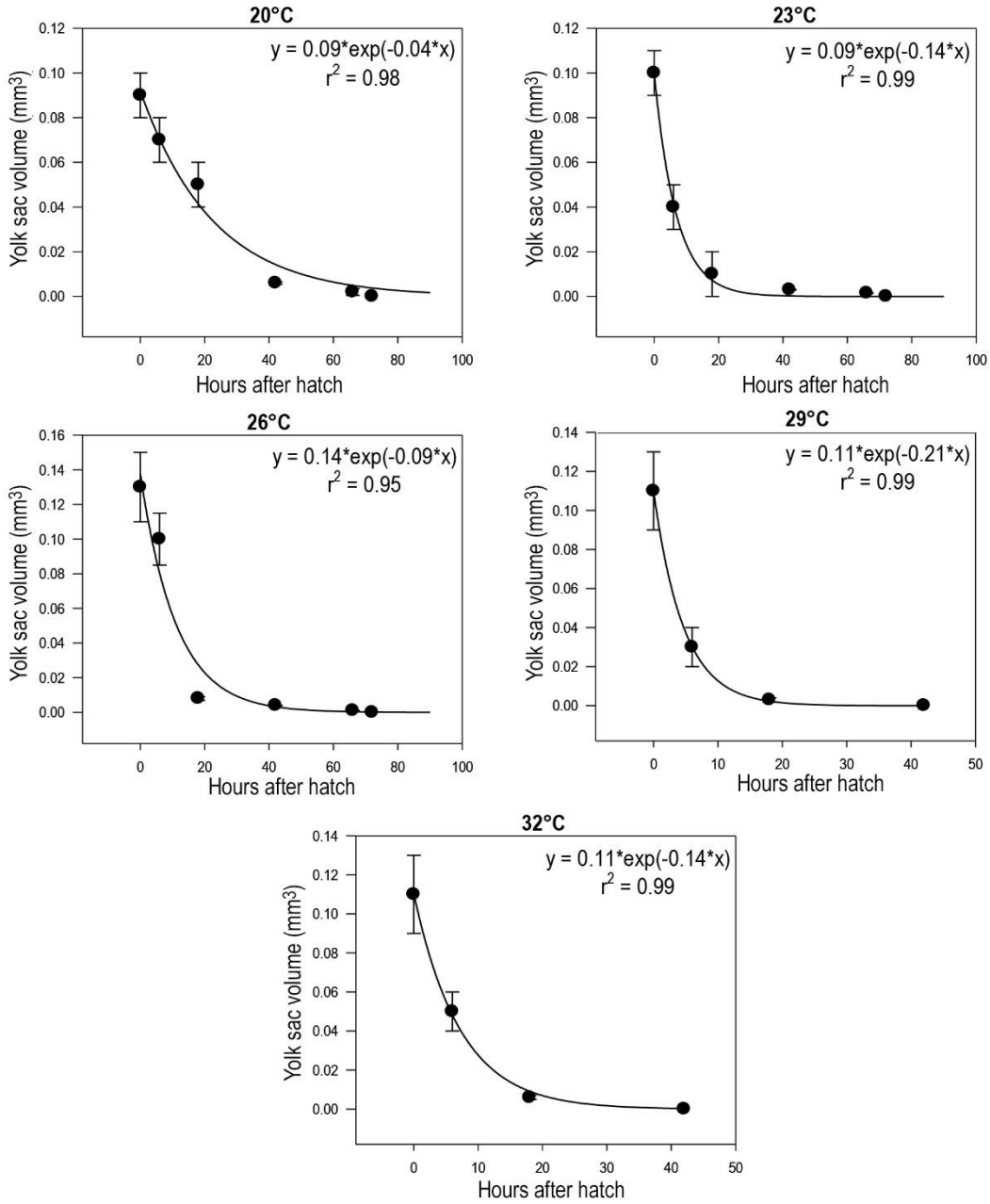

Figure 3. Yolk sac consumption of Pacific red snapper larvae, *L. peru*, at different temperatures. Regression analysis defined the relationship between yolk sac volumes (mm^3) with temperature (°C).

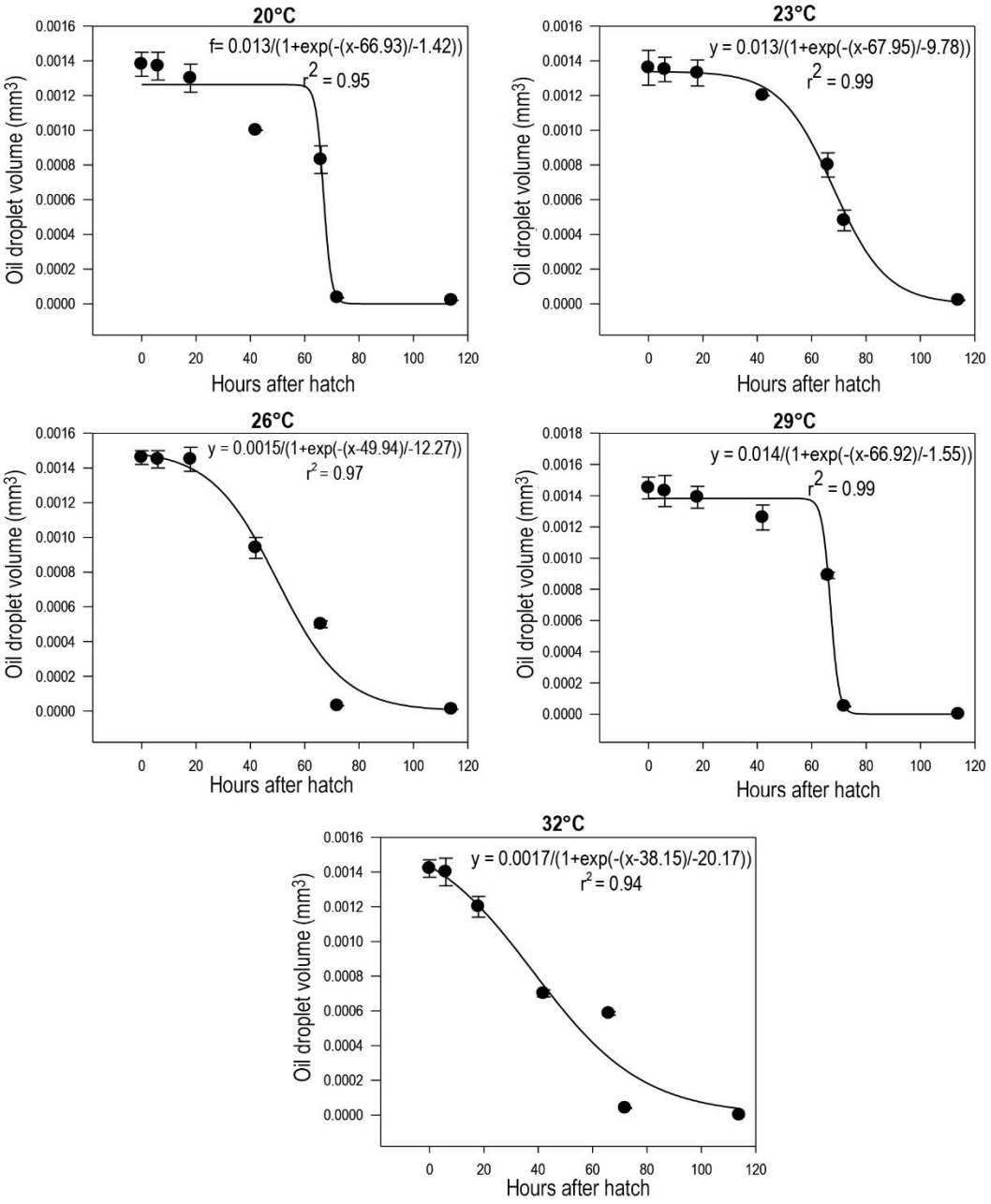

Figure 4. Oil droplet consumption of Pacific red snapper larvae, *L. peru*, at different temperatures. Regression analysis defined the relationship between oil droplet volumes (mm^3) with temperature (°C).

corded in this paper regard recorded by hormonal treatments are mainly due to the nutritional status of the broodstock, because in this work, the brooders were only fed with fresh sardine and squid like Dumas *et al*. (2004) reported, but these authors prepared a mix of vitamins which was added to the food and offered to the fish once a week (1% kg^{-1} food), improving the eggs quality on this way. Therefore, it is important to provide an adequate nutrition to the broodstock because the nutritional status may affect significantly the reproductive performance and the egg quality in marine fishes (Izquierdo *et al*., 2001; Watanabe & Vassallo-Agius, 2003; Álvarez-Lajonchere, 2011).

The spawning season for the Pacific red snapper is from April to June in the Gulf of California, with water temperatures ranging 20-30°C (Díaz-Uribe *et al*., 2004). In this paper, hatching rates higher than 90% were achieved at similar temperatures to that found under natural conditions. Similarly, hatching rates about 90% at temperatures from 28 to 30°C have been

Table 3. Survival rate (%) and survival time (HAH) of Pacific red snapper larvae, *L. peru*, at different experi-mental temperatures (°C). HAH: hours after hatch. Different letters shows significant differences among treatments (ANOVA, Tukey's test $P < 0.05$).

Temperature (°C)	Survival rate (%)	Survival time (HAH)
20	85 ± 4.3[a]	96
23	88 ± 5.1[a]	168
26	86 ± 5.8[a]	120
29	85 ± 6.1[a]	96
32	81 ± 6.3[a]	96

Table 4. Pacific red snapper, *L. peru*, yolk sac absorption (HAH), digestive system formation, and eye pigmentation in larvae of at different experimental temperatures (°C). HAH: hours after hatch. BDSD: beginning of the digestive system development, BEP: beginning of the eye pigmentation, CDSF: complete digestive system formation, CEP: complete eye pigmentation.

Temperature (°C)	Yolk sac consumption (HAH)	18 HAH	30 HAH	42 HAH	66 HAH	72 HAH
20	66			BDSD	BEP/CDSF	BEP
23	66			BEP/BDSD	CEP/CDSF	
26	66		BEP/BDSD	CEP/CDSF		
29	18	BEP/BDSD	CEP/CDSF			
32	18	BEP/BDSD	CEP/CDSF			

reported for the rose spotted snapper, *L. guttatus* (García-Ortega *et al*., 2005). Nevertheless, Pintos-Terán *et al*. (2003) and Dumas *et al*. (2004), reported slightly lower hatch rates (>85%) in eggs of *L. peru*, incubated at 25°C and obtained by hormonal induction. These results reflect a possible effect of hormonal treatments on egg hatching rate in the Pacific red snapper.

Likewise, larvae of *L. peru* incubated at 23°C showed the larger notochord length at hatching time, reducing significantly as the temperature raised. However, the larvae at 26°C showed a larger notochord length at the end of the experiment, and also showed a greater yolk sac volume at hatching. Therefore, there is a direct relationship between the yolk sac volume at hatching with growth of the larvae, which is consistent with results reported in other species of snappers as the Northern snapper, *L. campechanus* (Rabalais *et al*., 1980; Williams *et al*., 2004), the common bluestripe snapper, *L. kasmira* (Suzuki & Hioki, 1979) and in the rose spotted snapper, *L. guttatus* (Álvarez-Lajonchere *et al*., 2011) and larvae of other species such as the flathead grey mullet, *Mugil cephalus* (Walsh *et al*., 1991), the Nassau grouper, *Epinephelus striatus* (Watanabe *et al*., 1995) and the leopard grouper, *Mycteroperca rosacea* (Gracia-López *et al*., 2004).

A direct relationship between the oil droplet diameter and temperature has been found in newly hatched larvae of leopard grouper, *M. rosacea*, since larvae incubated at higher temperatures had higher oil droplet volume because they had a shorter incubation period (Gracia-López *et al*., 2004). However, in this study, no significant differences in oil droplet volume were found in larvae of *L. peru* at different temperatures, indicating that the effect of temperature on oil droplet consumption and the efficiency in the use of the same, varies among species as previously established by Blaxter (1969).

Moreover, larvae maintained at 26°C were the first to complete the pigmentation of the eyes and the formation of digestive system, when counting with enough yolk sac reserves, coinciding with that reported by Zabala-Leal *et al*. (2009) in a previous work on this species. Those results means an advantage, since the larvae incubated at 26°C, have enough energy to start searching for prey, resulting in an improved feed efficiency. In the bluefin tuna, *Thunnus orientalis*, the onset of exogenous feeding coincides with the complete differentiation and thickening of the temporal area of the retina (Kawamura *et al*., 2003). In this sense, several authors have noted that when the digestive system is complete, which culminates with the opening of the mouth, the eyes are fully pigmented and consists of simple conical cells (Porter & Theilacker 1999; Roo *et al*., 1999; Moorman, 2001; Kawamura *et al*., 2003; Peña & Dumas, 2007).

In conclusion, our results suggest that the optimal temperature for incubation of eggs of *L. peru*, in laboratory conditions, is around 25°C, obtaining good growth and survival rates at 26°C. However, in addition to evaluating the effects of environmental factors on the embryonic and larval development in this species, in necessary to design and test diets suitable for the broodstock in order to improve the quality of the spawning and thus more production.

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