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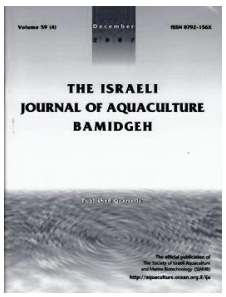

Timing of Puberty and Reproductive Performance in Wild-Reared and Hatchery-Reared Pacific Red Snapper *Lutjanus peru*

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Keywords: *Lutjanus peru*; puberty; reproduction; sex steroids

Abstract

A ten-year study (2005 to 2015) was conducted to examine the timing of puberty and reproductive performance in Pacific red snapper *Lutjanus peru*. Wild young juveniles reared in a 200 m^3 lined-pond from 2005 were captured and spawned over the next three consecutive years, starting in 2009. The study continued in hatchery-reared red snappers from the 2010 progenies that spawned in 2014. In wild-reared and hatchery-reared red snapper, puberty occurred in both sexes at three years and nine months. In a pond, eight wild-reared females had on average 54 days of broadcast spawning, which corresponded to 0.276 M viable eggs per day. Each female had on average 1.838 million (M) viable eggs, and a relative fecundity of 0.555 M eggs/kg yielding 0.010 M eggs/kg per spawn. All wild-reared males (n = 22) had running sperm. In progeny hatchery-reared red snapper, nine females had vitellogenic oocytes, but only three reproduced. Ninety percent of hatchery-reared males (n=31) had running sperm. The ratio of 11-ketosterone to estradiol allowed identification or corroboration of sex. Results suggest *L. peru* synchronizes reproduction according to photoperiod and water temperature. This species (2.3+ kg, 52.0+ cm) is a potential candidate for sustainable aquaculture, including rearing, and reproduction in captivity.

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Introduction

Snappers are economically important marine fish which belong to the family Lutjanidae, which comprises about 105 species and are found in tropical and subtropical waters (Allen, 1985; Nelson, 2006). The genus *Lutjanus* (Bloch) includes 65 species, of which, ten inhabit the eastern Pacific, from the southern coasts of the Baja California Peninsula in Mexico to the coast of Peru (Allen, 1985). Commercial fisheries, local fishermen, and recreational fishermen harvest snappers because of their high market value (Allen, 1985). Red snappers such as the Pacific red snapper *Lutjanus peru* (Nichols and Murphy), the Pacific colorado snapper *Lutjanus colorado* (Jordan and Gilbert), and the spotted rose snapper *L. guttatus* (Steindachner) (Boza-Abarca et al., 2008; Ibarra-Castro and Alvarez-Lajonchere, 2009) are in high demand in international markets and restaurants.

Commercial fishing of *L. peru* can only be sustained in the southern Gulf of California if measures are taken, limiting and controlling both local and commercial fishing (Díaz-Uribe et al., 2004). Research and private resources have been invested in this species for commercial fish farming along the Pacific coast.

Maturation in captivity of wild *L. peru* broodstock was first achieved through manipulation of temperature and photoperiod. Spawning was induced by intramuscular injections of human chorionic gonadotropin (Dumas et al., 2004). Further research showed that this and other lutjanid species such as *L. argentiventris* (Peters) (Guerrero-Tortolero et al., 2010), *L. aratus* (Günther), and *L. colorado* spawned periodically during summer in our aquaculture ponds at CIBNOR. There is no information on age, size, and weight at puberty of these wild snappers and their progeny. Therefore, this study analyzed the timing of puberty and the reproduction performance of wild-reared and hatchery-reared Pacific red snapper *L. peru*.

Materials and Methods

Fish handling. For ease of handling the red snapper were anesthetized using clove oil (Eugenol) stock solution (100 mg/mL) at 5–25 mg/L until fish were immobilized. They were then wrapped in wet towels and moved in a thick tarpaulin bag to avoid damage to eyes and skin.

Capture and rearing of wild L. peru. 120 young juvenile *L. peru* (body weight ~0.2 kg, body length ~22 cm) were captured off the coast of Isla Espiritu Santo and Isla Partida in the southern Gulf of California (24°29’ N, 110°24’ W) in June 2005. Fish were held in 1000 L aerated water tanks, and transported by truck to our research center where they were placed in an outdoor pond. Fish were maintained under ambient photoperiod and water temperature for seven years, where the summer solstice photoperiod is ~13.5 h and winter solstice is ~10.5 h. Water temperature in the pond fluctuated between 21-24°C during winter and spring, and increased gradually during early summer to 26–28°C, reaching 29–31°C during late summer. The temperature dropped rapidly after mid-October. Fish were reared in an outdoor pond (15 m × 8 m × 1.8 m), lined with plastic sheeting and covered with 60% shade cloth to provided refuge and reduce stress. Daily water exchange was 10% with constant aeration. Fish were fed chopped Humboldt squid *Dosidicus gigas* (D'Orbigny) to satiation three times a week by hand; occasionally, South American pilchard *Sardinops sagax* (Jenyns) was added.

Production and rearing of hatchery red snapper. In July of the second spawning year of wild-reared red snapper (age 5 in 2010), approximately 200,000 floating spawned eggs were collected from the pond. After larval rearing and nursing the fingerlings, 145 juveniles were transferred on day 54 to a pond and reared at a low density of ~1 fish/m (Leu et al., 2003).

Reproduction facilities. Wild-reared (2009-2011) and hatchery-reared (2014-2015) red snapper were allowed to spawn spontaneously under natural photoperiod and water temperature in order to record the timing of fish puberty and reproduction. Twenty-eight hatchery-reared 3 year old fish were randomly chosen and divided into two 7000 L tanks with a water recirculation system, a shadow roof net, and an egg collector at the water surface. Twelve fish were left in the pond.

Measurement of spawns. In wild-reared fish, the average number of eggs per female per annum was recorded. Relative fecundity was calculated as total number of eggs

divided by the biomass of females and expressed as eggs/kg and eggs/kg per spawn. In hatchery-reared fish, each female was tagged with an electronic tag (Avid Identification Systems, Norco, CA). Therefore, absolute fecundity was calculated as eggs/kg and eggs/kg per spawn.

Identification of sex. Since lutjanids have no sexual dimorphism, mature red snapper were anesthetized, and the urogenital papilla was observed by gently applying abdominal massage to obtain running sperm, or by an ovarian biopsy (Dumas et al., 2004), and by dissecting gonads in wild-reared dead fish. When neither sperm nor oocytes were observed, the gender of the fish was determined by determining the ratio of 11-ketosterone to estradiol (see section below). Blood samples were taken from two spawning females, four running males, and three unidentified hatchery-reared fish. For comparison, three wild vitellogenic females and three wild males showing a small amount of sperm were also analyzed. Peripheral blood was collected from the caudal vein with a heparin-coated 23-gauge needle attached to a 3.0 mL syringe. The samples were centrifuged at 750 × *g* for 15 min, and the plasma stored at –80 °C until analyzed.

Measurements of sex steroids. Sex steroids were extracted from 150 µL of plasma by adding 1.0 mL diethyl ether and incubated for 1 h with slow rotation at room temperature and then lyophilized and stored at 4°C. Kits from Cayman Chemical (Ann Arbor, MI) were used to measure levels of 17β-estradiol (E_2; #582251), 11-ketotestosterone (11-KT; #582751), and testosterone (T; #582701), which have 100% cross-reactivity of the antibodies.

Effect of monthly increase of temperatures in spawning. Water temperature was measured five times a week at 9:00 AM and 17:00 PM and the average provided the daily and monthly temperature in the pond and tanks. In wild-reared red snapper, the number of eggs per broadcast spawning was compared among the three reproductive years. The effect of average monthly water temperature increase from ~27 to ~31 °C from June to September on the number of eggs per broadcast spawning was then analyzed. The effect on the number of eggs per spawn in hatchery-reared female # 2 which spawned from June through August, and # 3, from July through September was recorded. Parameters included the mean diameter of the fertilized egg and the single oil droplet (n = 30 eggs per spawn). Diameters were obtained by using ImageJ software. A one-way ANOVA was used for each analysis, with a previous assessment of normality with the Kolmogorov–Smirnov test and homogeneity of variance with the Bartlett's test. Further differences between the means were assessed by Tukey's pair-wise comparisons, and statistical significance set at $P < 0.05$, using software SPSS 17.0 (IBM SPSS, Armonk, NY).

Results

Age, weight, and length at puberty. In wild-reared and hatchery-reared red snapper, puberty occurred in both sexes at three years and nine months in captivity. Spawning started by the summer solstice and occurred after dusk, from June (~27 °C) to September (~31 °C). Thirty wild-reared red snapper survived to age four (25%). Reproduction started in 2009 and was repeated in the following two years (2010-2011). From previous measurements of dead fish, a body weight of ~2.9 kg and a total length of ~58.0 cm was estimated in 2009. By gender, females measured ~2.6 kg, ~55 cm, and males ~3.0 kg; ~60 cm. Eight females and 22 males were recorded after seven years in captivity. Forty (17%) hatchery-reared red snapper survived to age four with a mean body weight of 2.7 ± 0.4 kg and a mean total length of 56 ± 3.0 cm. Nine females, and 31 males had a mean body weight of 2.4 ± 0.1 kg and 2.7 ± 0.5 kg, and a mean total length of 53.0 ± 2.0 cm and 56 ± 3.0 cm, respectively. The primary diseases detected were skin fluke (Monogenea: Capsalidae) (Ogawa and Yokoyama, 1998), and swollen eyes (pop-eyes) which led to blindness, and one male had scoliosis.

Analysis of spawning. The reproductive performance of red snapper fish is presented in Fig. 1. Fecundity data in wild-reared and hatchery-reared fish is shown in Tables 1 and 2 respectively. In wild-reared females, there was no significant difference in the number of eggs per broadcast spawning between the three reproductive years ($P > 0.05$). There

was however a significant difference when average monthly water temperature data were analyzed ($P < 0.01$) (Fig.1). In two hatchery-reared females (#2 and #3), the monthly water temperature effect was not significant ($P > 0.05$).

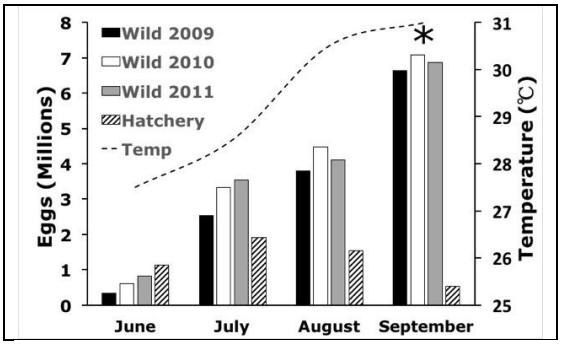

Fig. 1. Reproductive performance of eight wild-reared females and three hatchery-reared females *Lutjanus peru*. In wild-reared females, September (mean: 31.0 °C) was the month with the highest number of eggs per broadcast spawning (Asterisk; $P < 0.01$), while June (mean: 27.5 °C) and July (mean: 28.0 °C) were the lowest, and August (mean: 29.5 °C) was intermediate between these two periods. Additionally, September was the month with the highest number of days of spawning with 58, followed by 47 in July, 45 in August, and 14 in June.

Female #2 had 22 spawns, of which six were in June (mean: 27.5°C), nine in July (mean: 28.5°C), and seven in August (mean: 30.5°C), when held with 13 males in a tank. Courtship behavior occurred in daylight and continued till after dusk. Female #3 spawned in 2014, but these were not recorded. In 2015, it produced 17 spawns, of which seven were in July (mean: 28.0 °C), six in August (mean: 29.5 °C), and four in early September (mean: 30.0 °C).It was held in a pond with ten males (and one female that died in 2014). The mean egg diameters for these females were 803 ± 14 µm and 816 ± 10 µm, and the mean oil droplet diameters were 117 ± 5 µm, and 115 ± 4 µm, respectively.These diameters did not significantly vary by month ($P > 0.05$). Female #1 had five poor spawns, two by the end of May and three in mid-June (mean: 28.0 °C).It was held with five vitellogenic, but not-spawning females, and eight males in a tank.

Table 1. Fecundity in wild-reared Pacific red snapper *Lutjanus peru*.

	Females in pond (n = 8)			
	2009	*2010*	*2011*	*Average*
Mean female weight (Kg)	~2.60	~3.40	~4.20	3.40
Total biomass in the pond (Kg)	20.80	27.20	33.60	27.20
Days with broadcast spawning	55	59	47	54.0
Viable eggs (M)	13.302	15.485	15.352	14.713
Eggs per broadcast spawning (M)	0.242	0.262	0.326	0.276
Average of eggs per female (M)	1.662	1.935	1.919	1.838
Relative fecundity (M eggs/ kg)	0.639	0.569	0.457	0.555
Relative fecundity per spawn (M eggs/kg per day-spawn)	0.0116	0.0096	0.0097	0.0103

Table 2. Fecundity in hatchery-reared Pacific red snapper *Lutjanus peru*.

	Hatchery-reared female red snapper in tanks			
	Female 1 (Tank 1)	*Female 2 (Tank 2)*	*Female 3 (Pond)*	*Average*
Female weight (Kg)	2.3	2.4	3.3	2.6
No. of days with spawning	5	22	17	15
No. of eggs (M)	0.506	2.898	2.088	1.799
Eggs per day-spawn (M)	0.102	0.132	0.123	0.117
Absolute fecundity (M eggs/kg)	0.220	1.208	0.633	0.677
Absolute fecundity per spawn (M eggs/kg / spawn)	0.044	0.055	0.037	0.045
Viable eggs (%)	55	100	100	85

Sex steroids. At four years, estradiol, testosterone, and 11-ketosterone were detectable in plasma of sampled hatchery-reared and wild fish (Table 3). The average ratio of 11-KT to E_2 in two hatchery-reared spawning females was 0.093, and about half of this value in three-vitellogenic wild females (0.04); in contrast, in running sperm males it was 0.81. Three hatchery-reared red snapper not identified by observation of gametes had ratios about 20 times higher than the spawning females, and around three-quarters less than the average of males, ranging from 0.19 to 0.22. These were considered to be males, similar to wild males with a small amount of sperm (0.17). Therefore, in general, females had 11-KT to E_2 ratios below 0.1 and males above 0.16.

Table 3. Means and SD (ng/mL) of estradiol (E_2), testosterone (T), 11-ketosterone (11-KT), and 11-KT/E_2, in mature Pacific red snapper *Lutjanus peru*.

	E_2	*T*	*11-KT*	*11-KT* E_2^{-1}
Hatchery:				
Females (n =2)	47.8	18.0	4.4	0.093
Males (n = 4)	43.7 ± 07	54.1 ± 21	35.6 ± 12	0.81 ± 0.28
Unidentified (n = 3)	5.2 ± 06	0.09 ± 0.03	1.10 ± 0.14	0.21 ± 0.02
Wild:				
Females (n = 3)	13.2 ± 1.1	0.12 ± 0.02	0.66 ± 0.10	0.04 ± 0.01
Males (n = 3)	8.3 ± 3.5	0.09 ± 0.03	1.2 ± 0.30	0.17 ± 0.07

Discussion

Our study showed that the natural, spontaneous spawning of *L. peru* was feasible in captivity. The age at first maturation in wild *L. peru* was 3.5 years (Díaz-Uribe et al., 2004), which is similar to our results. Both wild-reared and hatchery-reared red snapper matured by the end of winter after 3.5 years and reproduction occurred four months later in the following summer solstice. As summer is the natural reproductive season for this species, photoperiod and temperature are synchronized (Bromage et al., 2001). The period of reproduction was similar to *L. argentimaculatus* (Forsskål), where the natural spawning season occurred from mid-May to mid-September (Leu et al., 2003). In their study, the spawning peak was May-June, whereas in our study, it was September. On average wild-reared females (3.4 kg) produced in an average of 1.8 M eggs per reproductive season, close to an average female (7 kg) of *L. argentimaculatus* with 2.3 M eggs (Leu et al., 2003), and both lower than hatchery-reared female # 2 (2.4 kg) that spawned 2.9 M eggs. The effect of increasing water temperatures on egg and larvae quality remains unknown. However, methods to increase survival during larval metamorphosis in Pacific red snapper are being studied (Zavala-Leal et al., 2013; 2015; Moguel-Hernández et al., 2015).

Once puberty occurs in fish, gametogenesis begins, and the reproductive system becomes functional, with the ability to reproduce for the first time (Okuzawa, 2002; Taranger et al., 2010). Only 3 of 9 hatchery-reared females spawned, and it was anticipated that the others would spawn in the following year(s). Although most males produced sperm, amounts varied from few to many. This is in agreement with the finding of Dumas et al. (2004), suggesting that only some contributed to fertilization because of a dominance hierarchy during reproduction. Further studies could determine the optimal proportion of females to males during courtship and spawning.

Lutjanids have exploited different environmental niches which define specific survival strategies and morphological as well as physiological traits. Among fish, including lutjanids, age and size at puberty are species-specific (Carrillo et al., 2015), (Table 4). Some species reach puberty between 3 and 4 years of age, weight between 0.9-2.3 kg, and lengthbetween 35-52 cm. Other lutjanids are more precocious with puberty occurring between 1.5- 2 years, weights from 0.5-0.8 kg, and lengths from 25-38.5 cm. In other species, puberty may occur later, between 4 and 7 years, with weight from 2.5-8.3 kg, and lengths from 49.6-77.0 cm. Puberty in males may occur one year earlier than in females (Emata et al., 1999). Species exhibiting delayed-puberty require high investment in terms of growing costs and it can take a decade to gather preliminary reproductive information. In summary, age at puberty in lutjanids varies, but may be controlled in farmed fish by photoperiod, feeding, and water temperature (Taranger et al. 2010).

Table 4. Timing of puberty in lutjanids.

Species	*Years/ months*	*Weight (kg)*	*length (cm)*	*Reference*
L. peru	3/9	2.3	52.0	This study
L. aratus	~4	?	?	Unpublished
L. colorado	~4	?	?	Unpublished
L. argentiventris	3/7	0.9–1.2	35.0–45.0	Guerrero-Tortolero et al., 2010
L. guttatus	~2	0.5–0.7	34.3–38.5	Boza-Abarca et al., 2008
L. argentimaculatus	7	6.4–8.3	67.1–77.0	Leu et al., 2003
L. argentimaculatus	4–5	2.5–3.2	49.6–57.0	Emata et al., 1999
O. chrysurus (Bloch)	~2	~0.50	25.0	Turano et al., 2000
L. analis (Cuvier)	3+	1.1–2.0	43.0–50.0	Watanabe et al., 1998
L. griseus (L.)	1/6	0.7–0.8	37.0–38.0	Cabrera et al., 1998
L. kasmira (Forsskål)	?	0.6	28.0	Suzuki and Hioki, 1979

Unexpectedly, most surviving red snapper after four years of rearing were males. However, this result is likely to occur when survival is low or occurs merely by randomly. It has been reported that differential mortality rates between the sexes occur in lutjanids (Grimes, 1987). Further observation related to survival of adult females is necessary.

The ratio of 11-ketosterone to estradiol identified or corroborated the sex in fish. However, these results were confined to the reproductive season and agreed with previous studies where this ratio has been used to identify sex in the South Pacific hapuku *Polyprion oxygeneios* (Schneider and Forster; Kohn et al., 2013), and the Amazonian pirarucu *Arapaima gigas* (Cuvier; Chu-Koo et al., 2009).

In conclusion, the reproductive cycle of *L. peru* took place twice in captivity. Puberty and reproduction occurred by year four when fish were longer than 52.0 cm. *L. peru* reproduction does not depend on wild broodstock, and hormone-induced spawning is not necessary. These are technological advantages that permit sustainability.

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