

Broodstock Management And The Method To Find The Spawning Temperatures Of Peruvian Rock Seabass *Paralabrax Humeralis* (Teleostei: Serranidae) Under Captive Condition

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Abstract

Paralabrax humeralis known as the Peruvian rock seabass, is a serranid regularly found in the southeast Pacific Ocean [1,2]. As a consequence of fishing pressure, its numbers have been declining since the 1980s. The objective of this study is to provide information about the spawning habit of *P. humeralis*, describing the relation between water temperatures and spawning habits. The study is one of the initiatives of the FONDEF-CONICYT ID-16110437 project is the repopulation of these species.

From November 2018 to the end of July 2021, 18 female broodstock weighing 14.4 kg laid 76.8 million eggs in 532 days and spawned at temperatures between 15.2 and 21.8 °C. The floating egg rate and hatch-out rate were 71.4% and 75.1% respectively. The fish gonad matured correctly almost double speed in 2020 compared to 2018 and 2019. The relation between average water temperature and eggs quantity per g of female in 30 days suggests the spawning and the temperature have positive relationships, that abundant between 17 to 19 °C with 200 to 500 eggs per g of body weight in 30 days. The relation of 2018 and 2019 was $Y=7.3739x^2-176.58x+930.85$ and 2020 was $Y=2.7855x^2+238.51x-3042.1$; Y =Spawned eggs per female body weight (g) per 30 days, X =Compensated average water temperature (°C). The possible reasons for the improvement of 2020 are suitable water temperature and nutritional advantage as a fed homemade pellet that contains high protein fishmeal (32 %), fish oil (5 %), and vitamins (5 %), instead of silverside in 2018 and 2019. Additionally the project completed life-cycle of the fish, the F1 are spawning several eggs, from December 18, 2021.

Keywords: Peruvian Rock Seabass, *Paralabrax Humeralis*, Early Life Stages, Reproduction, Serranidae, Marine Farm

Introduction

Oceans, along with coastal zones, are fragile ecosystems, which are constantly threatened by human activity, particularly the over-exploitation of resources. Marine biodiversity can be lost because of resource mismanagement. One strategy for conserving marine biodiversity is to create new technologies for blue economy development and to increase the production of food [3]. Marine farm is one example of this kind of technology, so long as it is developed in an economically viable, environmentally sustainable, and socially acceptable way.

Paralabrax humeralis known as the Peruvian rock seabass, is a serranid habitually found in the southeast Pacific Ocean [1,2]. It is a benthopelagic species that lives in rocky-sandy coastal areas among kelp forests [1]. *P. humeralis* is carnivorous with carcino-phagus and ichthyophagous tendencies, and hermaphrodite with batch spawning strategy [4].

Commercial aquaculture requires technology that produces a large quantity and high quality of juveniles at a reasonable cost. Therefore, spawning manipulation is a key technology for the constant production of large quantities of high-quality eggs. In addition, methods such as catching and acclimatizing broodstock, water and feed management, and sampling methods need to be established before the key technology can be developed. Basically, the fish start spawning naturally after acclimatizing to rearing conditions if meet the requirements of nutrition and water condition. Particularly photoperiod and temperature are considered vital factors controlling the maturation of gonads through the hormones in fishes [5]. Therefore the data of water temperature and spawning provide the ideal water temperature range that fish lay large quantities of high-quality eggs. The producer controls spawning by controlling water temperature and photoperiod with a water recycling system in order to produce fry efficiently.

This study contributes with the first natural and spontaneous spawning of *P. humeralis* antecedents. Specifically related to embryonic development, incubation period, egg morphology and vitelline larvae; contributing to the current state of knowledge of its biology. This information is key to plan culture initiatives and/or possible repopulation, and to implement adequate management plans for this coastal fish.

The objective of this study is to provide information about the spawning habit of *P. humeralis*, describing the relation between water temperatures and spawning habits.

Materials and Method

Wild specimens of *P. humeralis* were collected by spearfishing, ziggling, and trapping along the coast of Iquique in northern Chile (20°S-70°E). Of those captured 21 female breeders with body-weight between 294-1,794 g (mean 825±408 g) and 17 of males 256-1,396 g (mean 710±250 g) at the maximum biomass on December 12, 2018.

The fish are kept for a few days in a 2-ton transition tank after receiving the fish in order to reduce stress during captivity and acclimatize the rearing environment. Some fishes show expanded abdomen and float surfaces, can't swim properly, and die due to the air accumulated in the air bladder. Insert the needle of the syringe under the pectoral fin and press the abdomen and the air is removed (Figure. 1).



Figure 1: Insert the needle of the syringe under the pectoral fin and press the abdomen and the air is removed.

Sampling the fish after acclimatization, measuring total length (TL), standard length (SL), and body weight (BW) after soaking in anesthesia (Veterquímica BZ-20 0.4ml/L) with aeration. The fatness is figured out from BW and SL as $BW (g) / SL^3 (cm) \times 1000$ and estimated health conditions and maturity. Also inject a mini transponder (Virbac V800, 4x8.5 mm) at the back of the fish for monitoring growth and maturity individually. Additionally, pressing abdomen to confirm releasing sperm or eggs, or insert cannulation tube, Teflon tube 2m/m diameter, to a sexual hole, behind an anus, and suck out a part of gonad then observe by microscope

(Figure. 2). The fish are soaked in the chlorine-free freshwater with aeration for 2 minutes to remove parasites and then released into the broodstock tank finally.



Figure 2: Insert Teflon tube, 2m/m diameter, to a sexual hole behind an anus, and suck out a part of gonad.

The broodstock is housed in a 10-ton tank, 2.5 m depth, supplies unfiltered coastal water continuously, flow-through 200 % per day. Take water parameters, pH (HANA HI98127), water temperature, and dissolved oxygen (YSI 55/12 FT) at 09:00 and 14:00 every day. Breeders are fed with blue mussel meat (*Choromytilus chorus*) and Chilean silverside (*Odontesthes regia*) are fed under *ad libitum*, five days a week with a diet of 4 % of their body mass. From July 2020, we fed homemade pellet (consisting of fishmeal 32 %, fish oil 5 %, vitamin mix 5 %, mussel meat 40 %, and wheat flour 18 %) instead of the silverside.

In the middle of each month sampling broodstock and record TL, SL, BW and observe the growth and maturity individually as applied the same procedure of acclimatization.

The broodstock tank is connected to 100 L of egg collection tank with bag-net, 40 x 20 x 30 cm, 0.5 m/m nylon mesh, (Figure. 3). The rearing water passes through from the surface to the bag net every afternoon to the following morning. The eggs are collected every morning and count total egg numbers by volume method, Collected eggs are kept in a conical tank with 50 L of water and homogenized by aeration 10 minutes, and sampling 50 ml at four portions of surface diagonally. Are counted the sample eggs and estimate the number of the total eggs by volume method. Also, take photos of eggs under a microscope (Zeiss Axiostar plus) and figure out the average egg diameter. Then drain the sediment and dead eggs from the bottom, filled water and homogenized eggs by aeration. Take egg samples and estimate floating eggs rate. Floating eggs incubate with flow-through water and a 0.5 mm mesh filter with gentle aeration. The eggs hatch out the following day in the afternoon and estimate the hatch-out larvae number and rate as the same procedure. Evaluate the spawning batch according to floating egg rate, average egg diameter, and hatch-out rate.

The water parameters are obtained only during the day night temperature needs to be considered. A data logger (Elitech RC-5, Figure. 4) is installed at the bottom of the tank, and data is acquired every 30 minutes from 20:00 to 19:30 the next day. Then the data at 09:00 and 14:00 compensated with the data of logger.



Figure 3: The broodstock tank is connected to 100 L of egg collection tank with bag-net, 40 x 20 x 30 cm, 0.5 m/m nylon mesh,



Figure 4: A data logger (Elitech RC-5) is installed at the bottom of the tank, and data is acquired every 30 minutes from 20:00 to 19:30 the next day.

Result

Water analysis at broodstock tank showed from November 1st, 2018 to July 31, 2021, average and standard deviation, maximum and minimum of compensated water temperature were 17.7 ± 1.4 ,

21.8 and 15.2 °C, pH 7.8 ± 0.2 , 8.3 and 7.1, DO 6.34 ± 1.1 , 11.94 and 1.34 mg/L accordingly. The water temperature reached the maximum in January and the minimum in July.

The broodstock reached gonadal maturity when the water temperature rises, started spawning spontaneously. In the spawning season, a male operculum pigment to yellowish-orange and showed milt when pushed the abdomen (Figure. 5). The female showed ovulated eggs by the cannulation (Figure. 6). The courtship of the female by the male consisted in swimming merry-go-round fashion and splashed water in the late afternoon from 16:00 to 20:00 usually.



Figure 5: Showed milt when pushed the abdomen.



Figure 6: Showed ovulated eggs by the cannulation.

The spawning started on November 9th, 2018 when the water temperature rose. The spawning occurred between 16 to 20 °C, and the number of eggs and the water temperature showed a positive relationship generally. (Figure. 7).

From November 2018 to the end of July 2021, 18 female broodstock weighing 14.4 kg laid 76.8 million eggs in 532 days and spawning at temperatures between 15.2 and 21.8 °C. The aver-

age floating egg rate and hatch-out rates were 71.4% and 75.1% (Table 1). usually, *P. humeralis* habit was iteroparous because the number of spawning was larger than the number of females during the season.

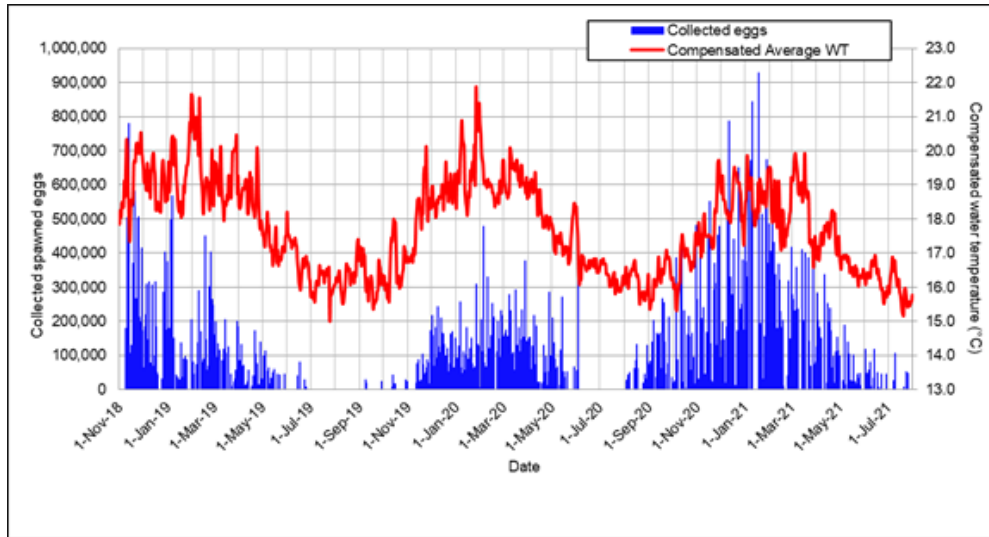


Figure 7: The spawning occurred between 16 to 20 °C, and the quantity of eggs and the water temperature showed a positive relationship generally

Table 1: Natural spawning, egg diameter, floating eggs and hatch-out larvae of Peruvian rock seabass (*Paralabrax humeralis*) by spawning season 2018 to 2021.

Season year (August - July)	BRSF*		Spawning period			Spawning				
	N	Kg	Date**	Days	Temp. (°C)	Days	Eggs	µm	Spawning/N***	Eggs/Day
2018 (Nov - Jul)	18	14.4	Nov 9, 2018 - Jun 25, 2019	229	15.9 - 21.8	144	19,961,800	978	8.0	138,624
2019 (Aug - Jul)	12	12.3	Sept 8, 2019 - Jun 3, 2020	270	15.4 - 21.8	167	18,093,600	964	13.9	108,345
2020 (Aug - Jul)	11	11.6	Aug 3, 2020 - July 26, 2021	358	15.2 - 19.9	221	38,727,600	966	20.1	175,238
Total				857	15.2 - 21.8	532	76,783,000			

* Female broodstock biomass at the spawning peak
 ** From first spawning to end spawning date
 *** Spawning days / Number of BRSF

Floating eggs		Hatch-out		Feeds
Eggs	%	Larvae	%	
12,780,100	64.0	8,977,800	70.2	Mussels and silverside
11,690,700	64.6	7,666,100	65.6	Mussels and silverside
30,345,100	78.4	24,499,200	80.7	Mussels and pellet
54,815,900	71.4	41,143,100	75.1	

In the 2018 season, 20.0 million eggs were collected over 144 days, with an average diameter of 978 µm, and 18 females weighing 14.4 kg spawned at temperatures between 15.9 and 21.8 °C. Therefore, on average, one female spawned 8.0 times during the

season, 139 thousand eggs per spawn. The floating egg rate and hatch-out rates were 64.0 % and 70.2 % usually.

In the 2019 season, 18.1 million eggs were collected over 167

days, with an average diameter of 964 μm, and 18 females weighing 12.3 kg spawned at temperatures between 15.4 and 21.8 °C. Therefore, on average, one female spawned 13.9 times during the season, 108 thousand eggs per spawn. The floating egg rate and hatch-out rates were 64.6 % and 65.6 %.

In the 2020 season, 38.7 million eggs were collected over 221 days, with an average diameter of 966 μm, and 11 females weighing 11.6 kg spawned at temperatures between 15.2 and 19.9 °C. Therefore, on average, one female spawned 20.1 times during the season, 175 thousand eggs per spawn. The floating egg rate and hatch-out rates were 78.4 % and 80.7 %.

The comparison of among the three seasons, the egg quantity, quality, and spawning frequency of 2020 are better than the other two seasons, some numbers are almost doubled, even the egg size was smaller. Thus, the fish gonad matured correctly almost double speed in 2020 compared to 2018 and 2019. Additionally the project completed life-cycle of the fish, the F1 spawning sevelar eggs, from December 18, 2021

Discussion

A fish has a particular water temperature range for maturation. The temperature activates hormones and gonad mature, some ova ovulated then eggs come outside the body [6]. However, in our rearing condition, the temperature fluctuated from 1 to 2 °C in a day and night. Also, even the temperature is suitable for mature the fish needs time for maturation to spawning; it takes hours to days depending on the species [7]. Therefore, it used long-term data to identify the relation between water temperature and maturation.

It is suggested to the following method in order to figure out the ideal water temperature for maturing the fish that compare in terms of 30 days data. At first figure out the average water temperature of 30 days from the first day of each month. Secondly, a sum of spawned eggs in 30 days is divided into the female biomass in each term. Lastly, the temperature compares with spawned eggs per g of body weight in 30 days [8-10].

The relation between average water temperature and eggs quantity per g of female in 30 days suggests the spawning and the temperature have positive relationships and that abundant between 17 to 19 °C with 200 to 500 eggs per g of body weight in 30 days. (Figure. 8). Additionally, the spawning quantity in the season 2020 improved compared to 2018 and 2019 as the formulation of 2018 and 2019 was $Y=7.3739x^2-176.58x+930.85$ and 2020 was $Y=2.7855x^2+238.51x-3042.1$; Y =Spawned eggs per female body weight (g) per 30 days, X =Compensated average water temperature (°C) (Figure.9). Moreover, the maturation of 2020 is more active than 2018 and 2019 as the trend line of 2020 is steeper than 2018 and 2019. Remarkably the spawning occurred in July 2020 at a water temperature of below 16 °C. The possible reason for the improvement of 2020 are suitable water temperature for maturation and a nutritional advantage as a fed homemade pellet that

contains high protein fishmeal (32 %), fish oil (5 %), and vitamins (5 %), instead of silverside in 2018 and 2019.

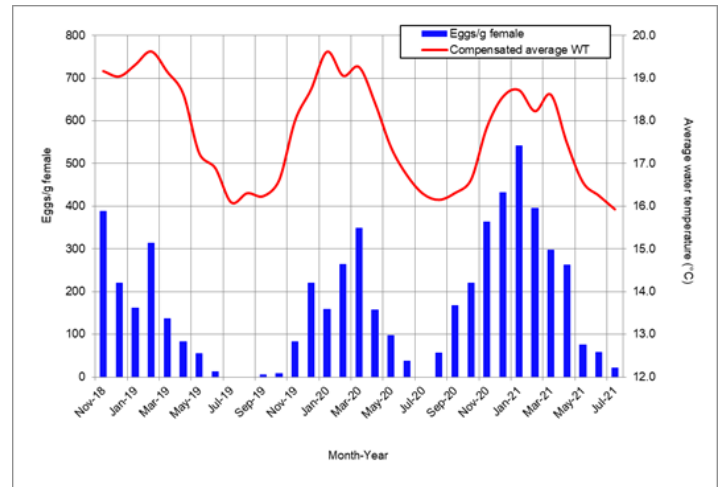


Figure 8: The relation between average water temperature and eggs quantity per g of female body weight in 30 days suggests the spawning.

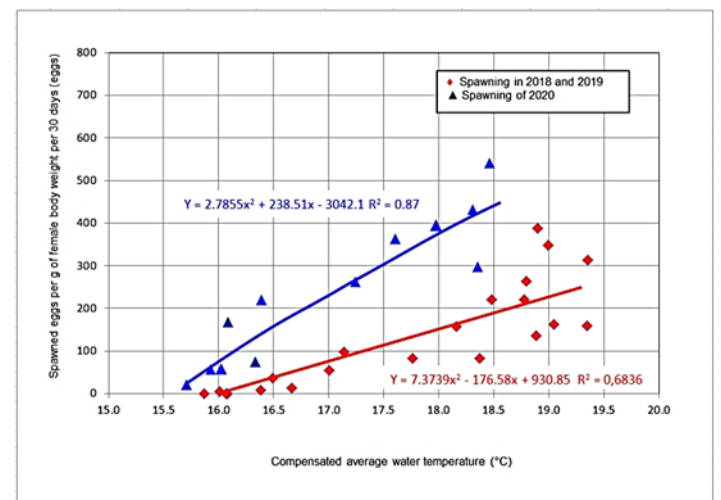


Figure 9: The formulation of 2018 and 2019 is $Y=7.3739x^2-176.58x+930.85$ and 2020 is $Y=2.7855x^2+238.51x-3042.1$; Y =Spawned eggs per female body weight (g) per 30 days, X =Compensated average water temperature (°C)

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