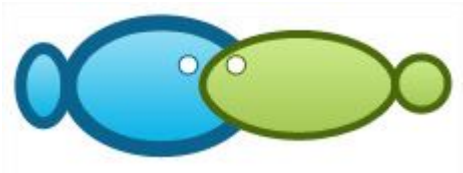

Induced reproduction of the sailfin pleco, *Pterygoplichthys gibbiceps* (Kner, 1854) (Pisces: Loricariidae)

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Abstract. The fish species *Pterygoplichthys gibbiceps*, better known as the sailfin pleco, is a loricarid of ornamental interest that inhabits the Orinoco and Amazon basins in Colombia. There is a basic information related to biology, natural environments where it lives, food habits, reproductive cycles, as well as information on spontaneous reproduction in confinement, but there is not enough information of the response of the species to hormonal inductions for reproduction. To approximate the response of the species to the use of reproductive hormones, sexually mature females were induced with carp hypophyseal extract (EHC) (Argent Inc.) and salmon gonadotropin-releasing hormone analogue to GnRhs plus Domperidone (Ovaprim®) (Sigma Chemical Co.). In the case of males hormonal induction was performed with EHC. The positive responses were given with the use of EHC, determining the latency time, fertility and incubation time, showing the reproductive viability of the species under hormonal inductions.

Key Words: carp pituitary extract, gonadotropin, reproductive fecundity, fertility, latency time, ornamental fish.

Introduction. In Colombia, the trading of native ornamental fish species is an activity that is sustained by extraction in natural environments, especially in the Orinoco and Amazon basins, being an important source of income for about 20 thousand families dedicated to this extractive activity (Arias 2013).

The fishing of continental ornamental fish as a small-scale economic activity began more than 60 years ago (Ortega-Lara 2015). Continue extraction for decades has generated negative impacts on ecosystems, attributed mainly to overfishing, which translates into a decline in the number of specimens and species captured (Sanabria 2004; Arias 2013; AUNAP 2013). A clear example of this is the decrease almost until the extinction of *Osteoglossum ferreirai* (blue arawana), an emblematic ornamental species in Colombia. This was a situation where drastic measures were adopted for its protection as closed all year round (Resolution 3704 of 2010 of the INCODER - Ministry of Agriculture and rural development from Colombia). The possibility of capturing ornamental fish is limited by several factors, such as the weather, where in the winter the high level of the water in rivers and ponds makes fishing impossible. Also, there are periods of time in which fishing is prohibited because of the reproductive season; and finally, problems of public order makes impossible to sample certain areas where ornamental species inhabit. In this situation and with the premise of protecting the environment and the fishing resource, given the fragility thereof, the possibility of producing these species in confinement is a valid option and also accepted by producers and fishermen.

Of the ornamental fish of interest in the export-oriented trade in Colombia, it corresponds to the Siluriform order, in which 208 species belonging to 10 families reported, with Loricariidae being the most abundant, accounting for 19.92% of the

commercialized species, above the Characidae family that contributed 12.64% with 66 species (Ortega-Lara 2015).

The species belonging to the family Loricariidae, are the most numerous of the siluriformes, with about 600 species in Central America and South America (Santos et al 1984) known as cuchas, corronchos, panaques, agujas, tablas etc., they being characterized by having the body covered with bony plates (Machado 1987).

The species *Pterygoplichthys gibbiceps* commonly called sailfin pleco or leopard pleco (Barreto et al 2015) is a species of large size, robust and elongated body reaching 50 cm, the body is covered with bone plates, dark brown with yellow spots all over the body and ventral mouth in the form of a sucker (Ureña et al 2005; Landines 2007), high dorsal fin with 14 rays, raised supraoccipital crest, abdomen with rounded spots (Galvis et al 2007). In Colombia it is distributed in the Orinoco (Ramírez & Ajiaco 2001; Ortega-Lara 2016) and Amazon basins (Weber 2003), where it lives in minor tributaries and is occasionally found in rivers, preferring waters of 26°C or more. Its habit is nocturnal with great activity at the beginning of the night where it explores substrates and feeds (Riehl & Baensch 1991). It is an omnivorous species with preference for algae, detritus and invertebrates of benthos being phytoplanktophagous and detritophagous (Riehl & Baensch 1991; Lasso et al 2004; Urueña et al 2005; Galvis et al 2007), consuming it avidly together with wood from submerged trees, which is obligatory for its digestive process (Riehl & Baensch 1991).

Regarding the reproductive biology, Aya-Baquero et al (2016) show that adult females of sailfin pleco in natural environments have globular and saccular ovaries, asymmetric in their maturation state (larger left ovary, of 20 to 30%) with a short oviduct culminating in a genital pore after the anus, cystovarians present a synchronic development by groups of oocytes with total spawning and an average fecundity of 1483±380 ovules/mature female. The same authors affirm that the highest value of the gonadosomatic relationship coincided with the mature states in both sexes during the rainy season in rising waters (2°37'52.7"N, 72°42'28.08"W, 196 m) during the months of March and April; in the case of males they have unrestricted spermatogonial testes, with spermatogenesis in the entire area of the gonad, occupying about 15% of the coelomic cavity, white and finger-shaped, where the lumen of each tubule is filled with free sperm in seminal liquid (Aya-Baquero et al 2016; Páez et al 2016a).

The embryonic development of the sailfin pleco is slow compared with other species of the siluriform order (Paez 2016b). From the fourth hour post-fertilization (hpf) at 26.9±0.7°C temperature, the first changes of cleavage 1 and 2 are perceived 24, 32 and 36 hpf; the initial blastula was observed high and late respectively, the closure of the blastopore occurs at about 56 hpf and hatching, at the temperature already reported, occurs at 106 hours, with complete ocular pigmentation and well-differentiated oral suckers (Páez 2016b).

They present apparent sexual dimorphism, where the females exhibit in the first radius of the pectoral fin steeper roughness than the males (Aya-Baquero et al 2016), the mature males build nests consisting of holes of 30 to 70 cm, selecting the walls in the channels from the rivers. Ureña et al (2005) report sizes and minimum weights of the broodstock, to contain in ponds on land, 24.5 cm and 270 g, keeping them at a density of 1.5 m^2 per breeder and a proportion of three females per male. Under the previously described females deposit the eggs of intense yellow color, being oviparous with demersal eggs (Rocha et al 2008).

However, the production in captivity of the sailfin pleco as a fish farming option has not been established, this to some extent due to the lack of information regarding its confined and controlled reproduction. The objective of this investigation was to evaluate the reproductive response of females of sailfin pleco to different hormonal inducers.

Material and Method. The experiments were carried out during May 2016 in the ornamental fish unit of the Institute of Aquaculture of the University of the Llanos - IALL (4º05'N and 73º37'W) in Colombia, at a height of 467 meters above sea level, with a relative humidity oscillating between 67 and 83% during the year, average temperature of 25.5°C, total annual rainfall average of 4383 mm.

Specimens of sailfin pleco were taken from a flock of broodstock that were kept in confinement in the IALL, which were transferred to concrete pools in density of one specimen per m^3, and fed once a day with a mixture of concentrated feed commercial of 25% crude protein and a supplementary food consisting of lettuce, spinach, broccoli and cucumber at a rate of 2% live weight, for eight months.

Taking into account that the maximum reproductive peak in this species occurs at the end of October (Aya-Baquero et al 2016), in this period 36 breeders were selected taking into account the external characteristics (pronounced and reddened urogenital papilla) and the result of the abdominal pressure made to each one of the specimens, which allowed to see the oocytes in the case of the females and barely perceptible a white liquid in the case of the males.

The following hormones and their combination were evaluated: carp pituitary extract (EHC) (Argent Inc.) and gonadotropin-releasing hormone analog of salmon aGnRhs plus Domperidone (Ovaprim®) (Sigma Chemical Co.), thus generating 6 treatments each with three replicas. In the case of the males, hormonal induction was performed only with EHC to eighteen specimens (Table 1).

The hormones were injected intramuscularly in the lower part of the dorsal fin between the juxtaposed plates, after weighing each specimen to determine the hormonal calculations.

Table 1

Hormonal treatments (Tm) for the spawn induction of the sailfin pleco

Sex	*Tm*	*Hormone*	*Induction protocol*		
			1st dose	*Interval*	*2nd dose*
♀	1	EHC	0.5 mg kg^{-1}	24 h	5 mg kg^{-1}
	2	EHC	0.7 mg kg^{-1}	24 h	7 mg kg^{-1}
	3	Ovaprim®	0.05 mL kg^{-1}	24 h	0.5 mL kg^{-1}
	4	Ovaprim®	0.07 mL kg^{-1}	24 h	0.7 mL kg^{-1}
	5	Ovaprim® + EHC	Ovaprim® 0.05 mL kg^{-1}	15 h	EHC 5 mg kg^{-1}
	6	Ovaprim® + EHC	Ovaprim® 0.07 mL kg^{-1}	15 h	EHC 7 mg kg^{-1}
♂	7	EHC	4 mg kg^{-1}	-	-

Obtaining the eggs and semen was done dry by squeezing, for this the fish were previously reassured with 2-phenoxyethanol (300 ppm), ten minutes after the gametes were mixed and once the eggs were hydrated, they were washed and taken to a horizontal flow incubator. All the experimentation was accompanied by permanent sampling and analysis of physical chemical parameters of the water; in the case of females, the reproductive responses, latency time, reproductive fecundity, fertility percentage and incubation time were evaluated; for males, semen samples were taken determining sperm motility.

Results and Discussion. The reproductive responses under the proposed hormonal protocols are presented in Table 2.

The parameters of water quality during the experimentation phase were within reported values in natural environments for the species (Aya-Baquero et al 2016): temperature 26.5±0.3°C, pH of 6.7±0.1 and oxygen saturation > 60%; under these conditions the females of treatments 1, 2 and 6 ovulated.

The ovulatory response or the latency time for treatments 1 and 2 was present at approximately 23.5 hours, at a temperature of 26.5±0.3°C. It should be noted that the females were in the same container, maintaining in this way the homogeneous water quality conditions. These results agree with those reported by Collazos-Lasso & Arias-Castellanos (2009) for the species *Ancistrus triradiatus*, loricariid of small size that after an environmental stimulus (manipulation of the electrical conductivity) presented positive reproductive responses in a period of 23 to 26 hours at a temperature between 26.8 and 27.8°C, similar to the result obtained in the present study for species of the same family.

Table 2

Reproductive performance of females of sailfin pleco under hormonal inductions at the Institute of Aquaculture of the Universidad de los Llanos

Tm	*weight (g)*	*Answer (%)*[1]	*Latency time*[2] *(h/°C)*	*Reproductive fertility*[3]	*Fertility (%)*	*Incubation time (h/°C)*
1	585.0±13.2	66.6	23.8±0.9/26.5±0.3	624±37[a]	25.5±7.8[a]	69±1.4/25.4±0.7
2	590.7±36.9	66.6	23.5±0.7/26.5±0.3	496.5±271[a]	19±1.41[b]	68.5±0.71/25.4±0.7
3	595.0±40.2	-	-	-	-	-
4	580.0±25.6	-	-	-	-	-
5	560.0±22.5	-	-	-	-	-
6	503.3±57.7	33.3	24.16/26.5±0.3	612*	5*	70*

Tm = treatment; [1] Answer = number of spawned couples/number of couples stimulated (%); [2] Latency time = response time to hormonal induction in hours/temperature in°C; [3] Reproductive fertility = number of total eggs spawning; *Individual value without statistical comparison. Different letters (a, b) mean significant differences between groups (values expressed as Mean±SD ($p < 0.05$)).

Regarding reproductive fecundity, no difference was found between the treatments with positive responses, being that the reproductive strategy of the species is K, this taking into account the number of eggs, the size of these (average of 2 mm) and parental care (MacArthur & Wilson 1967). The fertility reported by Aya-Baquero et al (2016) for the same species differs greatly from the present study, since the number of oocytes obtained (1483±380) in mature females with similar weights to those of natural environments was higher because the specimens were sacrificed to extract their ovaries and count the number of eggs. In this investigation the spawning was dry and therefore with this technique it is not possible to obtain the total number of eggs. However, the results are similar to that presented by Suzuki et al (2000) for species of the same family. There is no known literature with which you can compare the results reported, nor with information regarding the use of reproductive inducers in the species, which is why our work is an important contribution to the use of sex hormones for induced reproduction on sailfin pleco and a basis for work with other species belonging to the Loricariidae family.

In the case of the males, positive responses were obtained, collecting semen by squeezing with a volume of 50±9 µL, with a viability above 95%, as additional data we have that the spermatozoa were maintained with motilities greater than 50 minutes and that the same at the time of the spermiation already were active, which leaves a very large window of investigation for the species.

Conclusions. The females and males of sailfin pleco responded positively to the hormonal induction with reproductive ends, emphasizing the hypophyseal extract of carp for this purpose, being a contribution for the fish culture of the species. It highlights the unknown fact of sperm motility duration that there will be a new field of research of this species along with other loricariids.

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