Dams effects on potamodromous *Prochilodus magdalenae*: evidence from endocrine response

Daniela De Fex-Wolf Corresp., 1, 2, Silvia López-Casas 1, 3, Luz Fernanda Jiménez-Segura 1, Clara María Pérez-Gallego 4

1 Instituto de Biología, Universidad de Antioquia, Medellín, Antioquia, Colombia
2 Coordenação de Dinâmica Ambiental, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil
3 Northern Andes and South Central America Conservation Program, The Nature Conservancy, Bogotá, DC, Colombia
4 ISAGEN S.A E.S.P, Medellín, Antioquia, Colombia

Corresponding Author: Daniela De Fex-Wolf
Email address: danieladefex@gmail.com

**Background.** Prochilodus magdalenae is a neotropical fish that synchronizes reproduction with flood river pulses.

**Methods.** We used an endocrine method to test the influence of hydropeaking on hormone production related with reproduction. We analyzed *P. magdalenae* reproduction in individuals from two sectors of a Neotropical river basin: one with natural flow and the other with a regulated hydrological regime.

**Results.** In the section of the basin with natural flow we found that the production of hormones related with fish reproduction (FSH and LH) was correlated with the gonadosomatic index, while in fish experiencing hydropeaking due to hydroelectric operation no such correlation was detected.

**Discussion.** We conclude that potamodromous fish reproductive hormone production is sensitive to changes in water level/discharge, consequently, fish exposed to hydropower receive ambiguous stimuli that affect hormone production, reproduction synchronization with environmental cues, and ripening, which are essential for reproductive success.
Dams effects on potamodromous Prochilodus magdalenae: evidence from endocrine response

Daniela De Fex-Wolf, Silvia López-Casas, Luz Fernanda Jiménez-Segura and Clara María Pérez-Gallego

1 Instituto de Biología, Universidad de Antioquia, Medellín, Antioquia, Colombia
2 Coordenación de Dinâmica Ambiental, Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil
3 Northern Andes and South Central America Conservation Program, The Nature Conservancy, Bogotá, DC, Colombia
4 ISAGEN S.A. E.S.P, Medellín, Antioquia, Colombia

Corresponding Author:
Daniela De Fex-Wolf
Email address: danieladefex@gmail.com

Abstract

Background. Prochilodus magdalenae is a Neotropical fish that synchronizes reproduction with flood pulses.

Methods. We used an endocrine method to test the influence of hydropoeaking on hormone production related with reproduction. We analyzed P. magdalenae reproduction in individuals from two sectors of a Neotropical river basin: one with natural flow and the other with a regulated hydrological regime.

Results. In the section of the basin with natural flow we found that the production of hormones related with fish reproduction (FSH and LH) was correlated with the gonadosomatic index, while in fish experiencing hydropoeaking due to hydroelectric operation no such correlation was detected.
**Discussion.** We conclude that potamodromous fish reproductive hormone production is sensitive to changes in water level/discharge, consequently, fish exposed to hydropeaking receive ambiguous stimuli that affect hormone production, reproduction synchronization with environmental cues, and ripening, which are essential for reproductive success.

**Keywords**
Dams, Freshwater fish, Gonadotropic cells, Magdalena River Basin, Neotropical fish reproduction.

**Introduction**

Biology and ecology of fishes in tropical large rivers are linked to the hydrological regime in the main channel and regular flooding in associated floodplains (Welcomme, 1985; Junk et al., 1989). The hydrological cycle in these rivers is mainly determined by fluctuations in rainfall, which changes water level resulting in wet and dry periods (Lowe-McConnell, 1987; Wootton, 1999). Water level variations result in numerous environment changes that modify seasonally habitat and food availability, influencing reproduction, migrations, growth, ecology and regional fisheries (Agostinho et al., 2004). In tropical freshwater fishes, reproductive responses are associated with the flooding regime and discharge (Lowe-McConnell, 1987; Junk et al., 1989; Jiménez-Segura et al., 2010). In response to human demands - especially for energy- natural flow regimes of rivers suffer several modifications with the construction of dams. These changes might affect fish reproduction, specifically by changes in the natural amplitude and duration of river flows, creating physical barriers for migrations (Agostinho et al., 2001, 2003; Murchie et al., 2008) and affecting aquatic organisms’ behavior (Agostinho et al., 2000, 2001). At the population level, the transformations in regulated rivers involve alterations in physical and chemical characteristics and changes in aquatic habitat availability, that may disrupt environmental cues for reproduction that influence maturation, spawning and migrations (Macnaughton et al., 2015).

Reproduction is a multifactorial process involving interactions between environmental, social, neural, endocrinological and nutritional components (Urbinati, 2005). In fishes, reproduction starts by synchronised actions of the Hypothalamus-Pituitary-Gonadal (HPG) axis (Gonzalez et
al., 2012), which responds to environmental signals activating the production of specific 
hormones related with defined reproductive stages (Vongvatcharanona, 2006). The stimuli that 
influence hormone production are associated with gonadal maturation during rainy seasons 
(Donaldson, 1981; Donaldson and Hunter, 1983; Victoria et al., 2005; Murchie et al., 2008), and 
these hormones are synthesized in the adenohypophysis by gonadotrophs, pituitary cells that are 
developed in response to environmental factors (Donaldson and Hunter, 1983). Follicle-
stimulating hormone (FSH) contributes to early spermatogenesis and oogenesis, while 
luteinizing hormone (LH) is related with seasonal gonadal growth, sex steroid production, sperm 
production in males, and ovulation in females during spawning (Vongvatcharanona et al., 2006;
Gonzalez et al., 2012). Their concentrations and proportions in fishes bodies depend on 
reproductive stage (FSH concentration values are higher during immature stages and LH 
increases during maturity), which in Neotropical freshwater fishes are determined by the 
hydrologic regime (Donaldson, 1981; Donaldson and Hunter, 1983; Victoria et al., 2005;
Murchie et al., 2008). In reproductive biology, endocrinological tools have begun to be used to 
understand how reproduction is controlled and coordinated by the environment. Due to the 
existence of about 25,000 species of fishes, each with different reproductive strategies, the 
majority of studies are for just a few selected species such salmon or goldfish, that have 
representative characteristics which are used as a model of hormonal studies for other fish 
species (Melamed and Sherwood, 2005).

Change in reproductive response of tropical fishes due to hydropower is well documented, but 
conclusions are based mainly on shape and size of adult gonadal tissue (Murchie et al., 2008; 
González et al., 2010). There are many methodologies that can be employed to better understand 
effects of regulated rivers on freshwater fishes at the cellular level. Endocrinology, for example, 
brings a series of tools that can be used to support ecological interpretations related with 
reproduction, such as the use of anti-chum salmon FSHβ and LHβ (specific antisera) for 
immuochemical detection of FSH and LH gonadotrophs in fish like Odontestes bonariensis and 
Oreochromis niloticus (Vongvatcharanona et al., 2006). That approach could work for 
prochilodontids (pers. comm. Prof. Shunsuke Moriyama, 2011) to help explain how fishes 
respond when facing barriers such as dams. Until now, changes in environmental signals 
associated with modified river flow, and hormonal response related to spawning of a migratory 
freshwater fishes had not been confirmed. We tested the hypothesis that hydropoeaking due to
hydropower production negatively affects hormone production and the reproduction of *Prochilodus magdalenae* (Steindachner, 1879) individuals in the Magdalena River Basin. Our results show that changes in water lever due to hydropoeaking affect reproductive hormone response of this potamodromous fish.

1.1. Description of the study area

The Magdalena River (Fig. 1) is a sixth-order river that flows northwest, between the central and east branches of the Andes (3° and 11° N). It is 1,540 km long, discharges 7,500 m$^3$.s$^{-1}$ into the Caribbean Sea (Likens, 2010), and is considered a focal axis of Colombian economic development. The annual hydrological pulse is bimodal (two low water and two flooding periods). Due to geomorphology, many of its tributaries have been dammed for hydropower generation, and have an installed capacity of around 14,000 MW, which is the most important source of hydropower generation in Colombia (UPME, 2012). One of the Magdalena River’s tributaries is the La Miel River, which was dammed for hydropower in 2002. Mean flow is 84.3 m$^3$.s$^{-1}$ (Marulanda et al., 2003), but due to hydropower generation, the hydrological regime has been modified and daily hydropoeaking occurs downstream of the dam (Fig. 2).

FIGURE 1

Materials & Methods

To evaluate the effect of hydropoeaking on hormone production, we collected potamodromous fish under hydropoeaking conditions and under natural flow conditions. Individuals of *P. magdalenae* were captured at two different sites in the Magdalena River Basin: La Miel River (a regulated river influenced directly by dam hydropoeaking), and the main channel of Magdalena River at Puerto Berrío (a natural flow river with a natural hydrological regime). Although the Magdalena River has some dammed stretches (for example Betania Reservoir (2°41′06″N 75°26′24″O’)), the contributions of other tributaries cushion the effects of localized hydroelectric operation (see Fig. 2). Water levels (m) and discharge (m$^3$/s) for the natural river were obtained from the national hydrology database (Instituto de Hidrología, Meteorología y Estudios
Ambientes - IDEAM), and the ISAGEN S.A. E.S.P hydropower company supplied the information for the regulated flow river. This hydrologic data is collected daily in fixed points throughout the river basin. We used average daily water level (m) and discharge (m$^3$/s) (Fig. 2).

*FIGURE 2*

P. magdalenae from natural and regulated hydrological regimes rivers were bought monthly from fishermen. Fifty specimens were obtained each month, in the same week, from June 2010 to June 2011 from each sampling site. After capture, fish were fixed in 10% formalin *in situ* and transported to the Ichthyology Laboratory of the University of Antioquia.

For each fish we recorded: standard length ($L_s$, mm), total body weight ($W_T$, g), eviscerated body weight ($W_E$, g), liver weight ($L_W$, g), gonads weight ($G_W$, g), and sex (male or female).

Macrosopic observations were made of ovaries to determine sexual maturation stage of females, like color and vascularization or presence of oocytes in the ovaries. For males, testicles size, color and vascularization were observed. Reproductive stages for females and males were defined following Vazzoler scale (1996), which classified Stage A: Immature, B: Maturing, C: Mature, D: Spawned, and E: Resting.

The stage of reproductive activity was determined using gonad and liver weight relation with total weight, since the behavior of these organs is correlated with reproduction (Vazzoler, 1996).

We calculated gonadosomatic ($G_I$) indexes for gonad or liver weight proportion to total fish body weight, in a specific time or reproductive stage:

$$G_I = (G_w \div W_t) \times 100$$

Where $W_T$ is total body weight and $G_W$ is gonad weight. To test if these indices were different between sampling sites we used a non-parametric test in GraphPad Prism 7.

To estimate the relationship between discharge, gonadosomatic index, FSH and LH cell densities we used Spearman correlation.

**Hormonal analysis**
From each sampling site (natural and regulated flow river), we selected one pituitary gland of each macroscopic maturity state collected (one for stage A, one for B, one for C, one for D and one for E), from males and females, each month (12 months in each site). Each pituitary gland was embedded in paraffin wax and sectioned in, at least, 10 cuts (three to five-μm thickness) with a microtome in the par proximal distalis sector, where cellular density is higher compared with other pituitary sectors, and trying to cut in the same deep for all the pituitary gland collected. The response variable was the number of immunostained cells by mm².

Antisera used in this study were anti-chum salmon FSHβ and LHβ. Suzuki and collaborators (1988a, 1988b) (in Vongvatcharanon et al., 2006) previously described the origin and characteristics of these antibodies. We used a 1/5,000 dilution for anti-GTH-Iβ (FSH) and anti-GTH-IIβ antibody (LH) as suggested by Prof. Shunsuke Moriyama (pers. comm. 2011).

Monthly, pituitary glands were removed, fixed in 10% formalin for preservation and processed for paraffin embedding, cutting and placing in charged microscope slides. We used UltraVision Quanto Detection System HRP DAB kit (Thermo Scientific), and we follow the protocol described by Shi et al., (1999) for each sample:

The photographs were taken with a digital camera (Nikon D500) installed in an optical binocular microscopic (Zeiss) and the number of stained cells by mm² were counted.

To determine if there were differences in hormone production between gonadal ripeness states we tested for significant differences in the number of immunostained cells for each gonadal development state with a one way ANOVA for each cells type: FSH cells and LH cells.

Because rainy season is known to influence hormone production and gonadal maturation (Mishra and Sarkar, 2013), we used Spearman correlation to test for a relationship between the type and variations of hormone production (FSH and LH) with modifications in the water level and discharge for individuals from the natural flow river and the regulated river.

Results
We analyzed 1271 individuals, of which 588 (332 males and 256 females) came from the natural flow river and 683 (376 males and 307 females) from the regulated flow river. Individuals in all maturity states were collected at both sampling sites and the majority were found in A, B and C maturity stage in both natural and regulated flow rivers (Table 1).

The gonadosomatic index ($G_I$) in males changed through time at both sample sites. In the natural flow river, differences were found in $G_I$ among months ($H = 187.824$, $DF = 11$, $p < 0.005$), where June, July, August and October showed the highest values. Individuals from the regulated flow river, also had differences among months ($H = 208.509$, $DF = 12$, $p < 0.005$), with the highest $G_I$ values from June to August 2010, October 2010 and from March to April 2011. There was no significant difference in $G_I$ values in males for the natural ($M = 0.309$, $SD = 0.298$) and regulated ($M = 0.481$, $SD = 0.377$) rivers ($t (23) = -1.259$, $p > 0.005$).

For females, we found differences in the $G_I$ values through time at both sites. In the natural flow river, we found differences among months ($H = 124.260$, $DF = 11$, $p < 0.005$), with highest GI values in June and August 2010. We found differences among months for females in the regulated flow river ($H = 151.490$, $DF = 12$, $p < 0.005$), with the highest $G_I$ values from August to October 2010. There was no significant difference in $G_I$ values in females for the natural (Mnd = 12) and regulated (M = 13) rivers ($U = 55$, $p > 0.005$) (Fig. 3).

A total of 113 pituitary glands was analyzed. The number of LH immunostained cells was different among maturity stages in males from the natural river ($F_{(2, 21)} = 5.49$, $p < 0.005$), as well from individuals from the regulated river ($F_{(3, 27)} = 26.652$, $p < 0.005$). There was no significant difference in the number of LH immunostained cells between males from the natural and regulated river ($t_{(7)} = -0.889$, $p > 0.005$). For females, the number of LH immunostained cells was different among maturity stages in individuals from the natural river ($F_{(2, 17)} = 10.684$, $p < 0.005$), as well from individuals from the regulated river ($F_{(3, 27)} = 24.401$, $p < 0.005$). There was no significant difference in the number of LH immunostained cells between females from the natural and regulated river ($t_{(7)} = 0.415$, $p > 0.005$).
For the number of FSH immunostained cells, we found differences among maturity stages in males from the natural river ($F_{(2, 21)} = 8.945, p<0.005$), as well from those from the regulated river ($F_{(3, 27)} = 9.462, p<0.005$). There was no significant difference in the number of FSH immunostained cells between males from the natural vs. the regulated river ($t_{(7)} = 1.874, p>0.005$). For females, the amount of FSH immunostained cells was different among maturity stages in individuals from the natural river ($F_{(3, 18)} = 4.318, p<0.005$), as well from individuals from the regulated river ($F_{(3, 27)} = 13.592, p<0.005$). There was no significant difference in the amount of FSH immunostained cells between females from the natural and regulated river ($t_{(6)} = 0.763, p>0.005$).

We found that immature and maturing specimens (A and B maturity stages) had higher number of FSH immunostained cells, while individuals in advanced maturity stage (C) had higher values of LH immunostained cells (Fig. 4).

In the natural river, we found a negative and significant relationship between discharge ($m^3/s$) and the gonadosomatic index ($r = -0.168, p<0.05$), between gonadosomatic index and immunostained FSH cell densities ($r = -0.324, p<0.05$), and a positive and significant relationship between gonadosomatic index and immunostained LH cell densities ($r = 0.404, p<0.05$), showing an expected behavior between discharge variations and gonadal response, and an association between hormonal response with reproductive stages.

In counterpart, in the regulated river we found a positive and significant relation between discharge ($m^3/s$) and gonadosomatic index ($r = 0.195, p<0.05$) and a negative and significant relationship between FSH and LH immunostained cell densities ($r = -0.460, p<0.05$). Which means that in this river there is no correlation between reproduction and hormonal response.

### Discussion

Our results show that flow pattern influences the hormone production of potamodromous fish in the Magdalena River Basin, with changes in migratory fish hormone response that is correlated
with modified flow pattern, exhibiting a lack of synchrony between reproductive hormones response and water level in the regulated river.

In the natural flow river, *P. magdalenae* synchronized their reproductive cycle with water level signals. Conversely, in the regulated river, reproductive response was not related to the river water level.

Just as flow pulse has been shown to be the principal cue to initiate most biological processes of aquatic biota, spawning and migration of potamodromous fish in the Magdalena River has also been linked to changes in water levels, and regional climatic events such as ENSO cycles (López-Casas et al., 2016) and their associated physiochemical variables (pH, temperature and dissolved solids) (Jiménez-Segura et al., 2010). Flow pulse of a regulated river with hydropower scheme flows (like La Miel River) undergoes changes that disturb the historical climate and water discharge patterns that fishes use to respond to environmental stimuli. We suggest that lack of synchrony between hormonal behavior and gonadosomatic index is a response to the daily hydropeaking behavior of the river, which modifies environmental information perceived by fishes (Payne, 1986). This might influence their spawning behavior, recruitment and modify community structure (Agostinho et al., 2004).

Our results for *P. magdalenae* are similar to those obtained by Santos et al., (2001), where levels of FSH in rainbow trout decreased before sexual maturity starts, and increased after ovulation. This was because FSH contributes to early spermatogenesis and follicular growth, while LH promotes gamete maturation and is involved in sperm production and ovulation (Vongvatcharanona, 2006). Therefore, higher levels of FSH were found in immatures and resting individuals, and lowest in mature and recovery individuals. This pattern was found in Salmonidae and another species with synchronous gonadal development where FSH levels increase during the beginning of gonadal growth, and decrease during spawning (Victoria et al., 2005). In a development experiment with captive specimens of the subfamily Serrasalmidae, Iseki et al., (2003) found an increase in FSH from resting to initial maturation and signals of decrease starting from gonadal maturation.

Our outcomes indicate that water level/discharge and hydrologic regime changes influenced hormone production related with reproduction of potamodromous fish in the Magdalena River.
In the Magdalena River Basin (natural flow river), mature migratory individuals were recorded in rainy seasons, from April to May and from October to December (Jiménez-Segura et al., 2010). Similarly, mature individuals were reported in La Miel River (a regulated river) from January to June and between September to October 2010 (Reinoso-Flórez et al., 2010; López-Casas et al., 2016). Fish from natural flow and regulated rivers behaved as previously reported during the first half of the year but during the second half fish were always ripe. This could be explained partially by the succession of ENSO events that occurred during the study period in the Magdalena River Basin: "La Niña" 2007/2009, "El Niño" 2009/2010 and again "La Niña" 2010/2011, this last La Niña ended around June of 2011. Thus, in a transition from La Niña to El Niño occurred in June 2009, and subsequently a change back to a La Niña event in mid-2010 (León, 2010). This was reflected in the individuals performing the 2010 migrations, especially the subienda (first reproductive migrations of the year). These fish may mainly belong to cohorts of 2008 and 2009, which grew under "La Niña" conditions, i.e. abundance of nursery habitats and food (López-Casas, 2015). At the same time increased food availability and rapid growth for adults and juveniles has also been reported (Gomes and Agostinho, 1997; Agostinho et al., 2004). An increase in nursery areas, food availability and spawning areas result in a stronger cohort of P. magdalenae that show larger size and weight and higher numbers (López-Casas, 2015), stimulating gonads to ripen and bringing a longer spawning period in response to these atypical environmental signals, explaining population patterns of P. magdalenae during 2011.

Changes in river flow modified the reproductive hormone response in individuals of P. magdalenae independently of river flow, thus, GI was higher when fish were ripe, during June to September 2010 in the natural flow river, and from May to October 2010 and for February to April 2011 in the regulated river. GI and sexual maturity are closely related variables, involving energy use obtained by feeding and accumulated as fat (Vazzoler, 1996). This relationship is controlled by a set of signals that precedes the beginning of reproductive events, including fish migrations (Vazzoler, 1996). In tropical rivers, spawning occurs during flows (Lowe-McConnell, 1987). In the Magdalena River Basin, two high water (flood) seasons have been recorded: The first one begins in March and ends in May, and the second one starts in October and finishes in November (Restrepo et al., 2005; Jiménez-Segura et al., 2010; López-Casas et al., 2016). All large rivers in northern South America show marked seasonality on the hydrograph; most of
them show a unimodal seasonal discharge pattern, but the Magdalena has a more complex
pattern including dual minima (January, August) (Lewis et al., 2006). With an increase in water
level due to regional increase of precipitation twice a year, all biotic and abiotic conditions for
reproduction processes occur two times per years, allowing two reproductive events for P.

magdalenae.

Hydropower production generates daily hydropeaking downstream from the dam, causing a
permanent alteration in flow pulse that may change reproduction process signals. In regulated
flow rivers, the presence of mature individuals during almost all sampling months, and hormonal
behavior without relation with river level signals suggested that continuous changes in water
levels produced a blurred and permanent reproductive signal for individuals of P. magdalenae,
hindering perception of changes in water level, affecting in this way the reproductive success of
individuals inhabiting rivers with these attributes.

Conclusions

We found that minimal changes in flow regime are strong enough to cause the pituitary gland to
start its reproductive hormonal response. Due to the daily hydropeaking of hydropower
production, the environmental signal (mainly water level) that stimulates hormone production for
the reproduction process is constantly altered, affecting the natural relation between
environmental signals and gonadal and hormonal responses, which is essential for the welfare of
fish like Prochilodus magdalenae and the perpetuation of the species.

Acknowledgements

The authors dedicate this work to Mauricio Arias-Gallo for his contribution to the initial idea of
this work and for his unforgettable presence. We are grateful to Professor Shunsuke Moriyama
(Kitasato University, Iwate, Japan) who kindly supplied the antisera used in this study, helped
with the laboratory process and interpretation of results, to the Pathology Laboratory of San
Vicente de Paul Hospital, to Alejandra De Fex Wolf for assisting map elaboration, to Prof.
Donald Taphorn for review and improve the English of this paper, and to ISAGEN S.A. E.S.P.
provided financial support (contract 46/3296).
References


(Prochilodus mariae) como agente inductor sustitutivo en la reproducción de su misma 

Nesfatin-1 Regulates the Hypothalamo-Pituitary-Ovarian Axis of Fish. *Biology of 

Iseki KK, Correa SA, Negrão JA, Castrucci AML. 2003. Seasonal changes in LH and 17β-
estradiol levels in the freshwater teleost *Piaractus mesopotamicus*. Salvador: World 
Aquaculture, Book of Abstracts.

species in the Magdalena River basin, Colombia. *Ecology of Freshwater Fish*, 19:178-
186. DOI: 10.1111/j.1600-0633.2009.00402.x

*Canadian Journal of Fisheries and Aquatic Sciences*, 106:110-127

León G. 2010. Aspectos de la circulación atmosférica de gran escala sobre el noroccidente de 
Suramérica asociada al ciclo ENSO 2009-2010 y sus consecuencias en el régimen de 
precipitación en Colombia. Bogotá: IDEAM.

Lewis L Jr., Hamilton SK, Saunders JF. 2006. Rivers of northern South America. In: Colbert E, 
Cushing KW, Cummins G, Minshall W, editors. *River and Stream Ecosystems of the 

Press.

Cambridge University Press.

hydrological regimes. [PhD thesis]. Medellín, Colombia: Universidad de Antioquia.


Table 1 (on next page)

Number of individuals of *P. magdalenae* by sex, maturity stage and site

Number of individuals of *Prochilodus magdalenae* by maturity stage (according to Vazzoler classification), by sex and by sample site (natural or regulated flow river), collected between June 2010 to June 2011 in the Magdalena River Basin.
<table>
<thead>
<tr>
<th>SITE</th>
<th>Total by sex</th>
<th>Stage A</th>
<th>Stage B</th>
<th>Stage C</th>
<th>Stage D</th>
<th>Stage E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂: 254</td>
<td>154</td>
<td>35</td>
<td>51</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Natural flow river</td>
<td>♂: 332</td>
<td>164</td>
<td>56</td>
<td>103</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>♂: 307</td>
<td>147</td>
<td>62</td>
<td>65</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Regulated flow river</td>
<td>♂: 376</td>
<td>127</td>
<td>77</td>
<td>162</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

Map of the study area and sampling sites

Location of the study area and sampling sites on the Magdalena River (Natural flow river) and sampling sites on the La Miel River Basin (Regulated flow river). The Magdalena River flows from south to north.
Figure 2

Water level (m) and discharge (m$^3$/s) by sampling sites

Water level (m) and discharge (m$^3$/s) from June 2010 to June 2011 for natural and regulated flow rivers
Figure 3

Mean value and SD of Gonadosomatic Index by site, month and sex for *P. magdalenae*

Mean value and standard deviation of Gonadosomatic Index (GI) by sampling site, month and sex for *Prochilodus magdalenae* from natural and regulated rivers of the Magdalena River Basin. Same letter or number means equals mean values.
Figure 4

Relation between gonadal maturity and number of immunostained FSH and LSH cells for *P. magdalenae*

Relation between gonadal maturity and number of immunostained cells for Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) for *Prochilodus magdalenae* by river and sex. Same letter or number means equal mean values.