Hydropower effects on *Prochilodus magdalenae* (Prochilodontidae) reproduction: evidence from endocrine response

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ABSTRACT

**Objective.** Describe the endocrine response associated with the reproduction of a tropical potamodromous fish under changes in the flow discharge produced by hydropower in an Andean. **Materials and methods.** We analyzed *Prochilodus magdalenae* reproduction in individuals from two sectors of a Neotropical river basin: one with a natural flow and another one with a regulated hydrological regime. **Results.** In the sector of the basin with the 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 hydropoaking due to hydroelectric operation no such correlation was detected. **Conclusions.** Hormone production associated to reproduction of the Potamodromous fish was sensitive to changes in water level and discharge. Then, fish exposed to hydropoaking would be receiving ambiguous stimuli that affect hormone production, reproduction synchronization with environmental cues, and ripening, which are essential for reproductive success.

Keywords: Fish reproduction, freshwater fish, gonadotropic cells, hydropower, Magdalena river basin, (Source: Tesauro Ambiental para Colombia).

RESUMEN

**Objetivo.** Describir la respuesta endocrina asociada con la reproducción de un pez potamódromo tropical ante cambios en el régimen de descarga de caudal producido por la generación de hidro-energía en un río Andino. **Materiales y métodos.** Se analizó la reproducción de *Prochilodus magdalenae* en individuos de dos sectores de una cuenca neotropical: uno con flujo hidrológico natural y otro con un régimen regulado. **Resultados.** En la sección de la cuenca con flujo natural, se encontró que la producción de hormonas relacionadas con la reproducción de peces (FSH y LH) estaba correlacionada con el índice gonadosomático, mientras que en peces bajo la influencia del pulso de agua producto de la operación hidroeléctrica esta correlación no fue detectada. **Conclusiones.** La producción de hormonas asociadas con la reproducción en peces potamódromos fue sensible a cambios en el nivel/caudal. En consecuencia, peces expuestos a las alteraciones en el pulso de caudal estarían recibiendo estímulos ambiguos que afectan la producción de hormonas, la sincronización de la reproducción con las señales ambientales y la maduración, lo cual es esencial para el éxito reproductivo.

**Palabras clave:** Células gonadotrópicas, cuenca del río Magdalena, peces dulceacuícolas, represas, reproducción de peces (Fuente: Tesauro Ambiental para Colombia).
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 (1). The hydrological cycle in these rivers is mainly determined by fluctuations in rainfall, which changes water level resulting in wet and dry periods (2). Water level variations result in numerous environment changes that modify seasonally habitat and food availability, influencing reproduction, migrations, growth, ecology and regional fisheries (3). In tropical freshwater fishes, reproductive responses are associated with the flooding regime and discharge (1,2,4). In response to human demands - especially for energy- natural flow regimes of rivers suffer several modifications by hydroelectric construction. These changes might affect fish reproduction, specifically by changes in the natural amplitude and duration of river flows, creating physical barriers for migrations (5,6,7) and affecting aquatic organisms' behaviour (5,8). 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 (9).

Reproduction is a multifactorial process involving interactions between environmental, social, neural, hormonal and nutritional components (10). In fishes, reproduction starts by synchronized actions of the Hypothalamus-Pituitary-Gonadal (HPG) axis (11), which response to environmental signals activating the production of specific hormones related with defined reproductive stages (12). The stimuli that influence hormone production are associated with gonadal maturation during rainy seasons (7,13,14,15), and these hormones are synthesized in the adenohypophysis by gonadotrophs, pituitary cells that are developed in response to environmental factors (14). 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 (11,12). 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 tropical freshwater fishes are determined by the hydrologic regime (7,13,14,15).

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 more than 30,000 fish species (16), each with different reproductive strategies, most 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 (12).

Change in the reproductive response of tropical fishes due to hydropower is well documented, but conclusions are based mainly on the shape and size of adult gonadal tissue (7,17). There are many methodologies that can be employed to better understand the 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 immunochemical detection of FSH and LH gonadotrophs in fish like Odontesthes bonariensis and Oreochromis niloticus (12). That approach could work for prochilodontids to help explain how fishes respond when facing hydrological regime changes caused by barriers such as dams. Until now, changes in hormonal response related to the spawning of migratory freshwater fishes and changes in environmental signals had not been confirmed. We tested the hypothesis that hydropoaking due to hydropower production negatively affects hormones production and the reproduction of Prochilodus magdalenae (Steindachner,1879) individuals in the Magdalena River Basin.

MATERIALS AND METHODS

Description of the study area. The Magdalena River Colombia (Figure 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 m³·s⁻¹ into the Caribbean Sea (18) and is considered a focal axis of Colombian economic development.

Figure 1. 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.
Magdalena river 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 around 9.7 GW, which is the most important source of hydropower generation in Colombia (19). 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$^{-1}$, but due to hydropower generation, the hydrological regime has been modified and daily hydropoeaking occurs downstream of the dam (Figure 2).

To evaluate the effect of hydropoeaking on hormone production, we collected potamodromous fishes under hydropoeaking conditions (La Miel River) and under natural flow conditions (Magdalena River). Individuals of P. magdalenae were captured at those sites each month. Although the Magdalena River has some dammed stretches (i.e. Betania (2°41′06″N, 75°26′24″W) and El Quimbo Reservoirs (1°30′0″N, 76°34′59.88″W)), the contributions of other tributaries cushion the effects of localized hydropower generation (Figure 2). Water levels (m) and flow (m$^3$/s) from June 2010 to June 2011 were counted. We used the average daily water level (m) and discharge (m$^3$/s) for the natural river were 145 km approx.). After capture, fish were fixed in 10% formalin in situ and transported to the Ichthyology Laboratory of the University of Antioquia, Colombia.

For each fish, we recorded standard length ($L_c$, mm), total body weight ($W_T$, g), eviscerated body weight ($W_E$, g), gonads weight ($G_w$, g), and sex (male or female).

Macroscopic observations were made of ovaries to determine sexual maturation stage of females, looking for characteristics as colour and vascularization or presence of oocytes in the ovaries. For males, testicles size, colour and vascularization were observed. Reproductive stages for females and males were defined following Vazzoler scale (20), which classified Stage A: Immature, B: Maturing, C: Mature, D: Spawned, and E: Resting.

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

Where $W_T$ is total body weight and $G_w$ is gonad weight.

To test if this index was different between sampling sites, we used a non-parametric test in GraphPad Prism 7.

To estimate the relationship between flow peaks, 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 (between 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 pituitary glands analyzed. The response variable was the number of immunostained cells by mm$^2$.

Antisera used in this study were anti-chum salmon FSHβ and LHβ, see references (21) and (22). We used a 1/5000 dilution for anti-GTH-Iβ (FSH) and anti-GTH-IIβ antibody (LH).

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 the UltraVision Quanto Detection System HRP DAB kit (Thermo Scientific), and we follow the protocol described by Shi et al (23) 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 mm$^2$ were counted.

To determine if there were differences in hormone production between gonadal ripeness stages, 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.

Taking into account that the rainy season is known to influence hormone production and gonadal maturation, 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.

**Ethical aspects.** The fishes were slaughtered without cruelty suffering or prolongation of the agony, according to the Law 84 of 1989 (Colombia).

**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 stages 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).

<table>
<thead>
<tr>
<th>SITE</th>
<th>Total by sex</th>
<th>Stage</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural flow river</td>
<td>♂: 254</td>
<td></td>
<td>154</td>
<td>35</td>
<td>51</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>♀: 322</td>
<td></td>
<td>164</td>
<td>56</td>
<td>103</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Regulated flow river</td>
<td>♂: 307</td>
<td></td>
<td>147</td>
<td>62</td>
<td>65</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>♀: 376</td>
<td></td>
<td>127</td>
<td>77</td>
<td>162</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

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 ($H=187.824$, $DF=11$, $p<0.005$), so that 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 the highest $G_I$ 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 (M=12) and regulated (M=13) rivers ($U=55$, $p>0.005$) (Figure 3). In both rivers, there were low values in the $G_I$ in the first semester of 2011.

A total of 113 pituitary glands were analyzed. The number of LH immunostained cells was different among maturity stages in males from the natural river ($F_{(3,21)}=5.49$, $p<0.005$), as well as from regulated river individuals ($F_{(3,27)}=26.652$, $p<0.005$). 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 the natural river ($F_{(3,17)}=10.684$, $p<0.005$), as well that from regulated river individuals ($F_{(3,27)}=24.401$, $p<0.005$). 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 as those from the regulated river ($F_{(2,27)}=9.462$, $p<0.005$). 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 ($F_{(3,19)}=4.318$, $p<0.005$), 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 female specimens (A and B maturity stages) had a higher number of FSH immunostained cells, while individuals in advanced maturity stage (C) had higher values of LH immunostained cells (see figure 4 for an example of immunostained pituitary cells). This situation does not apply to male individuals from the natural river (Figure 5).
Figure 4. Sections of female *Prochilodus magdalenae* pituitary gland, stained with anti-GTH Iβ and anti-GTH IIβ antibodies showing immunoreactivity (dark brown colour) in gonadotropes cytoplasm. Images 1A and 2A correspond to an immature specimen (stage A), presenting minimal immunoreactivity with anti-GTH Iβ antibody compared with anti-GTH IIβ antibody stained. Images 1C and 2C correspond to a mature specimen (stage C), presenting higher immunoreactivity with anti-GTH Iβ antibody compared with anti-GTH IIβ antibody stained.

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 relationship between discharge (m$^3$/s) and gonadosomatic index ($r=0.195$, $p<0.05$), and a negative and significant relationship between immunostained FSH cell densities and water level ($r=-0.090$, $p<0.05$). There was not a significant relationship between LH immunostained cell densities and water level ($r=-0.004$, $p<0.05$), meaning that in this river there is no correlation between reproduction and hormonal response.

**DISCUSSION**

The results showed that flow pattern influences the hormone production of potamodromous fish in the Magdalena River Basin, changing migratory fish hormones response correlated with flow pattern, possibly leading to a lack of synchrony between reproductive hormones response and water level. We suggest that the altered flow peaks result of hydropower generation could...

Figure 5. Relationship 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.
be considered as a possible factor behind reproductive hormones alteration in *P. magdalenae*.

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 (24) and their associated physiochemical variables (e.g., temperature and dissolved solids) (4). 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, being a possible factor to influence hormonal changes in *P. magdalenae*. We suggest that the lack of synchrony between hormonal behaviour and the gonadosomatic index is a response to unusual flow peaks, which modifies environmental information perceived by fishes (25). This might influence their spawning behaviour, and lately the recruitment, modifying the community structure (3).

The results of the present study are similar to those obtained by Santos et al (26), where levels of FSH in rainbow trout decreased before sexual maturity starts and increased after ovulation. This was because FSH hormone contributes to early spermatogenesis and follicular growth, while LH promotes gamete maturation and is involved in sperm production and ovulation (12). Therefore, higher levels of FSH were found in immatures and resting individuals, and lowest in mature and recovery individuals. This pattern was found individuals from the Salmonidae family and another species with synchronous gonadal development where FSH levels increase during the beginning of gonadal growth and decrease during spawning (15).

Time periods for gonads ripening may change associated to environmental conditions associated to ENSO events. In the Magdalena River Basin (natural flow river), mature migratory individuals were reported in rainy seasons (April to May and October to December) (4). Similarly, mature individuals were reported in La Miel River (a regulated river) from January to June and between September to October 2010. In this study, fish from natural flow and the regulated rivers behaved as previously reported, however in the first six months of 2011, female’s Gs values were lower possibly because La Niña activity during that months, reducing known stimulus perception (i.e., low levels in flow) for the maturation of the gonads.

Changes in the time periods of gonads ripening could be explained partly 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. 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 (24). At the same time increased food availability and rapid growth for adults and juveniles have also been reported (3, 27). 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 (24), 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.

It was found that minimal 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 (12). This relationship is controlled by a set of signals that precedes the beginning of reproductive events, including fish migrations (17). In tropical rivers, spawning occurs during flooding (2). 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 (4, 24, 28). 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) (24).

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 year, allowing two reproductive events for *P. magdalenae*.

Hydropower production generates daily flow peaks downstream from the dam, causing a permanent alteration that may cause problems for the reproductive cycle. 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, as a result of hydroelectric production added to ENSO effects, produced a blurred reproductive signals for individuals of *P. magdalenae*, interfering in the perception of changes in water level, affecting in this way the reproductive success of individuals inhabiting rivers with these attributes.

We found that minimal changes in flow regime, either as a result of hydropower production or/and ENSO effects, are strong enough to cause a response in the pituitary gland of *P. magdalenae*, creating a reproductive hormonal response, in some cases desynchronized with the other environmental factors (as conductivity or food availability) necessary for a successful reproductive process. In tropical rivers, when the water level (mainly environmental stimuli for this region) that stimulates hormone production for the reproduction process is constantly altered, affect the 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. In this sense, more studies are needed to specifically explore water level alteration effects over hormonal behaviour in fishes under hydropower influence.

**Conflict of interests.**

The authors declare no conflict of interests.

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