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

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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 hydropeaking receive ambiguous stimuli that affect hormone production, reproduction synchronization with environmental cues, and ripening, which are essential for reproductive success.

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2   **response**

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16

17 **Abstract**

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20 **Methods.** We used an endocrine method to test the influence of hydropeaking on hormone

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24 **Results.** In the section of the basin with natural flow we found that the production of hormones

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28 **Discussion.** We conclude that potamodromous fish reproductive hormone production is sensitive

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30 ambiguous stimuli that affect hormone production, reproduction synchronization with

31 environmental cues, and ripening, which are essential for reproductive success.

32 **Keywords**

33 Dams, Freshwater fish, Gonadotropic cells, Magdalena River Basin, Neotropical fish

34 reproduction.

35

36

37 **Introduction**

38 Biology and ecology of fishes in tropical large rivers are linked to the hydrological regime in the

39 main channel and regular flooding in associated floodplains (Welcomme, 1985; Junk et al.,

40 1989). The hydrological cycle in these rivers is mainly determined by fluctuations in rainfall,

41 which changes water level resulting in wet and dry periods (Lowe-McConnell, 1987; Wootton,

42 1999). Water level variations result in numerous environment changes that modify seasonally

43 habitat and food availability, influencing reproduction, migrations, growth, ecology and regional

44 fisheries (Agostinho et al., 2004). In tropical freshwater fishes, reproductive responses are

45 associated with the flooding regime and discharge (Lowe-McConnell, 1987; Junk et al., 1989;

46 Jiménez-Segura et al., 2010). In response to human demands - especially for energy- natural flow

47 regimes of rivers suffer several modifications with the construction of dams. These changes

48 might affect fish reproduction, specifically by changes in the natural amplitude and duration of

49 river flows, creating physical barriers for migrations (Agostinho et al., 2001, 2003; Murchie et

50 al., 2008) and affecting aquatic organisms' behavior (Agostinho et al., 2000, 2001). At the

51 population level, the transformations in regulated rivers involve alterations in physical and

52 chemical characteristics and changes in aquatic habitat availability, that may disrupt

53 environmental cues for reproduction that influence maturation, spawning and migrations

54 (Macnaughton et al., 2015).

55 Reproduction is a multifactorial process involving interactions between environmental, social,

56 neural, endocrinological and nutritional components (Urbinati, 2005). In fishes, reproduction

57 starts by synchronised actions of the Hypothalamus-Pituitary-Gonadal (HPG) axis (Gonzalez et

58   al., 2012), which responds to environmental signals activating the production of specific

59   hormones related with defined reproductive stages (Vongvatcharanona, 2006). The stimuli that

60   influence hormone production are associated with gonadal maturation during rainy seasons

61   (Donaldson, 1981; Donaldson and Hunter, 1983; Victoria et al., 2005; Murchie et al., 2008), and

62   these hormones are synthesized in the adenohypophysis by gonadotrophs, pituitary cells that are

63   developed in response to environmental factors (Donaldson and Hunter, 1983). Follicle-

64   stimulating hormone (FSH) contributes to early spermatogenesis and oogenesis, while

65   luteinizing hormone (LH) is related with seasonal gonadal growth, sex steroid production, sperm

66   production in males, and ovulation in females during spawning (Vongvatcharanona et al., 2006;

67   Gonzalez et al., 2012). Their concentrations and proportions in fishes bodies depend on

68   reproductive stage (FSH concentration values are higher during immature stages and LH

69   increases during maturity), which in Neotropical freshwater fishes are determined by the

70   hydrologic regime (Donaldson, 1981; Donaldson and Hunter, 1983; Victoria et al., 2005;

71   Murchie et al., 2008). In reproductive biology, endocrinological tools have begun to be used to

72   understand how reproduction is controlled and coordinated by the environment. Due to the

73   existence of about 25,000 species of fishes, each with different reproductive strategies, the

74   majority of studies are for just a few selected species such salmon or goldfish, that have

75   representative characteristics which are used as a model of hormonal studies for other fish

76   species (Melamed and Sherwood, 2005).

77   Change in reproductive response of tropical fishes due to hydropower is well documented, but

78   conclusions are based mainly on shape and size of adult gonadal tissue (Murchie et al., 2008;

79   González et al., 2010). There are many methodologies that can be employed to better understand

80   effects of regulated rivers on freshwater fishes at the cellular level. Endocrinology, for example,

81   brings a series of tools that can be used to support ecological interpretations related with

82   reproduction, such as the use of anti-chum salmon FSHβ and LHβ (specific antisera) for

83   immunochemical detection of FSH and LH gonadotrophs in fish like *Odontestes bonariensis* and

84   *Oreochromis niloticus* (Vongvatcharanona et al., 2006). That approach could work for

85   prochilodontids (pers. comm. Prof. Shunsuke Moriyama, 2011) to help explain how fishes

86   respond when facing barriers such as dams. Until now, changes in environmental signals

87   associated with modified river flow, and hormonal response related to spawning of a migratory

88   freshwater fishes had not been confirmed. We tested the hypothesis that hydropeaking due to

89   hydropower production negatively affects hormone production and the reproduction of

90   *Prochilodus magdalenae* (Steindachner, 1879) individuals in the Magdalena River Basin. Our

91   results show that changes in water lever due to hydropeaking affect reproductive hormone

92   response of this potamodromous fish.

93

94   *1.1. Description of the study area*

95   The Magdalena River (Fig. 1) is a sixth-order river that flows northwest, between the central and

96   east branches of the Andes (3° and 11° N). It is 1,540 km long, discharges 7,500 $m^3.s^{-1}$ into the

97   Caribbean Sea (Likens, 2010), and is considered a focal axis of Colombian economic

98   development. The annual hydrological pulse is bimodal (two low water and two flooding

99   periods). Due to geomorphology, many of its tributaries have been dammed for hydropower

100   generation, and have an installed capacity of around 14,000 MW, which is the most important

101   source of hydropower generation in Colombia (UPME, 2012). One of the Magdalena River's

102   tributaries is the La Miel River, which was dammed for hydropower in 2002. Mean flow is 84.3

103   $m^3.s^{-1}$ (Marulanda et al., 2003), but due to hydropower generation, the hydrological regime has

104   been modified and daily hydropeaking occurs downstream of the dam (Fig. 2).

105   FIGURE 1

106

107   **Materials & Methods**

108   To evaluate the effect of hydropeaking on hormone production, we collected potamodromous

109   fish under hydropeaking conditions and under natural flow conditions. Individuals of *P.*

110   *magdalenae* were captured at two different sites in the Magdalena River Basin: La Miel River (a

111   regulated river influenced directly by dam hydropeaking), and the main channel of Magdalena

112   River at Puerto Berrío (a natural flow river with a natural hydrological regime). Although the

113   Magdalena River has some dammed stretches (for example Betania Reservoir (2°41′06″N

114   75°26′24″O')), the contributions of other tributaries cushion the effects of localized hydroelectric

115   operation (see Fig. 2). Water levels (m) and discharge (m^3/s) for the natural river were obtained

116   from the national hydrology database (Instituto de Hidrología, Meteorología y Estudios

117 Ambientales - IDEAM), and the ISAGEN S.A. E.S.P hydropower company supplied the

118 information for the regulated flow river. This hydrologic data is collected daily in fixed points

119 throughout the river basin. We used average daily water level (m) and discharge (m^3/s) (Fig. 2).

120   FIGURE 2

121

122 *P. magdalenae* from natural and regulated hydrological regimes rivers were bought monthly

123 from fishermen. Fifty specimens were obtained each month, in the same week, from June 2010

124 to June 2011 from each sampling site. After capture, fish were fixed in 10% formalin *in situ* and

125 transported to the Ichthyology Laboratory of the University of Antioquia.

126 For each fish we recorded: standard length (L_S, mm), total body weight (W_T, g), eviscerated body

127 weight (W_E, g), liver weight (L_W, g), gonads weight (G_W, g), and sex (male or female).

128 Macroscopic observations were made of ovaries to determine sexual maturation stage of females,

129 like color and vascularization or presence of oocytes in the ovaries. For males, testicles size,

130 color and vascularization were observed. Reproductive stages for females and males were

131 defined following Vazzoler scale (1996), which classified Stage A: Immature, B: Maturing, C:

132 Mature, D: Spawned, and E: Resting.

133 The stage of reproductive activity was determined using gonad and liver weight relation with

134 total weight, since the behavior of these organs is correlated with reproduction (Vazzoler, 1996).

135 We calculated gonadosomatic (G_I) indexes for gonad or liver weight proportion to total fish body

136 weight, in a specific time or reproductive stage:

137 $$GI = (Gw \div Wt) \times 100$$

138 Where W_T is total body weight and G_W is gonad weight. To test if these indices were different

139 between sampling sites we used a non-parametric test in GraphPad Prism 7.

140 To estimate the relationship between discharge, gonadosomatic index, FSH and LH cell densities

141 we used Spearman correlation.

142 ***Hormonal analysis***

143 From each sampling site (natural and regulated flow river), we selected one pituitary gland of

144 each macroscopic maturity state collected (one for stage A, one for B, one for C, one for D and

145 one for E), from males and females, each month (12 months in each site). Each pituitary gland

146 was embedded in paraffin wax and sectioned in, at least, 10 cuts (three to five-μm thickness)

147 with a microtome in the *par proximal distalis* sector, where cellular density is higher compared

148 with other pituitary sectors, and trying to cut in the same deep for all the pituitary gland

149 collected. The response variable was the number of immunostained cells by mm^2.

150 Antisera used in this study were anti-chum salmon FSHβ and LHβ. Suzuki and collaborators

151 (1988a, 1988b) (in Vongvatcharanon et al., 2006) previously described the origin and

152 characteristics of these antibodies. We used a 1/5,000 dilution for anti-GTH-Iβ (FSH) and anti-

153 GTH-IIβ antibody (LH) as suggested by Prof. Shunsuke Moriyama (pers. comm. 2011).

154 Monthly, pituitary glands were removed, fixed in 10% formalin for preservation and processed

155 for paraffin embedding, cutting and placing in charged microscope slides. We used UltraVision

156 Quanto Detection System HRP DAB kit (Thermo Scientific), and we follow the protocol

157 described by Shi et al., (1999) for each sample:

158 The photographs were taken with a digital camera (Nikon D500) installed in an optical binocular

159 microscopic (Zeiss) and the number of stained cells by mm^2 were counted.

160 To determine if there were differences in hormone production between gonadal ripeness states

161 we tested for significant differences in the number of immunostained cells for each gonadal

162 development state with a one way ANOVA for each cells type: FSH cells and LH cells.

163 Because rainy season is known to influence hormone production and gonadal maturation (Mishra

164 and Sarkar, 2013), we used Spearman correlation to test for a relationship between the type and

165 variations of hormone production (FSH and LH) with modifications in the water level and

166 discharge for individuals from the natural flow river and the regulated river.

167

168 **Results**

169   We analyzed 1271 individuals, of which 588 (332 males and 256 females) came from the natural

170   flow river and 683 (376 males and 307 females) from the regulated flow river. Individuals in all

171   maturity states were collected at both sampling sites and the majority were found in A, B and C

172   maturity stage in both natural and regulated flow rivers (Table 1).

173   TABLE 1

174   The gonadosomatic index (G_I) in males changed through time at both sample sites. In the natural

175   flow river, differences were found in G_I among months (H= 187.824, DF= 11, p <0.005), where

176   June, July, August and October showed the highest values. Individuals from the regulated flow

177   river, also had differences among months (H= 208.509, DF= 12, p <0.005), with the highest G_I

178   values from June to August 2010, October 2010 and from March to April 2011. There was no

179   significant difference in G_I values in males for the natural (M= 0.309, SD= 0.298) and regulated

180   (M= 0,481, SD= 0.377) rivers (t (23) = -1.259, p>0.005).

181   For females, we found differences in the G_I values through time at both sites. In the natural flow

182   river, we found differences among months (H = 124.260, DF= 11, p: <0.005), with highest GI

183   values in June and August 2010. We found differences among months for females in the

184   regulated flow river (H = 151.490, DF= 12, p: <0.005), with the highest G_I values from August

185   to October 2010. There was no significant difference in G_I values in females for the natural

186   (Mnd= 12) and regulated (M= 13) rivers (U= 55, p>0.005) (Fig. 3).

187   FIGURE 3

188   A total of 113 pituitary glands was analyzed. The number of LH immunostained cells was

189   different among maturity stages in males from the natural river ($F_{(2, 21)}$= 5.49, p<0,005), as well

190   from individuals from the regulated river ($F_{(3, 27)}$ = 26.652, p<0,005). There was no significant

191   difference in the number of LH immunostained cells between males from the natural and

192   regulated river (t $_{(7)}$ = -0.889, p>0,005). For females, the number of LH immunostained cells was

193   different among maturity stages in individuals from the natural river ($F_{(2, 17)}$ = 10.684, p<0,005),

194   as well from individuals from the regulated river ($F_{(3, 27)}$ = 24.401, p<0,005). There was no

195   significant difference in the number of LH immunostained cells between females from the

196   natural and regulated river (t $_{(7)}$ = 0.415, p>0,005).

197 For the number of FSH immunostained cells, we found differences among maturity stages in

198 males from the natural river ($F_{(2, 21)}$= 8.945, p<0,005), as well from those from the regulated river

199 ($F_{(3, 27)}$= 9.462, p<0,005). There was no significant difference in the number of FSH

200 immunostained cells between males from the natural vs. the regulated river ($t_{(7)}$= 1.874,

201 p>0,005). For females, the amount of FSH immunostained cells was different among maturity

202 stages in individuals from the natural river ($F_{(3, 18)}$= 4.318, p<0,005), as well from individuals

203 from the regulated river ($F_{(3, 27)}$= 13.592, p<0,005). There was no significant difference in the

204 amount of FSH immunostained cells between females from the natural and regulated river ($t_{(6)}$=

205 0.763, p>0,005).

206 We found that immature and maturing specimens (A and B maturity stages) had higher number

207 of FSH immunostained cells, while individuals in advanced maturity stage (C) had higher values

208 of LH immunostained cells (Fig. 4).

209   FIGURE 4

210 In the natural river, we found a negative and significant relationship between discharge (m^3/s)

211 and the gonadosomatic index (r= -0.168, p<0.05), between gonadosomatic index and

212 immunostained FSH cell densities (r= -0.324, p<0.05), and a positive and significant relationship

213 between gonadosomatic index and immunostained LH cell densities (r= 0.404, p<0.05), showing

214 an expected behavior between discharge variations and gonadal response, and an association

215 between hormonal response with reproductive stages.

216 In counterpart, in the regulated river we found a positive and significant relation between

217 discharge (m^3/s) and gonadosomatic index (r=0.195, p<0.05) and a negative and significant

218 relationship between FSH and LH immunostained cell densities (r= -0.460, p<0.05). Which

219 means that in this river there is no correlation between reproduction and hormonal response.

220

221 **Discussion**

222 Our results show that flow pattern influences the hormone production of potamodromous fish in

223 the Magdalena River Basin, with changes in migratory fish hormone response that is correlated

224 with modified flow pattern, exhibiting a lack of synchrony between reproductive hormones

225 response and water level in the regulated river.

226 In the natural flow river, *P. magdalenae* synchronized their reproductive cycle with water level

227 signals. Conversely, in the regulated river, reproductive response was not related to the river

228 water level.

229 Just as flow pulse has been shown to be the principal cue to initiate most biological processes of

230 aquatic biota, spawning and migration of potamodromous fish in the Magdalena River has also

231 been linked to changes in water levels, and regional climatic events such as ENSO cycles

232 (López-Casas et al., 2016) and their associated physiochemical variables (pH, temperature and

233 dissolved solids) (Jiménez-Segura et al., 2010). Flow pulse of a regulated river with hydropower

234 scheme flows (like La Miel River) undergoes changes that disturb the historical climate and

235 water discharge patterns that fishes use to respond to environmental stimuli. We suggest that lack

236 of synchrony between hormonal behavior and gonadosomatic index is a response to the daily

237 hydropeaking behavior of the river, which modifies environmental information perceived by

238 fishes (Payne, 1986). This might influence their spawning behavior, recruitment and modify

239 community structure (Agostinho et al., 2004).

240 Our results for *P. magdalenae* are similar to those obtained by Santos et al., (2001), where levels

241 of FSH in rainbow trout decreased before sexual maturity starts, and increased after ovulation.

242 This was because FSH contributes to early spermatogenesis and follicular growth, while LH

243 promotes gamete maturation and is involved in sperm production and ovulation

244 (Vongvatcharanona, 2006). Therefore, higher levels of FSH were found in immatures and resting

245 individuals, and lowest in mature and recovery individuals. This pattern was found in

246 Salmonidae and another species with synchronous gonadal development where FSH levels

247 increase during the beginning of gonadal growth, and decrease during spawning (Victoria et al.,

248 2005). In a development experiment with captive specimens of the subfamily Serrasalmidae,

249 Iseki et al., (2003) found an increase in FSH from resting to initial maturation and signals of

250 decrease starting from gonadal maturation.

251 Our outcomes indicate that water level/ discharge and hydrologic regime changes influenced

252 hormone production related with reproduction of potamodromous fish in the Magdalena River

253 Basin. In the Magdalena River Basin (natural flow river), mature migratory individuals were

254 recorded in rainy seasons, from April to May and from October to December (Jiménez-Segura et

255 al., 2010). Similarly, mature individuals were reported in La Miel River (a regulated river) from

256 January to June and between September to October 2010 (Reinoso-Flórez *et al.* et al., 2010;

257 López-Casas et al., 2016). Fish from natural flow and regulated rivers behaved as previously

258 reported during the first half of the year but during the second half fish were always ripe. This

259 could be explained partially by the succession of ENSO events that occurred during the study

260 period in the Magdalena River Basin: "La Niña" 2007/2009, "El Niño" 2009/2010 and again "La

261 Niña" 2010/2011, this last La Niña ended around June of 2011. Thus, in a transition from La

262 Niña to El Niño occured in June 2009, and subsequently a change back to a La Niña event in

263 mid-2010 (León, 2010). This was reflected in the individuals performing the 2010 migrations,

264 especially the *subienda* (first reproductive migrations of the year). These fish may mainly belong

265 to cohorts of 2008 and 2009, which grew under "La Niña" conditions, i.e. abundance of nursery

266 habitats and food (López-Casas, 2015). At the same time increased food availability and rapid

267 growth for adults and juveniles has also been reported (Gomes and Agostinho,1997; Agostinho

268 et al., 2004). An increase in nursery areas, food availability and spawning areas result in a

269 stronger cohort of *P. magdalenae* that show larger size and weight and higher numbers (López-

270 Casas, 2015), stimulating gonads to ripen and bringing a longer spawning period in response to

271 these atypical environmental signals, explaining population patterns of *P. magdalenae* during

272 2011.

273 Changes in river flow modified the reproductive hormone response in individuals of *P.*

274 *magdalenae* independently of river flow, thus, GI was higher when fish were ripe, during June to

275 September 2010 in the natural flow river, and from May to October 2010 and for February to

276 April 2011 in the regulated river. GI and sexual maturity are closely related variables, involving

277 energy use obtained by feeding and accumulated as fat (Vazzoler, 1996). This relationship is

278 controlled by a set of signals that precedes the beginning of reproductive events, including fish

279 migrations (Vazzoler, 1996). In tropical rivers, spawning occurs during flows (Lowe-McConnell,

280 1987). In the Magdalena River Basin, two high water (flood) seasons have been recorded: The

281 first one begins in March and ends in May, and the second one starts in October and finishes in

282 November (Restrepo et al., 2005; Jiménez-Segura *et al.* 2010; López-Casas *et al.* 2016). All

283 large rivers in northern South America show marked seasonality on the hydrograph; most of

284   them show a unimodal seasonal discharge pattern, but the Magdalena has a more complex

285   pattern including dual minima (January, August) (Lewis et al., 2006). With an increase in water

286   level due to regional increase of precipitation twice a year, all biotic and abiotic conditions for

287   reproduction processes occur two times per years, allowing two reproductive events for *P.*

288   *magdalenae.*

289   Hydropower production generates daily hydropeaking downstream from the dam, causing a

290   permanent alteration in flow pulse that may change reproduction process signals. In regulated

291   flow rivers, the presence of mature individuals during almost all sampling months, and hormonal

292   behavior without relation with river level signals suggested that continuous changes in water

293   levels produced a blurred and permanent reproductive signal for individuals of *P. magdalenae*,

294   hindering perception of changes in water level, affecting in this way the reproductive success of

295   individuals inhabiting rivers with these attributes.

296

297   **Conclusions**

298   We found that minimal changes in flow regime are strong enough to cause the pituitary gland to

299   start it reproductive hormonal response. Due to the daily hydropeaking of hydropower

300   production, the environmental signal (mainly water level) that stimulates hormone production for

301   the reproduction process is constantly altered, affecting the natural relation between

302   environmental signals and gonadal and hormonal responses, which is essential for the welfare of

303   fish like *Prochilodus magdalenae* and the perpetuation of the species.

312

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Table 1(on next page)

Number of invidivuals 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.

SITE	Total by sex	Stage A	Stage B	Stage C	Stage D	Stage E
Natural flow river	♀: 254	154	35	51	12	4
	♂: 332	164	56	103	8	1
Regulated flow river	♀: 307	147	62	65	32	1
	♂: 376	127	77	162	8	2

1

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.
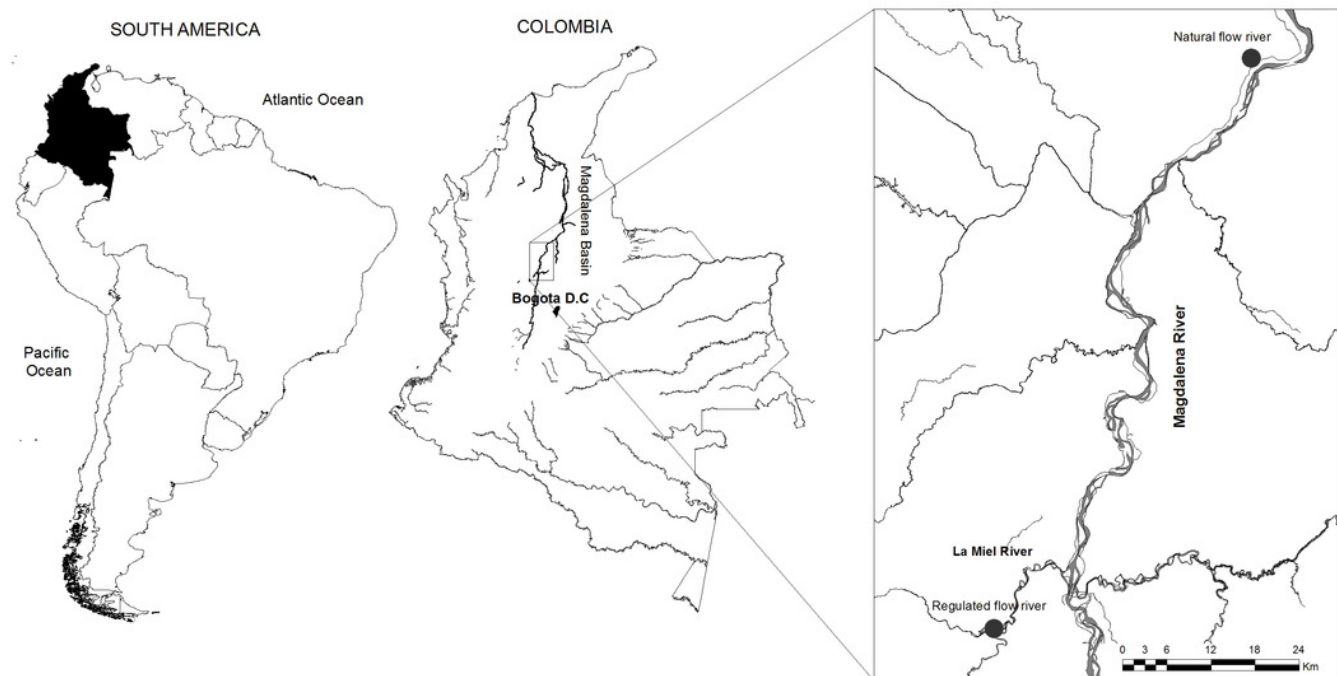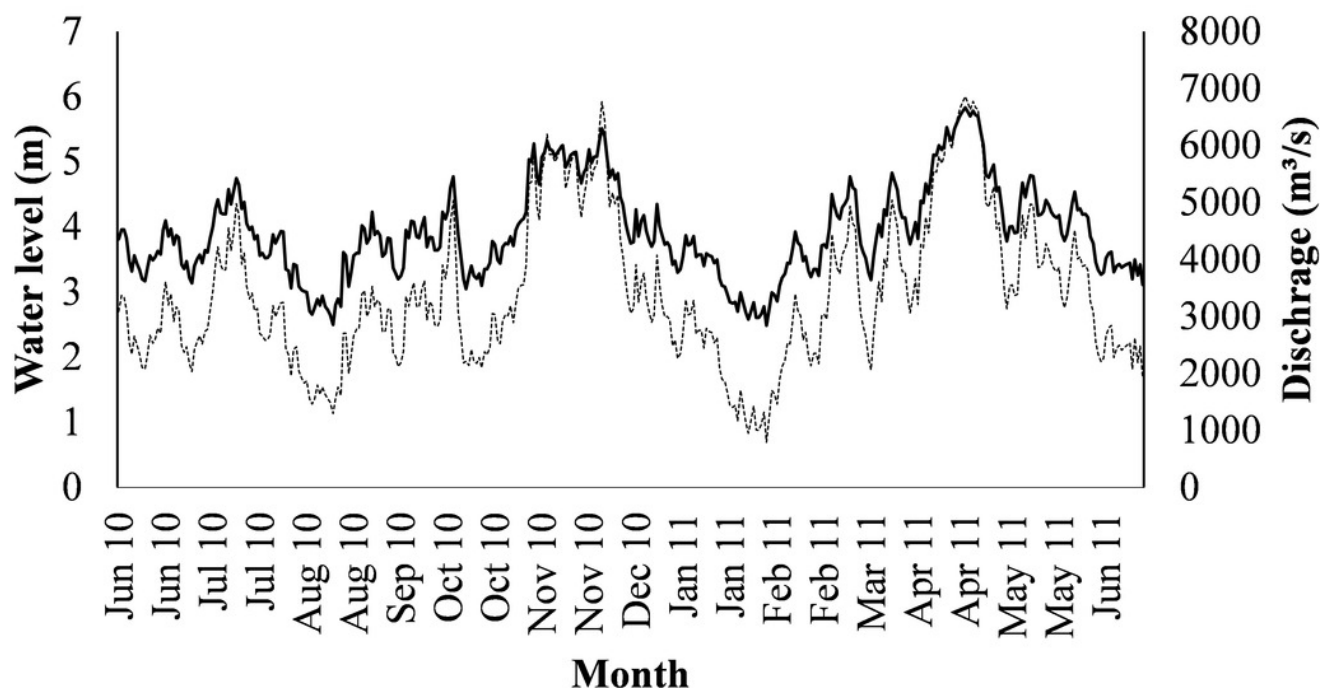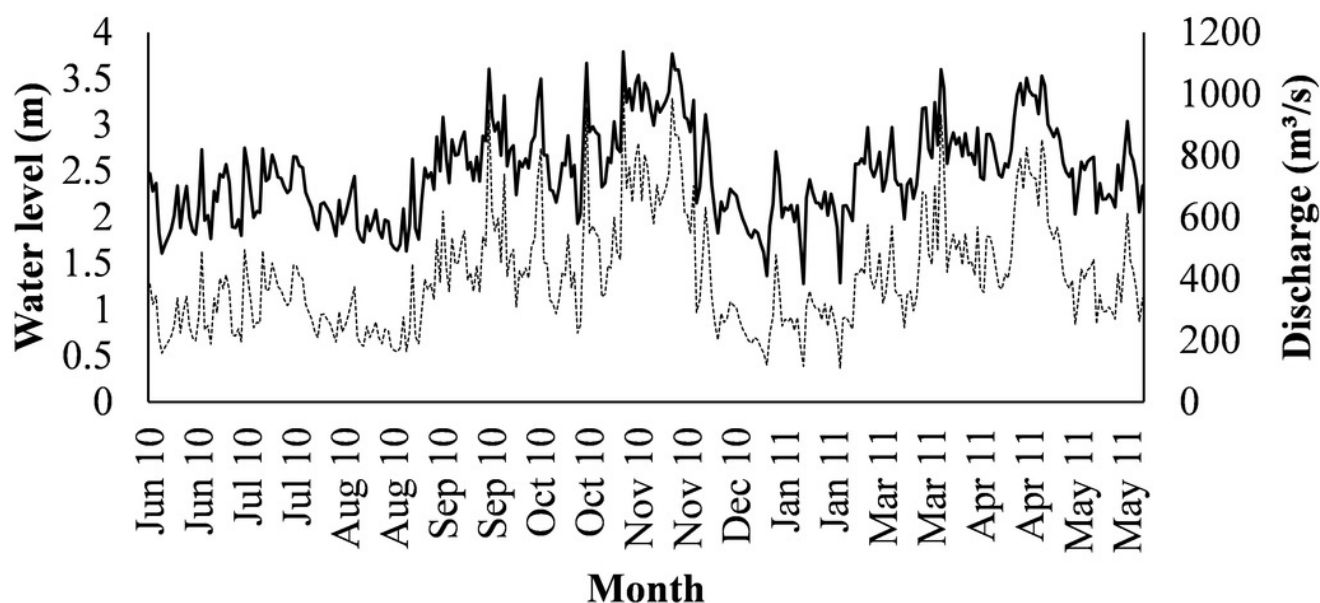
Figure 2

Water level (m) and discharge (m³/s) by sampling sites

Water level (m) and discharge (m³/s) from June 2010 to June 2011 for natural and regulated flow rivers

Natural river 2010 - 2011

Regulated river 2010 - 2011

—— Water level (m)   ---------- Discharge (m3/s)

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

Males between sites
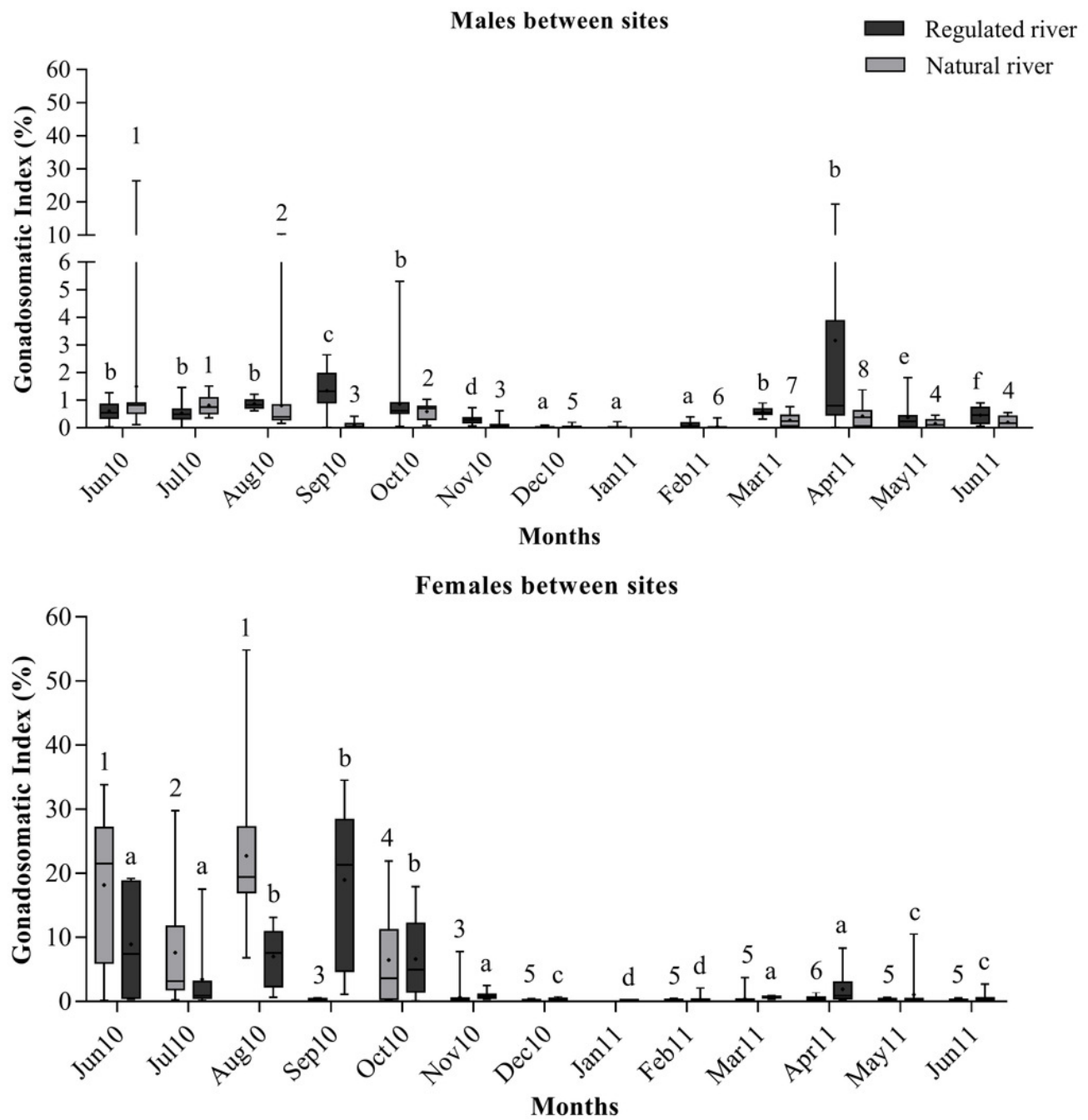
Females between sites

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.