



## Variations in reproductive strategy of the silver Arowana, *Osteoglossum bicirrhosum* Cuvier, 1829 from four sub-basins of the Peruvian Amazon

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### Summary

The aim of this study was to assess the variability of reproductive characteristics of the silver Arowana, *Osteoglossum bicirrhosum*, in four sub-basins of the Peruvian Amazon: the Amazonas, Ucayali, Napo and Putumayo rivers. An illustrated, detailed gonadal maturation scale is proposed for a clearer determination of the reproductive status of male and female Arowana. The breeding season occurred during the dry season and early rising waters in the Amazonas and Ucayali, whereas it was restricted to the dry season in the Napo and Putumayo. A same female can reproduce more than once during the breeding season. Age at maturity was reached during the second year, at sizes varying between 43–45 cm for males and 46–50 cm SL for females. Important variations in fecundity were observed between fish of the four sub-basins, whereas mean oocytes diameter and mass did not vary significantly, suggesting differences in reproductive investment. Together with previously reported variations of growth patterns in the same sub-basins, these results suggest the existence of distinct populations in the Peruvian Amazon, with important consequences for management and conservation, but also for future aquaculture development programs.

### Introduction

The silver Arowana, *Osteoglossum bicirrhosum*, is a relatively large (>1 m length), laterally compressed fish of the archaic bony-tongued fish Order Osteoglossiforms (Goulding, 1980), comprising, in South America, the blue Arowana, *Osteoglossum ferreirai* Kanazawa 1966, and the species of the genus *Arapaima* spp. (Stewart 2013a, 2013b), known as pirarucu in Brazil and paiche in the other Amazonian countries. The silver Arowana preferentially inhabits lentic waters of the Amazon, Rupununi and Oyapock rivers (Goulding, 1980; Bayley and Petrere, 1989; Saint-Paul et al., 2000; Reis et al., 2003). This species occupies a particular place in Amazonian fisheries as it is heavily harvested both as an adult for human

consumption and as a small juvenile (usually still with its yolk sac) for the ornamental trade (Moreau and Coomes, 2006; Alcántara et al., 2007). The Asian Arowanas (*Sleropages* spp.) or dragon fish, closely resemble the Amazonian silver Arowana. These are very popular and sought as pet fish in Asian markets, which has led natural populations to be so dramatically depleted that they have been listed under the Convention on International Trade in Endangered Species (CITES) Appendix I in 1975 (Moreau and Coomes, 2006). Since then, the Amazonian cousin, *O. bicirrhosum*, has replaced them in Asian markets and its exportation has increased alarmingly since the 1990s (Tello and Cánepa, 1991; Moreau and Coomes, 2006, 2007). Owing to its increasing exploitation in Amazonia for ornamental purposes, the species is considered threatened in Peru (Moreau and Coomes, 2006), has already been placed on the Red Book of Colombia (Mojica et al., 2002) and its trade for ornamental purposes is prohibited in Brazil (Lima and Prang, 2008; Prang, 2008).

The reproductive biology of the silver Arowana is relatively well described since the early studies of Goulding (1980, 1990) and Aragao (1989). Recently, patterns of reproductive investment were further investigated in the Mamirauá Reserve (Queiroz, 2008) and the potential role of male aggregations was explored (Verba et al., 2014). In Peru, its reproductive characteristics were studied in one lake of the Putumayo River (Cortegano et al., 2014) and further information also exists in unpublished dissertations (Ayala, 2001; Tang and Gomez Noriega, 2005). Despite extensive ornamental fisheries in almost all distribution areas of the species and growing concern about its conservation, however, no assessment of the geographic variability of reproductive traits has yet been attempted. Besides an obvious interest for conservation and fisheries management, the knowledge of whether geographic populations may display significant variations in specific reproductive traits such as the timing of maturation and egg production would be determinant for future aquaculture development. Indeed, the production of

Arowana juveniles in controlled conditions (aquaculture) appears to be the most immediate alternative to extraction of natural populations for ornamental purposes (Argumedo, 2005; Moreau and Coomes, 2006). Choosing breeders from a population with higher relative fecundity (egg production per unit body mass), for example, might increase chances of success. A recent study in the Peruvian Amazon demonstrated important variations in the growth patterns of the silver Arowana in four sub-basins, suggesting the existence of distinct populations that might require independent management (Duponchelle et al., 2012). The aim of the present study is to assess whether reproductive characteristics also vary, and to which extent, between and among these four sub-basins of the Peruvian Amazon.

## Materials and methods

### Fish collection

Fish were collected monthly between November 2006 and April 2009 at the Iquitos and Requena markets for the Amazonas, Ucayali and Napo sub-basins (Fig. 1). Requena is an important market located on the lower Ucayali River and Iquitos is the main landing market of the Peruvian Amazon, concentrating fishes from the Amazonas, Ucayali, Marañon, Napo, Huallaga, Tigre, Putumayo, Nanay, Yavari and Morona sub-basins (Garcia-Vasquez et al., 2009). For a better accuracy on specimen provenance, fish (of the largest possible size range) were bought from specific providers from each river basin for the Amazonas, Napo and Ucayali rivers. For the Putumayo River, fish were bought monthly, from March 2008 to April 2009, from local fishermen in El Estrecho and Huapapa communities (Fig. 1). The sub-basins were chosen on the basis of their hydrological cycle: the Amazonas and Ucayali have very similar hydrological cycles; the Putumayo and Napo also have similar hydrological cycles, but lag

by about 3 months from that of the Amazonas-Ucayali. Availability and reliability of specimen's provenance was also considered. Monthly water level means were provided by the SO-HYBAM (Observation Service – Geodynamical, hydrological and biogeochemical control of erosion/alteration and material transport in the Amazon basin) and SENAMHI (Servicio Nacional de Meteorología e Hidrología), Peru.

### Biological sample analysis

A total of 951 specimens of *O. bicirrhosum* were analysed. Total length ( $T_L$ ), standard length ( $S_L$ ) and body mass were measured for each fish to the nearest mm and g, respectively. Gonads were photographed and macroscopically checked for maturity stage. A gonad sample was preserved of each fish in Bouin's fluid for histological analyses. Fixed gonads were further processed under classical histological methods with Haematoxylin and Eosin staining for confirmation of macroscopic maturity stages. A gonadal maturation scale was then established, based on the macroscopic criteria detailed in Nuñez and Duponchelle (2009). Nevertheless, the proposed scale also provides the equivalence to the Brown-Peterson et al. (2011) terminology, which was developed in an effort to standardize the confusing number of terms referring to key phases of the reproductive cycle in fisheries science literature.

The breeding season was estimated from the monthly proportions of the females gonadal maturation stage 'spawning capable' (Brown-Peterson et al., 2011). To obtain a better representation of the annual cycle, data collected over the study period were pooled for each river basin.

The average size at first sexual maturity ( $L_m$ ) is defined as the standard length at which 50% of the individuals are at an advanced stage of the first sexual cycle during the breeding season (Legendre and Ecoutin, 1989). Practically, this is the size at which 50% of the fish have reached stage 2

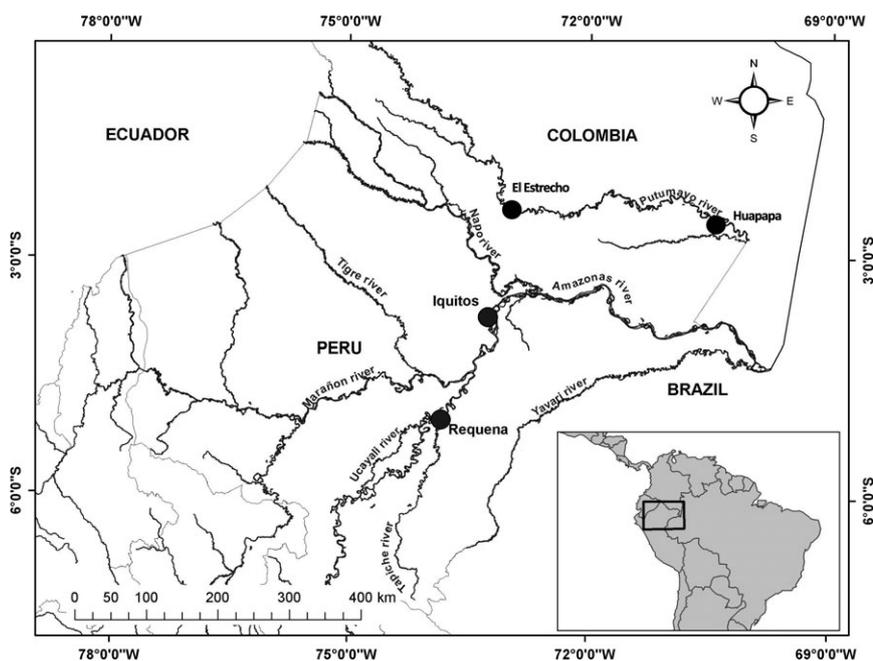


Fig. 1. Map with *Osteoglossum bicirrhosum* collection sites in the Peruvian Amazon (black circles). A total of 711 specimens were collected between November 2006 and April 2009 at the Iquitos and Requena markets for the Amazonas, Ucayali and Napo sub-basins. For the Putumayo, 240 fish were bought between March 2008 and April 2009 from local fishermen at El Estrecho and Huapapa communities

of the maturity scale for females and males (Nuñez and Duponchelle, 2009). The mean SL at first maturity ( $L_m$ ) was estimated by fitting the fraction of mature individuals per 50 mm  $S_L$  intervals to a logistic regression function weighted by the total number of individuals in each size class (Barbieri et al., 1994; Duponchelle and Panfili, 1998):

$$\%M = \frac{100}{1 + e^{(-a \times (L - L_m))}}, \quad (1)$$

where  $\%M$  = percentage of mature individual by size class,  $L$  = central value of each size class, and  $a$  and  $L_m$  = constants of the model.

Age at first sexual maturity ( $A_m$ ) was calculated from the von Bertalanffy growth function (VBGF) as follows:

$$A_m = (-\ln(1 - (L_m/L_\infty))/K) + t_0, \quad (2)$$

where  $L_m$  is the size at first sexual maturity previously calculated and  $t$ ,  $L_\infty$ ,  $K$  and  $t_0$  the parameters of the VBGF obtained from Duponchelle et al. (2012) on the same samples.

Batch fecundity (number of eggs to be potentially laid per spawning event, resulting from the maturation of fully developed intra-follicular oocytes) and oocyte diameter were estimated by image analysis (using NIH Image J software) from whole gonads in final maturation stage (stage 3). As the oocytes were not exactly circular in shape, the mean arithmetic diameter was used:  $\sqrt{d} \cdot D$ , where  $d$  is the minimum diameter and  $D$  the maximum diameter of each oocyte. Oocyte mean mass was calculated from each stage 3 gonad, by weighing 30 oocytes to the nearest 0.001 g.

### Statistical analyses

Differences in mean standard length and mass between sexes within each sub-basin were tested with a  $t$ -test. Differences of mean standard length or mass between sub-basins were tested with Kruskal–Wallis one-way ANOVA on ranks and Dunn's all pairwise *post-hoc* tests.

For each sub-basin, logistic regression models provided an estimate of the  $L_m \pm$  standard deviation (equation 1). Therefore, sizes at maturity between sexes or geographic locations were compared using the classical Student's  $t$ -test (degrees of freedom calculated from the number of size classes).

Analysis of covariance (ANCOVA) was used to test for differences in the fecundity-body mass relationships between geographic populations, using fecundity as the dependent variable, body mass as the covariate, and geographic population as categorical variable.

Comparisons of mean oocyte diameter and mass between geographic populations were carried out using one-way ANOVA.

For all comparisons, when more than two pairwise-tests were performed, the Bonferroni correction was applied to keep the experiment-wide error rate at the desired  $\alpha$  (0.05, 0.01 or 0.001).

### Results

A total of 290 specimens of *O. bicirrhosum* were analysed for the Amazonas, 276 for the Ucayali, 145 for the Napo

and 240 for the Putumayo (Fig. 2a). Mean standard lengths ( $S_L$ ) and masses did not differ among sexes in any population. There were, however, significant differences in mean length (Kruskal–Wallis one-way ANOVA on Ranks,  $H = 83.4$ ,  $P < 0.001$ ) and mass ( $H = 79.3$ ,  $P < 0.001$ ) between populations when females and males were pooled: the Ucayali population differed from all others in both mean length ( $Q = 8.6$ ,  $P < 0.05$  with the Putumayo,  $Q = 6.7$ ,  $P < 0.05$  with the Amazonas and  $Q = 4.8$ ,  $P < 0.05$  with the Napo) and mass ( $Q = 8.3$ ,  $P < 0.05$  with the Putumayo,  $Q = 6.8$ ,  $P < 0.05$  with the Amazonas and  $Q = 4.5$ ,  $P < 0.05$  with the Napo), whereas the other populations did not differ between or among each other in mean length nor in mean mass. Maximum lengths and even more masses, however, did vary considerably between populations, reaching barely 80 cm and about 3.8 kg in the Putumayo and more than 90 cm and 8 kg in the Amazonas (Fig. 2a). In the Amazonas, maximum observed body mass for the silver Arowana was 49, 75 and 113% heavier than in the Napo, Ucayali and Putumayo, respectively. These important differences in body mass, however, are not a mere reflection of the larger maximum lengths in the Amazonas. Indeed, although mass-at-length is relatively similar among populations until about 50 cm (~ size at first sexual maturity, see below), afterwards fish start to become progressively heavier at a given length in the Amazonas than in the Ucayali, Napo and Putumayo, in order of increasing difference (Fig. 2b). These differences reached ~ 500 g and ~1000 g at 70 and 80 cm, respectively, between the Amazonas and Putumayo sub-basins (Fig. 2b).

For comparison purposes with other studies, a relationship between standard length (SL) and total length (TL) is provided, calculated from all individuals in Fig. 2a, giving:  $SL = 0.9345 TL + 0.102$ .

### Gonadal maturity scale

Female and male silver Arowana only have a functional gonad on the left; the right gonad is atrophied. An illustrated, detailed gonadal maturity scale is presented in Fig. 3 for both sexes. The proposed maturity stages allow for a clear determination of the breeding season, size/age at maturity and fecundity.

The precise description of the gonadal cycle also revealed the silver Arowana as a multiple-spawner (*sensu* Nuñez and Duponchelle, 2009). Indeed, the presence of small, vitellogenic oocytes in between large oocytes in 'spawning capable' ovaries, both in stage 3 or 4 (Fig. 4a) and in stage 5-2 (recently spawned) ovary initiating another vitellogenic cycle (Fig. 4b) observed in all four sub-basins, proves that a same female can reproduce at least twice during a breeding season. Further evidence comes from the size frequency distribution of oocytes in 'spawning capable' ovaries from the Putumayo sub-basin, showing two clearly separated modes of vitellogenic oocytes: the largest (244 oocytes) that will be spawned shortly and a smaller one (221 oocytes) that will likely be released in another spawning bout during the same breeding season (Fig. 5).

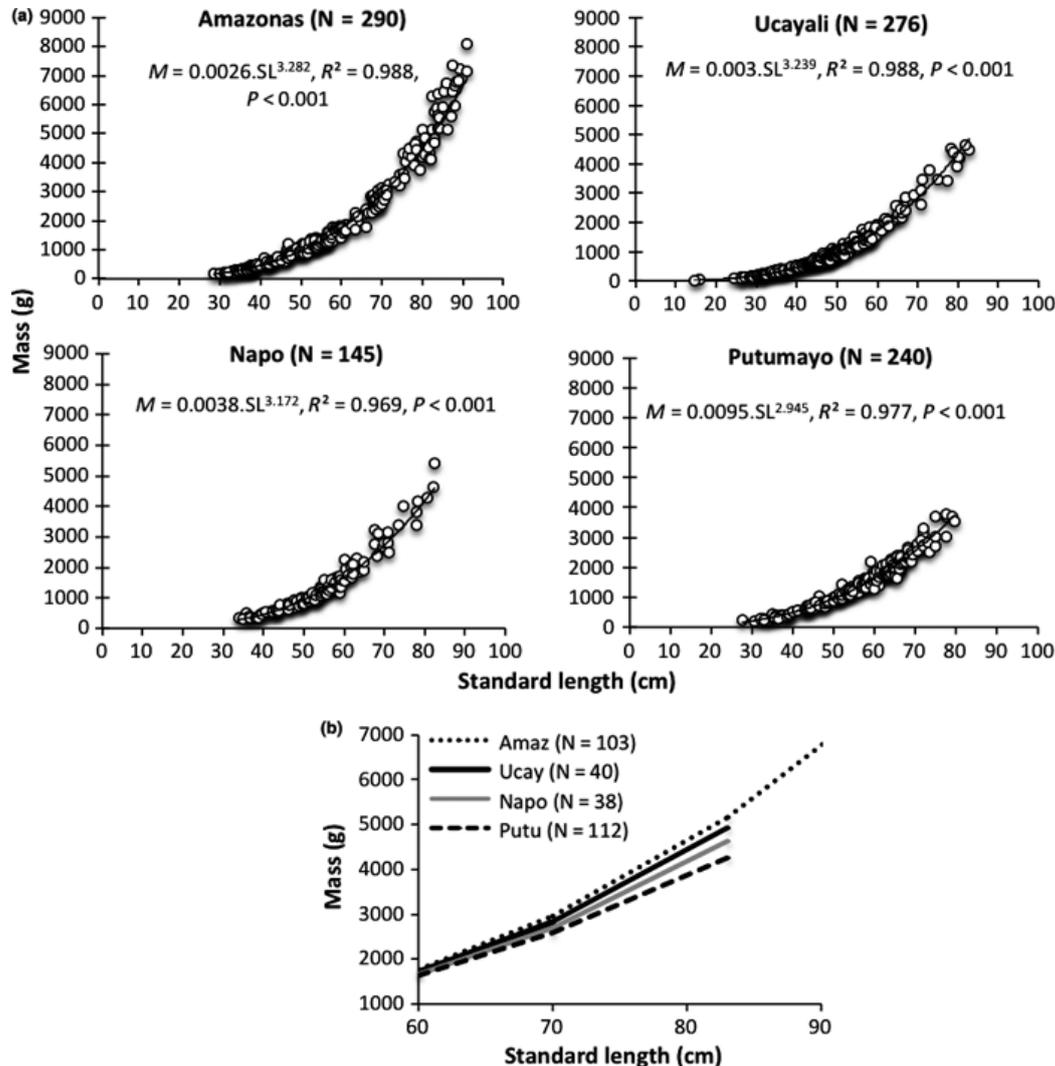


Fig. 2. Relationships between standard length (SL) and total body mass (M) of *Osteoglossum bicirrhosum* caught in four sub-basins of the Peruvian Amazon between 2006 and 2009 (a). Modelled regressions (M as a function of SL) between 60 and 90 cm SL, to illustrate inter-basin differences (b)

### Breeding season

There exists a relationship, albeit not a direct one, between the reproductive cycle of the silver Arowana (estimated from the monthly proportions of females in 'spawning capable' phase of the gonadal maturation scale) and the hydrological cycle (Fig. 6). In the Amazonas and Ucayali rivers, which have comparable hydrological cycles, females have similar breeding seasons. Although sampling could not be completed in some months, it can nevertheless be observed that the breeding season started during the dry season (August-September) and ended before the water level reached its maximum in both rivers. Some residual reproductive activity could be observed in the Ucayali in almost every month outside the main breeding season. Again, in the Napo and Putumayo rivers, which have comparable hydrological periodicity, the breeding season of the silver Arowana also tended to be similar (Fig. 6). Although samples could not be obtained in

December and January in the Putumayo, it is likely that, as in the Napo, the breeding season initiated in December to peak in February-March. The breeding season in the Napo and Putumayo, however, did not last as long as in the Amazonas and Ucayali and was restricted to the low water period.

### Size and age at sexual maturity

Females tended to have a slightly larger size at first sexual maturity (Fig. 7) than males in every sub-basin, but these differences were not significant (*t*-test,  $t = 0.292$ , d.f. = 24,  $P = 0.773$  for the Amazonas;  $t = 0.226$ , d.f. = 24,  $P = 0.823$  for the Ucayali;  $t = 0.630$ , d.f. = 18,  $P = 0.536$  for the Napo; and  $t = 0.327$ , d.f. = 24,  $P = 0.746$  for the Putumayo).

Size at maturity did not differ significantly between sub-basins for females nor males.

(a)

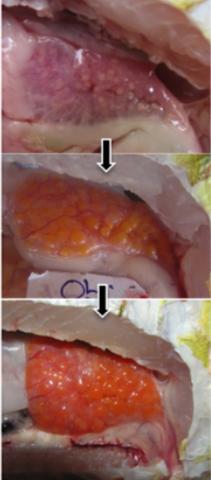
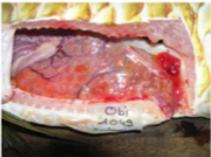
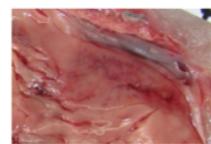
Description	Illustration (females)	Equivalence with Brown-Petersen et al. (2011)'s terminology
<p><b>Stage 1: immature individuals</b> Ovaries small and firm, opaque and whitish or slightly pinkish. No oocyte can be seen to the naked eye. A typical longitudinal canyon-type depression is clearly visible at the surface of the ovary.</p>		Immature
<p><b>Stage 2: maturing individuals</b> Ovaries are larger with more important vascularisation and clearly visible oocytes. Oocytes are whitish and sparse at the beginning of stage 2, and the canyon-like depression is still visible. Progressively, the oocytes turn yellow and the canyon disappears. At the end of stage 2, the oocytes are orange and fill the ovary, but are not of a homogeneous size: all intermediaries between small whitish oocytes and large orange ones are visible. This last point is a clear distinction with stage 3.</p>		Developing
<p><b>Stage 3: individuals in final maturation</b> The ovary now occupies about ¼ of the abdominal cavity. Most oocytes are large, circular, orange, and homogeneous in size. Small oocytes are still visible in between the larger ones, but there is now a very clear size difference between the large and small ones.</p>		
<p><b>Stage 4: individuals ready to spawn/ripe</b> The ovary occupies almost the whole abdominal cavity and appears even fuller. The ovary's membrane is very thin and transparent. A slight pressure on the abdomen can expulse the ovules, that are now free (out of their follicles) in the ovary.</p>		Spawning capable
<p><b>Stage 5-2: recently spawned individuals initiating another cycle</b> The appearance is close to that of a stage 2 gonad, but the ovary is still relatively flaccid, larger and more vascularised. Developing oocytes of all colours are filling the ovary and some remaining large atretic oocytes can sometimes be observed. This stage is characteristic of females that have just spawn and that are initiating a new reproductive cycle.</p>		
<p><b>Stage 5: individuals that have just spawned</b> The ovary is of similar size to a stage 4 ovary, but with an empty, flaccid appearance and a well developed vascularisation. Some un-spawned, large oocytes can still be seen together with others already in re-absorption process (atresia) and early vitellogenic ones.</p>		Regressing
<p><b>Stage 5-1: resting individuals</b> The ovary looks like a stage 1 ovary (with no visible oocyte), but is larger, with a thicker membrane, a more important vascularisation and a generally redder colour than a stage 1. This stage is characteristic of adult females resting in between two breeding seasons.</p>		Regenerating

Fig. 3. Macroscopic gonadal maturity scale for females (a) and males (b) *Osteoglossum bicirrhosum*, following Nuñez and Duponchelle (2009). Equivalence with the Brown-Petersen et al. (2011) terminology is provided

(b)

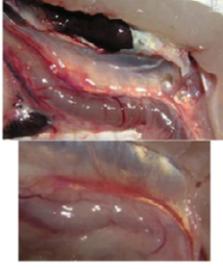
Description	Illustration (males)	Equivalence with Brown-Petersen et al. (2011)'s terminology
<p><b>Stage 1: immature individuals:</b> The testis is a pinkish, translucent curved tube, thinner and longer than stage 1 ovary. It is often bordered by a golden colour towards the genital pore.</p>		Immature
<p><b>Stage 2: maturing or resting individuals:</b> Testis wider, thicker, of a more opaque pinkish to reddish colour. The golden colour towards the genital pore is often still visible. Resting adults are usually at this stage.</p>		Developing/ Regenerating
<p><b>Stage 3: ripe individuals:</b> Testis even larger, wider and really thick, with dark red opaque appearance. The golden colour is usually not visible at this stage.</p>		Spawning capable
<p><b>Stage 4: individuals after ejaculation:</b> Testis of a similar size to stage 3, but with a flaccid, more translucent appearance.</p>		Regressing

Fig. 3. (Continued).

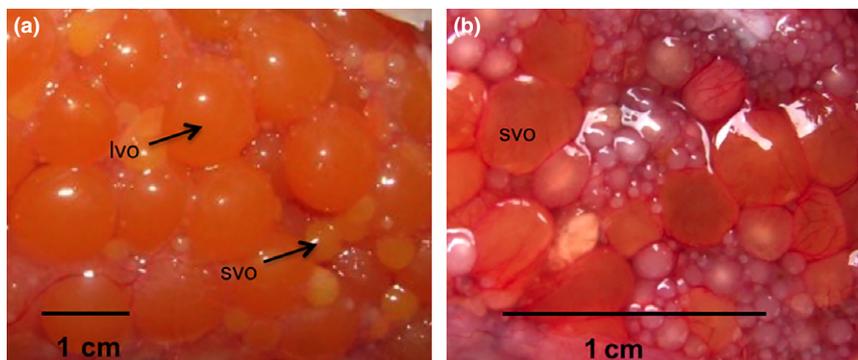


Fig. 4. Small vitellogenic oocytes (svo) in 'spawning capable' ovaries of female *Osteoglossum bicirrhosum*: (a) in between large vitellogenic oocytes (lvo) in a ripe – stage 4 – gonad and (b) in a post-spawning gonad reinitiating another vitellogenic cycle – stage 5-2

Age at first sexual maturity was reached during the second year for both males and females in all four sub-basins (Fig. 7).

In all four sub-basins, the size at which 100% of both males and females were sexually mature was slightly less than 60 cm  $S_L$  (Fig. 7).

#### Fecundity

The number of oocytes per spawning event (batch fecundity) significantly varied (ANCOVA,  $D_{3,41} = 6.610$ ,  $P < 0.001$ ) between fish of the four sub-basins (Fig. 8, Table 1). Batch fecundity ranged from 93 to 256 oocytes for females of 1300 and 4960 g, respectively, in the Amazonas, from 94 to 311

oocytes for females of 2080 and 3480 g, respectively, in the Ucayali, from 92 to 197 oocytes for females of 1460 and 3220 g, respectively, in the Napo and from 125 to 360 oocytes for females of 1770 and 2980 g, respectively, in the Putumayo.

The Putumayo population differed significantly from the Amazonas (ANCOVA,  $D_{1,32} = 25.9$ ,  $P < 0.001$ ), Napo ( $D_{1,30} = 9.7$ ,  $P < 0.05$ ) and Ucayali ( $D_{1,33} = 53.9$ ,  $P < 0.001$ ) populations, whereas the Amazonas, Ucayali and Napo populations did not differ significantly among each other ( $D_{2,12} = 1.4$ ,  $P > 0.05$ ).

Relative fecundity (batch fecundity per unit body mass), oocyte diameter and oocyte mass are presented in Table 2. Relative fecundity differed significantly between fish of the four

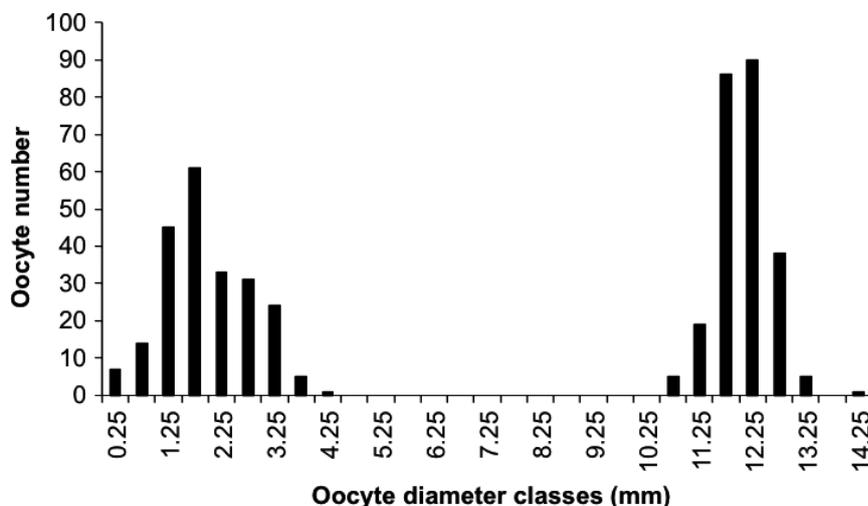


Fig. 5. Size frequency distribution of oocytes in a 'spawning capable' ovary of *Osteoglossum bicirrhosum* from the Putumayo River showing two clearly separated modes of vitellogenic oocytes

sub-basins (One-way ANOVA,  $F_3 = 11.6$ ,  $P < 0.001$ ). Holm-Sidak *post-hoc* test indicated that fish from the Putumayo significantly differed from the Amazonas ( $t = 4.812$ ,  $P < 0.001$ ), the Ucayali ( $t = 3.614$ ,  $P = 0.004$ ) and the Napo ( $t = 2.732$ ,  $P = 0.037$ ), whereas fish from the Amazonas, Ucayali and Napo did not differ significantly among each other.

There was no significant difference in mean oocytes diameter or mass among fish of the four sub-basins (Table 2).

## Discussion

Important length and even more important body mass differences were observed between the four sub-basins, as already noted while studying age and growth patterns of these same populations (Duponchelle et al., 2012). After reaching sexual maturity, fishes in the Amazonas become progressively heavier than those from the Ucayali, Napo and Putumayo, in order of increasing difference. Beside inter-basin differences, silver Arowana from the Peruvian Amazon grow relatively heavier than fish from the Mamirauá Reserve in Brazil. An individual of 80 cm  $T_L$  (~ 75 cm  $S_L$ ) weighs ~ 3000 g in the Mamirauá Reserve (Cavalcante, 2008), whereas it weighs on average 3161 g in the Putumayo, 3369 g in the Napo, 3552 g in the Ucayali and 3702 g in the Amazonas. The better body condition of Arowana in the Peruvian Amazon might result from better trophic conditions than in the Mamirauá Reserve, something which would have to be tested.

## Gonadal maturity scale

The descriptions of the different maturity stages were consistent with the Aragao (1989) descriptions for both females and males, although the number of stages was reduced to fit the Nuñez and Duponchelle (2009) scale. This illustrated gonadal maturity scale provides a clear reference for comparative works on the reproductive biology of this species.

Previous studies suggested, from the observation of different sizes of oocytes in ripe gonads (but without providing evidence) that the same female could reproduce more than

once during the breeding season (Ayala, 2001; Tang and Gomez Noriega, 2005). In the present study, the presence of small, vitellogenic oocytes in all stages of 'spawning capable' ovaries and the size frequency distribution of oocyte in 'spawning capable' ovaries provide strong evidence that the same individual female can reproduce more than once during the breeding season in the Peruvian Amazon. This result, however, differs from other studies in the Brazilian Amazon; Aragao (1989) in Lake Janavaca and more recently Queiroz (2008) in the Mamirauá Reserve, both reported that this species probably spawns only once during the breeding season, but without demonstrating evidence.

## Breeding season

In Central Amazonia, Brasil, where most published studies on the silver Arowana breeding cycle were carried out, the species was reported to reproduce during the dry season and in early rising waters (Goulding, 1980; Aragao, 1989; Cavalcante, 2008; Queiroz, 2008). This is consistent with the observations from the present study in the Amazonas and Ucayali sub-basins, or with previous observations in Peru, in the Pacaya Samiria Reserve (Tang and Gomez Noriega, 2005) and in the Ucayali River (Ayala, 2001). This pattern, however, does not seem to hold for all silver Arowana of the Peruvian Amazon, as the breeding season seems restricted to the dry season in the Napo and Putumayo sub-basins. This shorter breeding season might be an adaptation to the much shorter flooding season in the Napo and Putumayo (Fig. 5). Once females have spawned, males guard the eggs and then the fry in their mouths for 5–7 weeks (Goulding, 1990; Argumedo, 2005; Queiroz, 2008), after which free swimming juveniles of over 6 cm are released from parental care to fend for themselves. It is well known that in Amazonian environments, the high water season with its extended floodplains and inundated vegetation, providing food and shelter from predators is particularly advantageous for the development and survival of young stages of fish (Lowe-McConnell, 1964, 1987; Gould-

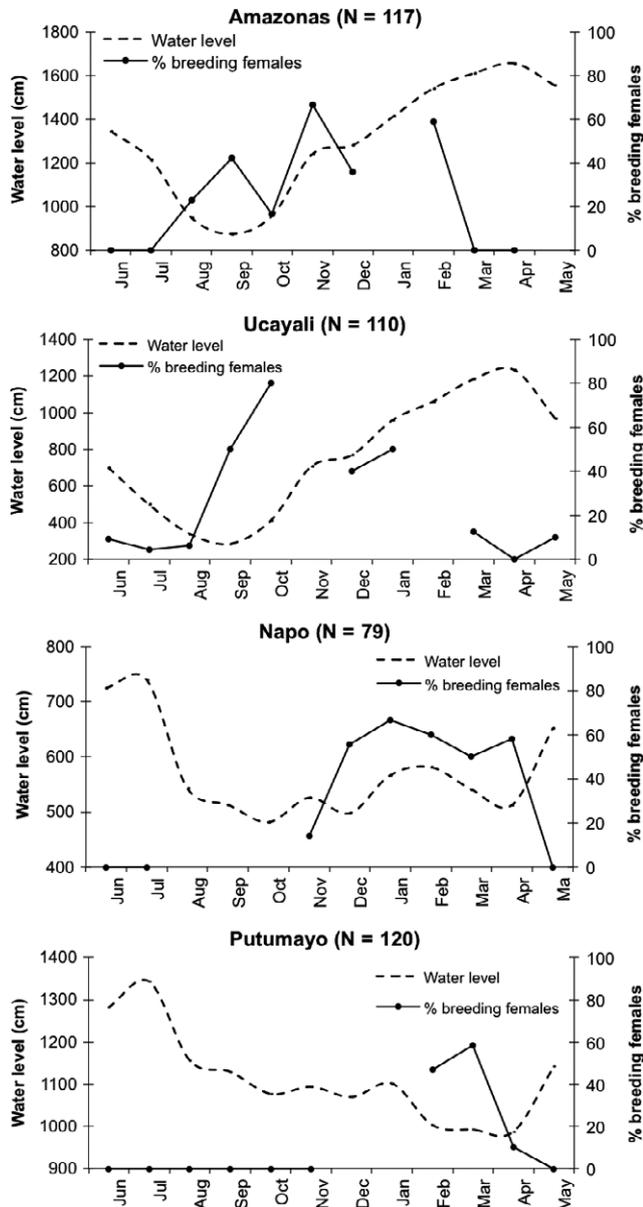


Fig. 6. Proportions of breeding *Osteoglossum bicirrhosum* females in relation to the mean water level in four sub-basins of the Peruvian Amazon

ing, 1980). In order for males to release their free-swimming young in time to make the most of these advantageous environmental conditions – barely lasting three months in the Napo and Putumayo sub-basins (Fig. 5), while allowing for the long incubation, the spawning period might have to be completed when the water begins to rise. Nevertheless, size frequency distribution of oocytes in ripe gonads (Fig. 4c) and the presence of small vitellogenic oocytes in all stages of ‘spawning capable’ ovaries support the idea that individual females may have time to produce a second brood during the spawning period, despite its shorter duration in the Putumayo. This suggests that the time needed for the small vitellogenic oocytes (Fig. 4a,b) to complete

their growth is relatively short (only a few weeks). These results also imply polyandry in the species: indeed, the short breeding season in the Putumayo and Napo and the long paternal mouthbrooding period do not allow the same male to carry successively two broods of a same female. On the other hand, in the Amazonas and Ucayali sub-basins, where the high water season lasts nearly twice as long (December to May), females that spawn when the water has already begun to rise may still ensure their progeny will find favourable environmental conditions long enough to survive, hence ensure reproductive success. This hypothesis could be tested by additional studies on these two specific sub-basins and others with similarly short high water seasons. As Arowana do not live in rivers but rather in adjacent lakes and lagoons, testing this hypothesis would also require having water level data in specific lakes where Arowana concentrations are high.

#### Age and size at maturity

In the Mamirauá Reserve, size at first sexual maturity was ca. 52 cm  $T_L$  (~ 49 cm  $S_L$ ) for both sexes and reached in their second year (Cavalcante, 2008). These values were relatively similar to those observed in the Peruvian Amazon, where silver Arowana also reached maturity during their second year at sizes varying from 43–45 cm for males and 46–50 cm for females. These values were relatively smaller than the ~ 60 cm  $T_L$  (~ 56 cm  $S_L$ ) reported in the El Dorado Lake in the Pacaya Samiria Reserve, Peru (Tang and Gomez Noriega, 2005). In a preliminary study in the Ucayali River, Peru, Ayala (2001) reported sizes at first maturity of 65.9 and 73.9 cm  $T_L$  (~ 62 and 69 cm  $S_L$ ) for females and males, respectively. These obviously over-estimated values, however, were likely due to the fact that the author erroneously considered individuals in vitellogenesis (stage II on gonadal maturation scale) as still immature. Cortegano et al. (2014) reported sizes at maturity of ~58 and 62 cm for females and males, respectively, in Lake Grande, close to Huapapa in the Putumayo. These large differences with our results from the Putumayo (47 and 43 cm for females and males, respectively) are particularly surprising, as about a third of our own sampling also came from Lake Grande. Most of the observed differences might result from the interpretation of what is considered ‘sexual maturity’ in the Cortegano et al. (2014) study, which was not properly defined. It is likely, when looking at their logistic curves that reach 100% only close to the maximum observed sizes, that they considered as ‘sexually mature’ female at the end of stage 2 rather than at the beginning (Fig. 3a). Likewise, they likely considered males in late stage 2 or stage 3 rather than in stage 2 (Fig. 3b).

It is worth noting that while the other reproductive traits (breeding season and fecundity) largely varied between sub-basins, size at maturity was relatively conserved and that the slight differences observed were not significant. This is particularly surprising as size at maturity is a plastic trait that readily responds to environmental variation (Stearns and Crandall, 1984; Stearns and Koella, 1986; Duponchelle and Panfili, 1998; Duponchelle et al., 2007) or to fishing pressure

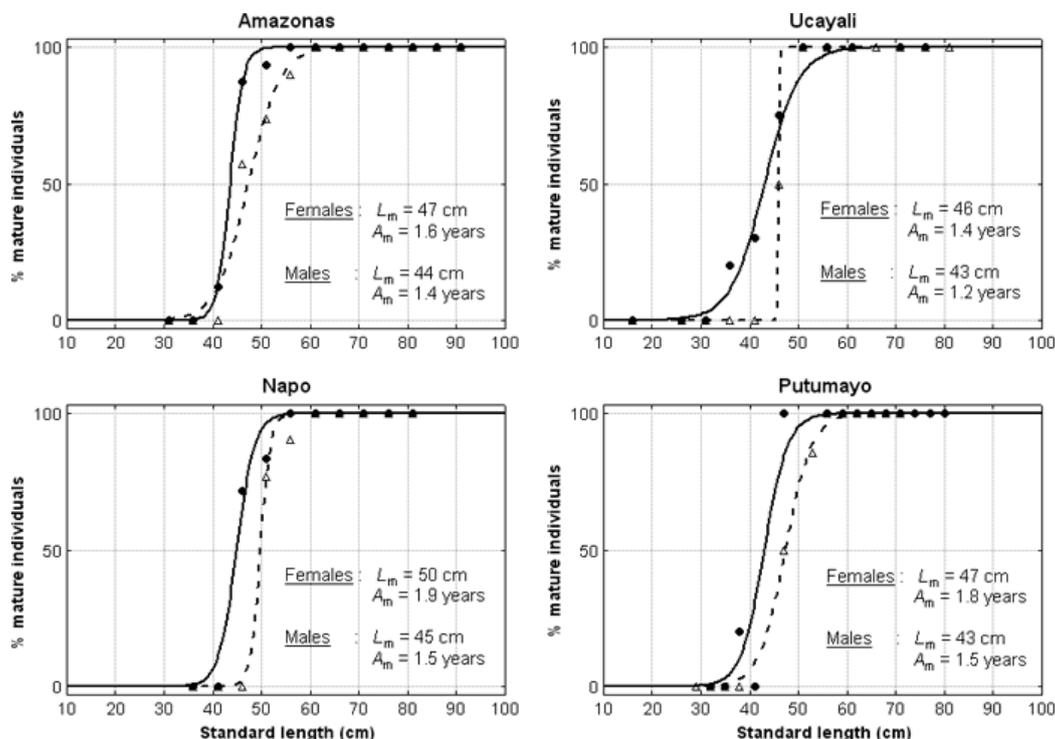


Fig. 7. Age ( $A_m$ ) and size ( $L_m$ ) at first sexual maturity of both males (black circles and solid lines) and females (white triangles and dashed lines) *Osteoglossum bicirrhosum* in four sub-basins of the Peruvian Amazon

(Olsen et al., 2004). This observation will have to be confirmed in other geographic populations.

It is important to keep in mind that size at first sexual maturity ( $L_m$ ) is the size at which 50% of the individuals have reached maturity during the breeding season, meaning the other 50% have not. Considering the size at first sexual maturity as the legal minimum size at capture, as is customary in Amazonian fisheries regulations, we implicitly allow that half the fish be caught as immature, hence before they can reproduce (growth-overfishing). In order to ensure a healthy spawning stock, however, fisheries should ensure that all captured individuals have had a chance to reproduce at least once (Myers and Mertz, 1998; Froese, 2004). This goal can only be attained by fixing the minimum size at capture not at the size at which 50% of the individuals in a population are mature ( $L_m$ ), but at the size at which 100% are mature. The size at which 100% of the fish are mature, considering all four sub-basins of the Peruvian Amazon, is slightly below 60 cm  $S_L$  (Fig. 6). Therefore, considering the approx. 5 cm difference between total and standard lengths, we recommend that the minimum size at capture should be fixed at 60 cm  $T_L$  in the Peruvian Amazon.

#### Fecundity

The silver Arowana reputedly produces very few eggs for its size (Goulding, 1980; Aragao, 1989; Queiroz, 2008). Fecundity variations between geographical samples, however, have never been thus far reported. In the present study, important differences in batch fecundity were observed among the silver

Arowana of the four sub-basins. The Putumayo population significantly differed from the other three populations that did not differ from each other. The absence of significant differences among the Amazonas, Napo and Ucayali populations, however, was likely due to the low number of gonads analysed and to the large variance observed in the Ucayali population (Fig. 7). Such a large variance could result from the mixing of individuals from distinct populations within the Ucayali sub-basin. Indeed, fish were sampled from fishermen operating in large portions of the Ucayali basin, which could hold populations with distinct reproductive characteristics. This indicates that further studies on this species are necessary and with on-site sampling rather than market sampling. Nevertheless, as an illustration of the important fecundity variation among fish of the four sub-basins, despite the absence of statistical significance the batch fecundity in two representative female body masses (1500 and 3500 g) was calculated for each sub-basin from the regressions among batch fecundity and body mass (Table 1). Batch fecundity differences among populations ranged from 33 to 104% for females weighing 1500 and 3500 g, respectively. Given the energetic cost of gamete production, food is considered one of the most important environmental factors involved in the regulation of fecundity (Wootton, 1979, 1998). One would thus expect the fish with the better body condition (in decreasing order those of the Amazonas, then Ucayali, Napo and Putumayo, see Fig. 2b), which usually reflects better trophic conditions, to also display the highest relative fecundity. Yet, relative fecundity was inversely proportional to body condition (Table 2), suggesting contrasted energy

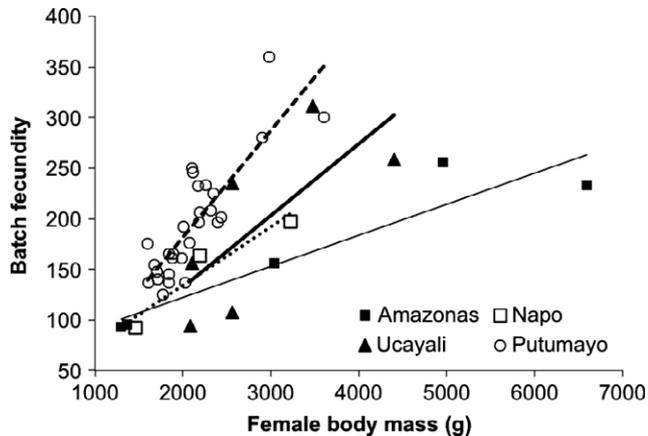


Fig. 8. Linear regressions between batch fecundity and total female body mass of *Osteoglossum bicirrhosum* in the Amazonas (N = 5,  $F = 0.0307 \cdot M + 60.922$ ,  $R^2 = 0.875$ ,  $P < 0.001$ ), Napo (N = 3,  $F = 0.0578 \cdot M + 18.547$ ,  $R^2 = 0.907$ ,  $P < 0.001$ ), Ucayali (N = 6,  $F = 0.0708 \cdot M - 9.1942$ ,  $R^2 = 0.537$ ,  $P < 0.001$ ) and Putumayo (N = 28,  $F = 0.1055 \cdot M - 29.253$ ,  $R^2 = 0.714$ ,  $P < 0.001$ ) rivers, Peruvian Amazon

Table 1

Comparison of *Osteoglossum bicirrhosum* batch fecundity at a given female body mass in four sub-basins, Peruvian Amazon (calculated from regressions between batch fecundity and total body mass presented in Fig. 8)

Female body mass (g)	Batch fecundity			
	Amazonas	Napo	Ucayali	Putumayo
1500	107	105	97	129
2500	138	163	168	234
3500	168	221	239	340

allocation (towards body growth vs reproduction) strategies between fish of the four sub-basins. Additionally, these large variations in fecundity were not compensated by variations of oocyte size or mass, suggesting differences in reproductive effort among fish of the four sub-basins. These hypotheses, however, will need further validation with an increased number of ripe gonads analysed in the Amazonas, Napo and Ucayali sub-basins.

As a comparison, in the Mamirauá Reserve females of 65 cm  $T_L$  (~ 61 cm  $S_L$  and 1500 g) and 75 cm  $T_L$  (~ 70 cm  $S_L$  and 2500 g) produce ~ 200 and 230 oocytes, respectively (Queiroz, 2008; using the length-mass relationship provided in Cavalcante, 2008). This would be approx. twice as much as the fecundity of a 1500 g female in the Peruvian Amazon and in the range of the Putumayo fecundity for a 2500 g female (Table 1), suggesting a higher relative fecundity in small females progressively decreasing with length in the Mamirauá Reserve. Again, fish from the Mamirauá, which have lower body condition than fish from the Peruvian Amazon, also have higher relative fecundity, contrary to expectations. Surprising information comes from the mean diameter (9.75 mm) and mass (1.8 g) of oocytes in the Mamirauá Reserve, which are 25% smaller but 100% heavier than the largest and heaviest oocytes in the Peruvian Amazon

Table 2

Mean relative fecundity (oocytes per kg total body mass), oocytes diameter (mm) and oocytes mass (g)  $\pm$  SD of *Osteoglossum bicirrhosum* in four sub-basins, Peruvian Amazon

	Amazonas (N = 5)	Napo (N = 3)	Ucayali (N = 6)	Putumayo (N = 28)
Relative fecundity	56 $\pm$ 15	66 $\pm$ 7	67 $\pm$ 22	91 $\pm$ 14
Oocyte diameter	10.8 $\pm$ 0.29	10.6 $\pm$ 0.36	11.8 $\pm$ 1.46	12.2 $\pm$ 0.95
Oocyte mass	0.67 $\pm$ 0.05	0.64 $\pm$ 0.01	0.77 $\pm$ 0.17	0.89 $\pm$ 0.24

(12.2 mm and 0.98 g in the Putumayo), resulting in a density over 3.5 g/cm<sup>3</sup>, (vs 1.03 g/cm<sup>3</sup> in the Putumayo), which appears much too dense for an egg.

In the Pacaya-Samiria Reserve, Peru, Tang and Gomez Noriega (2005) observed that females of 1500 and 2500 g would produce on average 215 and 266 oocytes, respectively, which is above the mean values observed in the present study. A recent study in Lake Grande on the Putumayo River, reported batch fecundities ranging from 115 oocytes for a female weighing 1100 g to ~240 oocytes for a female of ~3000 g (Cortegano et al., 2014), within the range of the values observed for the Putumayo in the present study.

The only other information on the silver Arowana fecundity is from Aragao (1989) in the Solimões River basin (Lake Janauacá), but his range of female body masses (551–1750 g, with only two females above 1500 g) barely overlap with ours, hampering a direct comparison. Nevertheless, a 1500 g female would produce approx. 182 oocytes, also above the mean values observed in the present study.

## Conclusions

The important variability observed in reproductive characteristics and body condition of the silver Arowana from the four sub-basins is consistent with the important growth variations previously reported in these same localities (Duponchelle et al., 2012). This variability might result either from the phenotypic plasticity of the species due to environmental heterogeneity between the sub-basins or from the existence of several distinct genetic populations within Peru, that may need further characterization and be managed independently. The important differences in breeding season, condition, growth and fecundity, however, strongly suggest that fish from the different sub-basins are distinct populations. The different breeding seasons and sedentary nature of the species should result in a reduced gene flow between sub-basins, hence inducing genetic structuring. Although microsatellite markers are available for the silver Arowana (Silva et al., 2009), they have only been used to test for multiple paternity in the species (Verba et al., 2014) and no population genetic study has thus far been carried out. Yet, as previously pointed out (Duponchelle et al., 2012), the growing concerns regarding *O. bicirrhosum* conservation (Moreau and Coomes, 2006, 2007) emphasize the need for comparative life history and genetics studies at the inter- and intra-basin levels (with on-site sam-

pling) in order to understand the population structuring of this species and to define appropriate management strategies.

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