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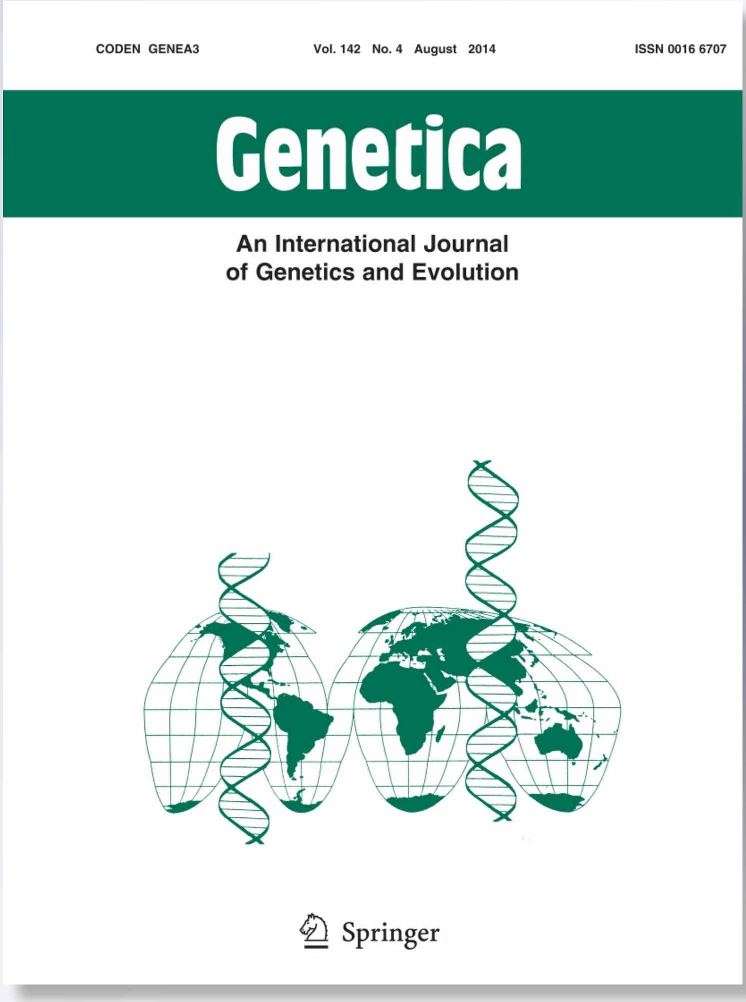

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Genetic structure in the Amazonian catfish *Brachyplatystoma rousseauxii*: influence of life history strategies

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Abstract The Dorado or Plateado (Gilded catfish) *Brachyplatystoma rousseauxii* (Pimelodidae, Siluriformes) is a commercially valuable migratory catfish performing the largest migration in freshwaters: from the Amazonian headwaters in the Andean foothills (breeding area) to the Amazon estuary (nursery area). In spite of its importance to inform management and conservation efforts, the genetic variability of this species has only recently begun to be studied. The aim of the present work was to determine the population genetic structure of *B. rousseauxii* in two regions: the Upper Madera Basin (five locations in the Bolivian Amazon) and the Western Amazon Basin (one regional sample from the Uyucalí–Napo–Marañon–Amazon basin, Peru). Length polymorphism at nine microsatellite loci (284 individuals) was used to determine genetic variability and to identify the most probable panmictic units (using a Bayesian approach), after a significant departure from Hardy–Weinberg equilibrium was observed in the overall dataset (Western Amazon + Upper Madera). Bayesian analyses revealed at least three clusters in admixture in the five locations sampled in the Bolivian Amazon, whereas only two of these clusters were observed in the Western Amazon. Considering the migratory behaviour of *B. rousseauxii*, different life history strategies, including homing, are proposed to explain the cluster distribution. Our results are discussed in the light of the numerous threats to the species survival in the Madera basin, in particular dam and reservoir construction.

Keywords Bolivia · Madera River · Population structure · Microsatellites · Siluriformes · Pimelodidae

Introduction

Brachyplastystoma rousseauxii (Castelnau 1855) is a large (>1.5 m) migratory catfish of the family Pimelodidae, commonly known as Dorado in Bolivia, Colombia and

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Peru, as Dourada in Brazil and as Plateado in Bolivia (Carvajal-Vallejos et al. 2011). It is one of the most emblematic species of the Amazon basin, owing to its economic importance and exceptional life cycle, involving the largest known migration in freshwater (Barthem and Goulding 1997, 2007; Alonso 2002; Araujo-Lima and Ruffino 2003). The Dorado is widely distributed throughout the white-water tributaries and main channels of the Amazon River (Araujo-Lima and Ruffino 2003; Barthem and Goulding 2007), and also can be observed sporadically in clear- and black-water rivers (e.g. Negro, Tocantins—Goulding 1980). There are few studies on this species to date, despite the interest that arose among ichthyologists as a result of Barthem and Goulding's (1997) hypothesis about its life cycle. These authors followed the size distribution of the Dorado among the main fishing ports along the Amazon and Lower Madera River channels, from the estuary (Belem) all the way to the headwaters of an Amazon tributary in Peru (Pucallpa, 4,500 km from the estuary). Fishery statistics and experimental fishing data collected over several years showed a clear change in size distribution of the Dorado between the estuary and the headwaters, with only small and medium-sized fish in the estuary, almost exclusively large specimens in the headwaters of Peru or Colombia, and intermediate size-classes in between. They also observed that juvenile Dorado were absent and pre-adults and adults were rarely found in the flood plains. Alonso (2002), using age determination from otolith readings, reconstructed the age-size distribution of the species along the main axis of the Amazon River. His results confirmed that while mostly juveniles were present in the Amazon estuary, almost exclusively adults were observed in the Iquitos area and mostly pre-adults were caught in between. Providing new information regarding age, growth, reproductive biology, and species' dependence on fluvial dynamics along the Amazon main stem, studies of García et al. (2009a) and Agudelo et al. (2013) confirmed the existence of a complex life cycle involving long-distance migration between the Amazon estuary and the headwaters. In the Peruvian and Colombian Amazonia, where these studies were carried out, *B. rousseauxii* reaches sexual maturity at about 4 years old and 90 cm SL and has longevity of at least 15 years (García et al. 2009; Agudelo et al. 2013).

Batista and Alves-Gomez (2006) suggested that the Dorado displays spatial genetic variability along the Amazon's mainstem that could be explained by a homing behaviour. These authors observed that haplotypic diversity decreases from the estuary westwards and suggested that this could be due to preferential recruitment of differentiated populations to certain tributaries as the fish move upstream. While an interesting hypothesis, the variability observed in this study also could have alternative explanations since the sampling design and molecular markers used (the maternally-inherited Control Region—CR at mtDNA) did not provide sufficient information or consistent evidence of a philopatric migration model (see Campos Telles et al. 2011) and were unable to define the reproductive units related to a possible homing behaviour. More recently, Batista (2010), using nuclear (microsatellites) and mitochondrial (CR) DNA markers, concluded without testing departure from Hardy–Weinberg equilibrium (HWE) that the large number of individuals collected from different tributaries of the Amazon basin (including the Madera River) were parts of a single panmictic unit. The possibility of a homing phenomenon, however, was not totally dismissed, as some degree of structure was observed for mtDNA among some large tributaries. These conclusions, suggesting the existence of a single population of Dorado in the Amazon basin, could have dramatic consequences for species conservation. Indeed, the Dorado is considered overexploited in the Lower Amazon (Petrere et al. 2004; Alonso and Pirker 2005) and heavily exploited in some areas of the Upper Amazon in Peru (García et al. 2009a, b) and Colombia (Agudelo et al. 2013). If this species is composed of a large, single panmictic population of homogeneous individuals throughout its distribution area, then over-exploiting the species in some tributaries would not be particularly problematic at regional scale, as recruitment would be ensured from other, less exploited tributaries. On the other hand, if there is genetic structure within the species, then the total allowable catches have to be set and enforced for each population. Additionally, the growing number of hydroelectric power plants under construction in the Amazon basin are further threatening the species by disturbing or interrupting its migration routes (Junk et al. 2007; Van Damme et al. 2011b; Finer and Jenkins 2012). But again, if the single panmictic population hypothesis is verified, a depletion of the breeding individuals in the Upper Madera following the Madera hydroelectric impoundments of Santo Antônio and Jirau, might be regarded by dam supporters as not so dramatic (even though reducing the range of a species could be problematic), as recruitment for the population would be ensured from the Upper Amazon–Solimões system. Considering that the Dorado is an economically (e.g. Goulding 1979; Agudelo et al. 2000) and ecologically (Angelini et al. 2006) important, yet relatively poorly studied resource with a complex life cycle occurring over a continental scale while subject to differential fishing pressures (on juveniles in the Central Amazon Basin and on adults in the Western Amazon Basin), it is crucial to better understand its biology and genetic structure and shed new light on a potential homing behaviour.

The Amazon Basin is over 6,000,000 km^2, and its main tributaries (e.g. Madera, Ucuyalí) have distinct hydrological

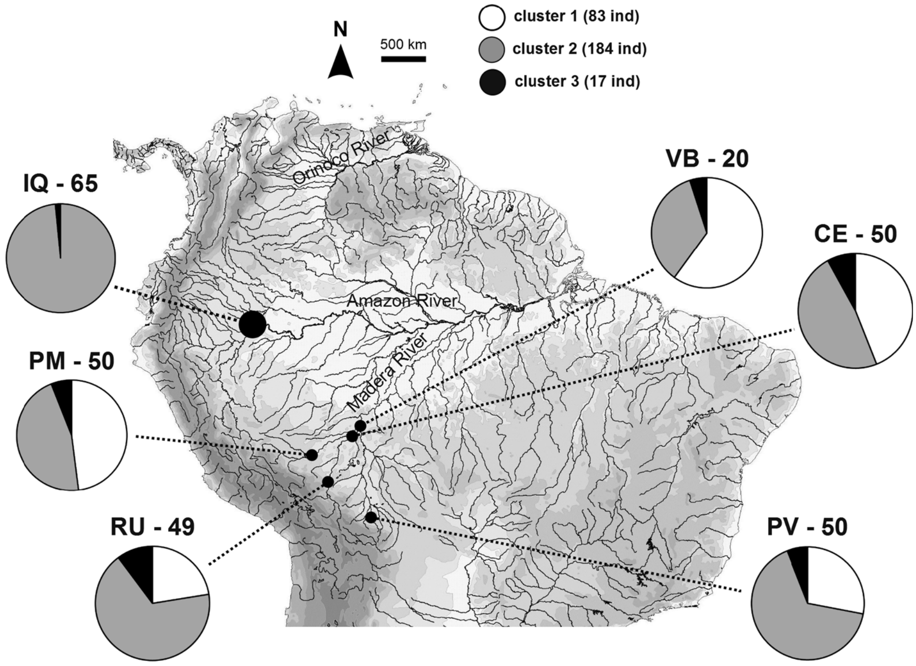

Fig. 1 Composition and distribution of the clusters identified by BAPS for each geographic location studied in the Upper Madera (VB, CE, PM, RU, PV) and Western Amazon (IQ) (Bolivian and Peruvian Amazon). Numbers next to the location names refer to the number of individuals of Dorado *Brachyplatystoma rousseauxii* examined from each location. Locations correspond to VB: Villa Bella; CE: Cachuela Esperanza; PM: Puerto Maldonado; RU: Rurrenabaque; PV: Puerto Villarroel; IQ: Iquitos—larger *black circle* means a regional sampling. Base map of drainages and elevations provided by Conservation Science Program, World Wildlife Fund US

and geological characteristics. The Madera is one of the largest sub-basins of the Amazon, and among those that contribute the most in terms of water and sediment volumes (Barthem and Goulding 2007; Wilkinson et al. 2010; Molina and Vauchel 2011). Most of the upper portion of the Madera Basin is in Bolivian territory, fed by several tributaries with distinct physicochemical characteristics (Navarro and Maldonado 2002). These tributaries converge into one channel (the Madera River proper) that is separated from the Lower Madera portion by a series of rapids spread over 300 km (Molina 2011a). The history of this basin and the rapids (principally the Teotônio rapids in Porto Velho) have had an important influence as a dividing boundary on the structure of the aquatic organism and fish communities (Farias et al. 2010; Torrente-Vilara et al. 2011; Carvajal-Vallejos and Zeballos 2011; Tavera et al. 2011), and likely also on specific populations of species of migratory fishes able to pass the rapids (Goulding 1980; Araujo-Lima and Goulding 1997; Barthem and Goulding 1997), such as the Dorado. While little, apart from some preliminary information (Coronel et al. 2004), is known about the biology of *B. rousseauxii* in the Madera Basin, it could be expected that its migratory dynamics would be similar here to those described for the Amazon main channel. Given the size and particular characteristics of the Upper Madera, the species might present some degree of genetic structure, associated with specific geographic zones or sub-basins, with fish returning to and breeding in the area where they were born (i.e. homing).

This study aimed at generating new genetic information on geographic populations of Dorado present in the upper basin of the Madera River (Bolivian and Peruvian Amazon), and to compare this with information obtained from fish in the Peruvian Amazon system. Sampling design and microsatellite loci analysis were organized and selected specifically to assess genetic populations at different geographical scales (local and regional), and to test whether homing takes place towards the tributaries of the Upper Madera Basin.

Methods

Study area

The study area included the upper basin of the Madera River in Bolivia and Peru (Puerto Maldonado) (locations VB, CE, PM, RU, PV—Fig. 1), and the Ucayalí–Marañon–Napo–Amazon system around Iquitos (Peru) (location IQ—Fig. 1), defined hereafter as the Western Amazon. The Western Amazon system accounts for at least 22 % of the total water volume and represents almost 10 % of the total Amazon watershed area (Goulding et al. 2003). Its lower reaches have characteristics similar to those seen in the Central Amazon, and there is no apparent barrier separating them.

Sampling design

Considering existing information on the biology of *B. rousseauxii* and the variability of aquatic systems in the Upper Madera Basin (mainly the Bolivian Amazon), the sample collection strategy encompassed two geographic scales for analysis: a local-scale (main tributaries of the Upper Madera River), and a regional-scale (main Amazon tributaries—Madera and Western Amazon systems).

Sample locations for the Upper Madera were defined according to the hydrological network and information gathered through personal interviews with fishermen between 2005 and 2006. Additionally, reference information on the biology and distribution of large pimelodids in the Madera Basin (e.g. Goulding 1979, 1980; Barthem et al. 1991, 2003; Barthem and Goulding 1997, 2007; Lauzanne et al. 1999) also was considered.

Each major, turbid-water tributary was sampled in its upper area. In order to assess the possibility that the species swims up-river in a selective manner and that major tributaries may contain distinct panmictic units, we also sampled in two spots close to the confluence of the major tributaries, Cachuela Esperanza and Villa Bella (Fig. 1).

All the samples from the Upper Madera were obtained from local fishermen directly on the fishing ground (which often required several weeks of travel with fishermen), and catch location was recorded. For the broader regional-scale analysis, samples were obtained from the market in Belén (Iquitos, Peru), where fisher families sold fish caught in an area of ~300 km around Iquitos, which includes the Amazon (Upper Solimões), Ucayalí, Napo and Marañon river systems. In total, 284 individuals of *B. rousseauxii* were collected between January 2005 and March 2009. Two hundred and nineteen Dorados were captured using gillnets (20–24 cm mesh size) in the main channels of the Madera (20 in Villa Bella—VB with a mean standard length-SL of 54 ± 14.5 cm estimated from only seven individuals), Upper Beni (49 in Rurrenabaque—RU), Lower Beni (50 in Cachuela Esperanza—CE, 91.8 ± 10.1 cm SL), Madre de Dios (50 in Puerto Maldonado—PM), and Ichilo or Upper Mamoré (50 in Puerto Villarroel—PV, 89.2 ± 8.2 cm SL) rivers. As size at first sexual maturity is ~75 cm in the Madera (M. Hauser UNIR—Brazil and F. Duponchelle, unpublished data), hence smaller than in the Peruvian and Colombian Amazonia (García et al. 2009a; Agudelo et al. 2013), most sampled individuals could be considered as adults, except the seven small specimens caught in VB. Sixty-five samples were purchased from the central fish market in Belén (Iquitos—IQ). For each individual caught or purchased, approximately 1 cm^3 of muscle was preserved in 98 % ethanol.

For the Upper Madera and the Western Amazon, each locality is represented by samples from different periods of the year and different years.

DNA extraction

Genomic DNA was extracted from ethanol-preserved muscle following modifications of the CTAB procedure developed by Doyle and Doyle (1987): proteinase K (10 µL/100 mg sample, 10 mg/mL) was added for DNA extraction for 10 h (60 °C), chloroform (1 mL) was used instead of chloroform-isoamyl alcohol to extract proteins and cell debris (8,000 rpm by 5 min), and a final 15 min of washing (13,000 rpm) with 70 % ethanol (750 µL), and resuspension of the nucleic acid pellet in water.

Microsatellite amplification

Microsatellite amplification was performed at 9 loci using fluorescently labeled (FAM, HEX, NED) primers. The primers used were designed for *Pseudoplatystoma corruscans* (Pcor1, Pcor2, Pcor 7) by Revaldaves et al. (2005) and *B. rousseauxii* (BR37, BR38, BR44, BR45, BR47, BR49) by Batista et al. (2009).

Microsatellite amplification by polymerase chain reaction (PCR) was carried out on a Mastercycler thermocycler (Eppendorf AG 2231, Hamburg). Fragments amplified with Pcor1, Pcor2 and Pcor7 primers were produced in a total volume of 10 µL consisting of 2.0 µL of 5× reaction buffer (Colorless GoTaq Promega, 7.5 mM $MgCl_2$ at pH 8.5), 0.6 µL of $MgCl_2$ (25 mM), 1.0 µL of dNTPs (1 mM each dNTP), 0.4 µL of each primer (forward and backward, 10 µM), 0.4 U of Taq polymerase (GoTaq, Promega), approximately 50 ng DNA extract and 4.02 µL Milli-Q water. The temperature profile to which the mixture was subjected consisted of an initial denaturation at 94 °C (2 min); followed by 35 denaturation cycles at 94 °C (30 s), annealing at 57 °C (Pcor1), 58 °C (Pcor2), 62 °C (Pcor7) (30 s), elongation at 72 °C (40 s); and one final elongation at 72 °C (30 min), followed by final refrigeration of the products at 4 °C.

Amplifications with the remaining primers (BR) were designed and executed in duplex 1 (BR37-BR38), duplex 2 (BR44-BR45), and duplex 3 (BR47-BR49). Compatibility and grouping of the primers for the formation of the duplexes were evaluated with the program MULTIPLX version 1.2 (Kaplinski et al. 2004). All duplex reactions were carried out in a final volume of 10 µL, composed of 2 µL of 5× reaction buffer (Colorless GoTaq, Promega, 7.5 mM $MgCl_2$ at pH 8.5), 0.6 µL of $MgCl_2$ (25 mM), 2.0 µL of dNTPs (10 mM each dNTP), 0.2 µL of each primer for duplex 1, 0.07 µL (BR44) and 0.12 µL (BR45) for duplex 2, 0.08 µL (BR47) and 0.1 µL (BR49) for duplex 3 (all primers at 10 µM), 0.5 U of Taq polymerase (GoTaq, Promega), approximately 50 ng of DNA extract, and the corresponding difference with Milli-Q water to complete the volume for each duplex. The temperature profile consisted of initial denaturation at 94 °C (2 min); followed by 32 cycles of denaturation at 94 °C (30 s), annealing at 62 °C (duplex 1 and 2) or 60 °C (duplex 3) (35 s), elongation at 72 °C (40 s); and a final elongation at

72 °C (30 min) prior to rapid cooling to 4 °C. Every PCR reaction run performed included a negative control reaction, containing all of the reagents except for the DNA template.

The PCR products were electrophoretically separated and visualized in an ABI 3130 (Applied Biosystems) automated sequencer. Allele sizes were defined in relation to the standard size marker GeneScan^{TM}–500 ROX^{TM} (Applied Biosystems) (primers Pcor) or GeneScan-500(-250) ROX^{TM} (Applied Biosystems) (primers BR) using analyses from the program PEAKSCANNER^{TM} version 1.0 (Applied Biosystems). Program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to detect evidence for null alleles in the data sets for each locus.

Comparison between geographic samples

First, the departure from HWE expectations was tested for all the individuals together (284 specimens spread over the six locations studied) to test whether the hypothesis of a single global genetic unit could be rejected using the f estimator (Wright's F-statistics, 1978; Weir and Cockerham 1984) of the endogamy index F_{IS} implemented in the program GENETIX version 4.05.2 (Belkhir et al. 1996–2004). If departure from HWE expectations occurs, a population structure involving a Wahlund effect, among other factors, will give rise to deficiency of heterozygotes and a positive value of f.

Then, tests of departure from HWE for each geographic sample (f estimator) and the degree of genetic differentiation among them, using Weir and Cockerham's (1984) θ, a widely-used analogue of the fixation index F_{ST} (Wright's F-statistics, 1978), were implemented in the program GENETIX version 4.05.2 (Belkhir et al. 1996–2004). The significance of the f and θ estimators was calculated using 1,000 random permutations from the original data.

Identification of panmictic populations (cluster analysis)

As *B. rousseauxii* undergoes great migrations throughout its life cycle, and samples were collected in different years and seasons, each geographic sample could be constituted of individuals belonging to different genetic units. Hence, panmictic units were investigated without a priori categorization of individuals. Population genetic structure then was investigated in the total sample using a Bayesian approximation. This approach consisted of a cluster analysis on various levels of hierarchy with Bayesian Analysis of Population Structure (BAPS) version 5.2 (Corander et al. 2003, 2004) and STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). The BAPS algorithm is known to correctly identify subpopulations with F_{ST} values as low as 0.02–0.03, with accuracy similar to STRUCTURE, one of the most common programs for population analysis (e.g., Latch et al. 2006).

The procedure in BAPS for estimating the optimal number of subpopulations (K, groupings of individuals) with the highest probability consisted of executing 10 independent runs of the program with a vector made up of 10 repetitions of each value for $K = 2$–8. Probability was computed on the basis of the best partitions that were visited during all runs, and the number of clusters inferred (K) was evaluated by the modal value of the replicates. Once the most probable numbers of inferred clusters were identified, separate runs consisting of 100 and 1,000 repetitions were executed. This procedure helped choosing the best partition according to the logarithm of the highest marginal probability [Log(mp)] value.

The assignments in STRUCTURE consisted of a burn-in of 5×10^5 iterations followed by a Markov Chain Monte-Carlo procedure of 10^6 steps. STRUCTURE and BAPS try to group individuals into clusters that conform to the best HWE and linkage equilibrium. Average posterior probabilities, were calculated from 10 independent runs for $K = 1$ to $K = 8$. The estimation of the most probable K (number of biological subgroups in the entire sample) was identified following recommendations by the Evanno et al. (2005) method through STRUCTURE HARVESTER (Earl and von Holdt 2012). The admixture ancestry model and correlated allele frequencies were used, as this configuration is considered the best (Falush et al. 2007).

The most probable K value was chosen by testing departure from HWE in the cluster assignments performed by BAPS and STRUCTURE. That is, the best assignment provided clusters having the smallest departure from Hardy–Weinberg equilibrium. Program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004), was used to detect evidence for null alleles in clusters.

Intra-populational genetic diversity, test of HWE and linkage disequilibrium

The genetic variability within each identified cluster was quantified and evaluated with standard descriptive statistics: number of alleles per locus (A), unbiased heterozygosity (H nb—Nei 1978), observed heterozygosity (H obs). Additionally, the program GENALEX version 6.4 (Peakall and Smouse 2006) was used to obtain the distribution of private alleles for each identified cluster. Tests for genotypic disequilibrium among all locus pairs (independence among loci), according to Black and Krafsur's (1995) algorithm, also was carried out with GENETIX program. Linkage disequilibrium in a subdivided population can result from a natural epistatic selection within subpopulations creating a frequency correlation among the linked alleles (Lewontin 1974). However, with limited migration among subpopulations and small effective size, random drift can increase linkage disequilibrium frequency. To differentiate between random and selective equilibrium effects, Ohta (1982a, b) devised a method to divide the variance of a coefficient inferred from two loci in disequilibrium into components that occur within and among subpopulations. Ohta's method is based on the hypothesis that when selection produces specific combinations of alleles, they should appear consistently among the subpopulations. Variability in the observed frequency of allele combinations among subpopulations must therefore be smaller under selection than under drift (Black and Krafsur 1995). Five 'D' statistics describe the variance in observed and expected frequencies of allelic combinations, and such statistics are calculated for pairs of loci. The presentation of the results follow Ohta's notation, and the statistics are indicated with the subscripts I (individuals), S (subpopulations), and T (total population).

Differentiation among genetic populations

To determine the differentiation among inferred clusters, the variance of allelic identity was used [*F*-statistics, F_{ST}; measured by the analogue Weir and Cockerham 's θ (1984)]. The work was carried out using F_{ST} values rather than R_{ST} values which consider allelic size variation (Slatkin 1995) due to the better estimate performance of F_{ST} when divergence among units is expected to be low (Balloux and Goudet 2002). The θ was estimated as for the geographical samples.

Results

Comparison of geographic samples

The evaluation of the multilocus estimator *f* value (9 loci, 129 alleles) for the global sample (284 individuals, six locations from Upper Madera and Western Amazon) indicated a strong and significant deviation from HWE ($f = 0.031$; $p < 0.001$). In the single-locus analysis, BR45 was the only locus that showed a significant departure from HWE. However, the observed deviation was maintained even when a new global analysis was performed on eight loci, excluding BR45 ($f = 0.023$; $p = 0.021$). Therefore, the overall deviation from HWE depended on a multiple-loci combination, and not only on the information contained at one single locus.

Considering each geographic sample, Cachuela Esperanza—CE ($f = 0.095$, $p = 0.001$) and Rurrenabaque—RU ($f = 0.042$, $p = 0.034$) were the only samples with a significant departure from HWE (Table 2). At the regional scale, HWE was rejected in the Upper Madera sample (220 individuals, five geographic samples, $f = 0.033$, $p = 0.001$), but not in the Western Amazon sample (64 individuals, one regional sample, $f = -0.011$, $p = 0.724$).

The regional comparison revealed a significant differentiation ($\theta = 0.018$, $p = 0.010$) between the Western Amazon and Upper Madera samples. Pair-wise θ comparisons showed that the sample from the Western Amazon (Iquitos—IQ) was significantly different ($p < 0.001$) from each of the local geographic samples from the Upper Madera (Table 1), and indicated a significant differentiation between Villa Bella—VB and Puerto Villarroel—PV ($\theta = 0.009$, $p = 0.029$) within the Upper Madera.

Identification of panmictic populations (cluster analysis)

Given the significant overall deviation from HWE ($p < 0.001$), more specifically in the Upper Madera ($p < 0.001$) and the migratory behaviour of *B. rousseauxii*, an admixture of populations was likely in our geographic sampling collection, resulting in a Wahlund effect (Hartl and Clark 1997). Therefore, STRUCTURE and BAPS analyses were carried out to find the most probable reproductive units (clusters). Whereas the estimator θ differentiated the regional sample of Iquitos from all the others, STRUCTURE did not identify different units within the Western Amazon (Iquitos) and Upper Madera. On the other hand, three clusters were identified by BAPS ($K = 3$, marginal −9,231.41 and posterior 0.97 probabilities), with a distribution of allelic frequencies and genetic parameters (Hnb, Hobs, F_{IS}) presented in Table 2. Consequently, considering the overall deviation from HWE for the whole data set, the BAPS assignment ($K = 3$) was considered as the most biologically plausible partition, as a reproductive unit is defined by panmixia in a sexual reproducing species.

This partition into three units consisted of two large clusters of 83 (Cluster 1) and 184 (Cluster 2) individuals, and a smaller one of 17 individuals (Cluster 3), with a specific geographic distribution (Fig. 1). Individuals assigned to the small Cluster 3 were basically the same (apart for two individuals) when analyses were performed with 100 and 1,000 replicates, respectively, for $k = 3$. No correlation was observed among cluster conformation and standard length of the individuals ($p > 0.05$).

According to population genetics theory, loci showing large F_{ST} values between populations should be the loci for which F_{IS} are highest in population mixtures (Wahlund effect). In a first step, clusters 1 and 2 (83 and 184 individuals, respectively) were compared, computing θ (F_{ST}) values between the two clusters at each locus and the F_{IS} values combining the two clusters at each locus, and then

Table 1 Pair-wise θ (F_{ST}) values (Weir and Cockerham 1984) for the geographic samples of Dorado (*Brachyplatystoma rousseauxii*) from the Western Amazon and Upper Madera

	IQ (65)	VB	CE	PM	RU	PV
IQ						
VB (20)	**0.020** ($p = 0.000$)					
CE (50)	**0.023** ($p = 0.000$)	−0.004 ($p = 0.825$)				
PM (50)	**0.021** ($p = 0.000$)	−0.002 ($p = 0.616$)	−0.001 ($p = 0.525$)			
RU (49)	**0.015** ($p = 0.000$)	0.005 ($p = 0.121$)	0.000 ($p = 0.425$)	0.002 ($p = 0.242$)		
PV (50)	**0.0170** ($p = 0.000$)	**0.009** ($p = 0.029$)	0.004 ($p = 0.070$)	0.001 ($p = 0.377$)	0.001 ($p = 0.241$)	

Numbers in parenthesis next to locality abbreviations represent the size sample

VB Villa Bella, *CE* Cachuela Esperanza, *PM* Puerto Maldonado, *RU* Rurrenabaque, *PV* Puerto Villarroel, *IQ* Iquitos

Significant values ($p < 0.05$) highlighted in bold. Negative θ values represent zero

plotting the F_{IS} and θ (F_{ST}) values across the nine loci analysed. No correlation was observed ($r^2 = 0.086$). However, as the test is very sensitive to the mixture proportions (Waples 2011), in a second step the analysis was run again after having randomly eliminated 101 individuals in Cluster 2 in order to compare two clusters of equal size (83 individuals each). In this case also, no correlation ($r^2 = 0.00005$) was observed. These results emphasize the fact the information provided by each locus is not enough to explain the structuring in clusters or to explain the deviation from HWE when clusters are in admixture, whereas the combination of multi-loci allelic frequencies explains both the deviation from HWE in the Upper Madera sample and its structuring in clusters.

Cluster 1 was not observed in the Western Amazon samples (Iquitos), whereas it was identified in all five locations from the Upper Madera (Bolivian Amazon sites + Puerto Maldonado in Peru). Individuals from the upper-most locations in the basin (Rurrenabaque and Puerto Villarroel) were in larger proportion in Cluster 2 than in clusters 1 and 3. Cluster 2, the largest, was distributed both in the Upper Madera and in the Western Amazon. It included all but one of the individuals from the Western Amazon (Iquitos) system (64 out of 65), and a substantial proportion (45 %) of the fish caught in the lower reaches of the Upper Madera River (Villa Bella, Cachuela Esperanza and Puerto Maldonado). Finally, Cluster 3, the smallest and most differentiated, included individuals from all sampled locations, although just one specimen from the Western Amazon (Iquitos) system was assigned to this group (Fig. 1).

The genetic diversity within each cluster was moderate (Table 2), with an average observed heterozygosity (H obs) ranging from 0.66 (Cluster 3) to 0.71 (Cluster 2), and numbers of alleles per locus ranging from 6.67 (Cluster 3) to 13.11 (Cluster 2). Cluster 2 had the highest number of unique alleles (39) and Cluster 1 the lowest (4). Generally, the frequencies of these alleles were low (<6 %). The locus with the highest number of private alleles per cluster was Pcor1, with 14 private alleles in Cluster 2.

Theta values between clusters 1–2, 1–3 and 2–3 were 0.028, 0.043 and 0.038, respectively.

Linkage disequilibrium analysis between pairs of loci showed a non-systematic pattern, where D^2_{ST} (0.006–0.041) > D^2_{IS} (0.002–0.009) and D'^2_{IS} (0.016–0.095) > D'^2_{ST} (0.001–0.004) (minimum and maximum values of variance components of disequilibrium coefficients), consistent with the hypothesis that variation within loci depends more on genetic drift than on selection (Ohta 1982a, b).

Discussion

Clear evidence of genetic differentiation between regional samples of *B. rousseauxii* from the Upper Madera and the Western Amazon was observed, and therefore the hypothesis of a single panmictic population across the Amazon basin suggested by Batista (2010) should be rejected. Furthermore, we found evidence for three clusters of multilocus genetic variation, suggesting three differentiated populations within the Upper Madera and Western Amazon systems.

Although Latch et al. (2006) suggested that the accuracy of BAPS is as good as STRUCTURE at low levels of differentiation (F_{ST} values as low as 0.02–0.03), BAPS was later shown to perform better than STRUCTURE in identifying subsets (clustering) (e.g., Corander et al. 2008; Wilkinson et al. 2011; Neophytou 2014). This was also the case for *B. rousseauxii*, where BAPS identified panmictic clusters with low levels of differentiation.

The degree of differentiation observed with microsatellites between the clusters was low relative to that reported in other groups of migratory fishes in the Neotropics, as for example *Pseudoplatystoma* (see Abreu et al. 2009: θ = 0.229; Pereira et al. 2009: θ = 0.034–0.164) and *Piaractus* (Calcagnotto and DeSalle 2009: θ = 0.052–0.065)

Table 2 Average heterozygosity at nine microsatellite loci, and values of the estimator *f* of the endogamy index F_{IS} calculated with multilocus data, for the three Dorado (*Brachyplatystoma rousseauxii*) clusters (Clus) as identified by BAPS and geographic samples in the Upper Madera and Western Amazon (Bolivian and Peruvian Amazon)

	Clus 1	Clus 2	Clus 3	VB	CE	PM	RU	PV	IQ	Global
N	83	184	17	20	50	50	49	50	65	284
Pcor1										
A	11	26	11	12	20	12	19	15	24	32
H nb	0.735	0.870	0.832	0.830	0.821	0.802	0.848	0.863	0.892	0.847
H obs	0.759	0.875	0.824	0.800	0.760	0.860	0.816	0.880	0.892	0.842
F_{IS}	−0.032	−0.005	0.011	0.037	0.076	−0.073	0.038	−0.019	−0.001	0.007
p				0.486	0.137	0.908	0.315	0.476	0.588	0.347
Pcor2										
A	8	15	5	7	11	10	13	11	12	17
H nb	0.700	0.768	0.652	0.830	0.821	0.802	0.848	0.863	0.892	0.745
H obs	0.663	0.772	0.647	0.800	0.760	0.860	0.816	0.880	0.892	0.732
F_{IS}	0.053	−0.005	0.008	**0.312**	0.017	0.078	−0.023	−0.095	−0.012	0.017
p				0.013	0.477	0.205	0.460	0.116	0.515	0.282
Pcor7										
A	5	9	7	5	6	7	6	5	6	9
H nb	0.442	0.259	0.415	0.554	0.364	0.353	0.261	0.188	0.327	0.326
H obs	0.410	0.266	0.412	0.550	0.320	0.360	0.245	0.200	0.339	0.313
F_{IS}	0.073	−0.029	0.009	0.007	0.123	−0.021	0.062	−0.066	−0.035	0.039
p				0.665	0.965	0.657	0.859	1.000	0.743	0.106
BR37										
A	6	11	4	5	5	6	6	8	11	12
H nb	0.638	0.689	0.668	0.623	0.685	0.653	0.712	0.666	0.646	0.673
H obs	0.627	0.701	0.353	0.450	0.640	0.640	0.633	0.680	0.739	0.655
F_{IS}	0.017	−0.018	0.480	0.283	0.066	0.021	0.112	−0.021	**−0.144**	0.027
p				0.055	0.269	0.470	0.131	0.466	0.022	0.210
BR38										
A	15	20	9	11	16	16	16	17	18	20
H nb	0.895	0.917	0.854	0.899	0.898	0.901	0.910	0.912	0.916	0.913
H obs	0.855	0.924	0.824	0.800	0.860	0.900	0.918	0.940	0.908	0.898
F_{IS}	0.044	−0.007	0.037	0.112	0.043	0.001	−0.010	−0.032	0.009	0.016
p				0.152	0.230	0.568	0.545	0.332	0.337	0.148
BR44										
A	7	9	7	6	9	8	9	9	9	9
H nb	0.791	0.799	0.711	0.790	0.809	0.807	0.806	0.801	0.774	0.800
H obs	0.121	0.079	0.029	0.900	0.680	0.820	0.755	0.780	0.769	0.771
F_{IS}	0.040	0.007	0.177	−0.144	**0.161**	−0.017	0.064	0.026	0.006	0.036
p				0.149	0.016	0.492	0.185	0.423	0.511	0.091
BR45										
A	9	12	6	7	9	10	9	8	11	12
H nb	0.823	0.843	0.804	0.815	0.829	0.857	0.846	0.835	0.825	0.844
H obs	0.783	0.772	0.765	0.750	0.680	0.780	0.816	0.840	0.769	0.775
F_{IS}	0.049	0.085	0.050	0.082	**0.181**	0.090	0.036	−0.006	0.068	**0.082**
p				0.300	0.007	0.069	0.326	0.538	0.121	0.000
BR47										
A	4	7	5	4	6	6	7	5	6	8
H nb	0.583	0.496	0.658	0.530	0.585	0.554	0.555	0.583	0.479	0.550
H obs	0.554	0.467	0.765	0.500	0.460	0.560	0.469	0.600	0.462	0.507

Table 2 continued

	Clus 1	Clus 2	Clus 3	VB	CE	PM	RU	PV	IQ	Global
F_{IS}	0.050	0.057	−0.169	0.057	**0.216**	−0.012	0.156	−0.029	0.036	**0.079**
p				0.364	0.025	0.551	0.069	0.470	0.423	0.025
BR49										
A	10	9	6	9	7	8	9	10	8	10
H nb	0.822	0.776	0.672	0.863	0.827	0.811	0.816	0.799	0.754	0.815
H obs	0.843	0.815	0.765	0.950	0.820	0.840	0.816	0.800	0.785	0.820
F_{IS}	−0.026	−0.051	−0.143	−0.104	0.009	−0.037	−0.001	−0.002	−0.041	−0.007
p				0.211	0.507	0.361	0.610	0.572	0.329	0.549
Multilocus										
A	8.3	13.1	6.7	7.3	9.9	9.2	10.4	9.8	11.7	129
H nb	0.714	0.713	0.696	0.736	0.723	0.719	0.719	0.715	0.710	0.724
H obs	0.695	0.710	0.660	0.689	0.656	0.716	0.689	0.731	0.716	0.702
F_{IS}	0.027	0.005	0.054	0.066	**0.095**	0.005	**0.042**	−0.023	−0.009	**0.031**
p				0.942	0.000	0.425	0.034	0.858	0.705	0.000

N number of individuals, *A* Number of alleles, *H nb* heterozygosity calculated without bias (Nei 1978), *H obs* observed heterozygosity, *VB* Villa Bella, *CE* Cachuela Esperanza, *PM* Puerto Maldonado, *RU* Rurrenabaque, *PV* Puerto Villarroel, *IQ* Iquitos

Significant values ($p < 0.05$) highlighted in bold

Table 3 Comparisons of Weir and Cockerham's (1984) θ (F_{ST}) values (for microsatellites) among clusters of Dorado (*Brachyplatystoma rousseauxii*) defined by BAPS in the Upper Madera and Western Amazon (Bolivian and Peruvian Amazon), and those for other freshwater, brackish and marine migratory fish species

Order	Family	Species	Environment	Behaviour	Loci	θ (F_{ST}) ($p < 0.05$)	Reference
Siluriformes	Pimelodidae	*Brachyplatystoma rousseauxii*	Continental	Migratory-amphidromous	9	0.028–0.043	Present study
Siluriformes	Pimelodidae	*Pseudoplatystoma corruscans*	Continental	Migratory-potamodromous	7	0.034–0.164	Pereira et al. (2009)
Siluriformes	Pimelodidae	*Pseudoplatystoma reticulatum*	Continental	Migratory-potamodromous	7	0.229	Abreu et al. (2009)
Characiformes	Serrasalmidae	*Piaractus mesopotamicus*	Continental	Migratory-potamodromous	8	0.052–0.065	Calcagnotto and DeSalle (2009)
Cypriniformes	Cyprinidae	*Coreius guichenoti*	Continental	Migratory-potamodromous	11	0.022–0.047	Zhang and Tan (2010)
Cypriniformes	Balitoridae	*Barbatula barbatula*	Continental	Migratory-potamodromous	5	0.010–0.110	Barluenga and Meyer (2004)
Salmoniformes	Salmonidae	*Salmo salar*	Marine, brackish, continental	Migratory-anadromous	8	0.027–0.072	McConnell et al. (1997)
Perciformes	Scombridae	*Thunnus thynnus*	Marine	Migratory-oceanodromous	9	0.009	Carlsson et al. (2004)
Perciformes	Scombridae	*Thunnus thynnus*	Marine	Migratory-oceanodromous	8	0.005–0.012	Carlsson et al. (2007)
Gadiformes	Gadidae	*Gadus morhua*	Marine	Migratory-oceanodromous	10	0.0013–0.005	Knutsen et al. (2003)
Gadiformes	Gadidae	*Gadus morhua*	Marine	Migratory-oceanodromous	13	0.002–0.006	Knutsen et al. (2011)
Anguilliformes	Anguillidae	*Anguilla anguilla*	Marine	Migratory-catadromous	22	0.005–0.015	Pujolar et al. (2011)
Carchariniformes	Carcharinidae	*Carcharinus limbatus*	Marine, brackish	Migratory-amphidromous	8	0.004–0.067	Keeney et al. (2005)

(Table 3). Nevertheless, these values were equal or much greater (up to ten times) than those observed for other continental and marine migratory species with low F_{ST} values that nevertheless reflect a biological reality (e.g. *Salmo salar*—McConnell et al. 1997: $\theta = 0.027–0.072$; *Gadus morhua*—Knutsen et al. 2011: $\theta = 0.002–0.006$) (Table 3). It can therefore be considered that our results indicate the existence of more than one genetic unit, and probably at least three in the Amazon.

Structured populations

The presence of *B. rousseauxii* along the main channel of the Amazon and its main tributaries has raised speculation that commercial fisheries exploit a single stock that moves continuously between the headwaters and the estuary (Barthem and Goulding 1997, 2007). This hypothesis recently was asserted by Batista (2010), who analysed 483 individuals from 13 locations using eight microsatellites. This study focused on the main channel of the Solimões-Amazon, but also considered several tributaries, including the Madera River (60 specimens from four localities) and the Madre de Dios River (tributary of the Madera, with 43 specimens from Puerto Maldonado). In spite of this overlap with our sampling areas, Batista (2010) inferred general panmixia over the whole *B. rousseauxii* distribution area, albeit without giving any value for their departure from HWE nor for the overall F_{IS}. On the contrary, our work revealed the existence of three clusters, two of them principally localized in the Upper Madera (Cluster 1 and Cluster 3). Furthermore, the differentiation between the Upper Madera and the Western Amazon samples was strong.

The discrepancy between the results of the present study and those of Batista (2010) may lie in the numbers and geographic origins of the samples examined from the Upper Madera basin. Batista (2010) analyzed mainly samples from the Amazon River's main channel, and considered 103 samples from two locations in the Upper Madera basin (43 from the Madre de Dios and 60 from Porto Velho), which represented less than half our sampling number. BAPS analyses revealed that Cluster 1 appears to be exclusive to the Upper Madera River, whereas the ubiquitous Cluster 2 (largest in number) is dominant in the Iquitos (Ucayali-Amazon) sample (only one out of 64 individuals did not belong to Cluster 2), and Cluster 3, the most differentiated, consists of a small number of individuals, all but one (from Iquitos) belonging to the Upper Madera. The degree of differentiation observed between the three clusters was weak ($\theta = 0.028–0.043$), albeit significant, and therefore its signal in the total grouping is low. It is therefore possible that the signal from clusters 1 and 3 (the least abundant) was not detected in Batista (2010)' sampling, which contained only a small number of individuals from the Upper Madera basin. Additionally, the ubiquitous and large Cluster 2, predominant in the Iquitos samples (all individuals but one), may also be numerically dominant along the main channel of the Amazon River (Peru and Brazil) and in the lower reaches of its main tributaries, masking the signal of other, less abundant populations, such as clusters 1 and 3, which may be present in the lower Amazon (below the confluence with the Madera), given the migratory life cycle of the species. The observation that two of the clusters identified in the Upper Madera are absent, or scarce, in the Upper Amazon (Western Amazon) suggests the existence of a homing behaviour at the scale of large sub-basins (Madera *vs.* Amazon). This homing hypothesis, however, requires further evidence, including use of complementary methodologies such as mark-recapture, isotopic signatures on otholiths or telemetry. Alternatively, a potential explanation for the population structure in the Upper Madera is the co-existence of more than one life-history strategy in the species. *B. rousseauxii* could be composed of long-distance migrants, as suggested by earlier work, and by one or more populations that migrate shorter distances or residualize within river systems. Co-existence of multiple life-history strategies with residualized populations have been shown in other migratory fish species, especially salmonids, when migration leads to high mortality, when migration is cut off or when various factors act synergistically to create complex patterns of movement within populations (Wood and Foote 1996; Chapman et al. 2011, 2012; Brodersen et al. 2014). Hence Clusters 1 and 3 could correspond to more resident populations and Cluster 2 to a long-distance migratory population. Testing this hypothesis would require the sampling and genetic characterization of spawning assemblages.

The existence of three distinct clusters (genetic units) in a same geographical area suggests that differential reproductive behaviour among the three populations occurs either by temporal and/or spatial segregation. Breeding seasons vary among tributaries in the Upper Madera: between October and December, with a peak in October in the Upper Madre de Dios (Cañas and Pine 2011; Cañas and Waylen 2011), between February and May in the Upper Mamoré River (Van Damme et al. 2011a), whereas females with ripe gonads were observed from October to May in the Upper Beni River (pers. obs. F. Duponchelle, Institut de Recherche pour le Développement—France, unpublished data). We also know that *B. rousseauxii* larvae are present almost year-round in the Madera (R. Leite, Instituto Nacional de Pesquisas da Amazônia—Brazil, pers. com.), and so are ripe adults in specific tributaries (R. Barthem, Museu Paraense Emílio Goeldi—Brazil, and M. Goulding, consultant of Wildlife Conservation Society—USA, pers. com.). Taking into account our biological knowledge of the species, the presence of three distinct genetic populations in admixture in every sampled tributary may be explained either by the fact that individuals from these populations would reproduce at the same places but at different periods (temporal segregation hypothesis) with some overlap (gene flow), or may reproduce only in specific tributaries (spatial segregation hypothesis associated with a homing behaviour among the Upper Madera tributaries). Most known cases of homing are associated with some degree of straying, which could explain the presence of an individual from Cluster 3 in the Western Amazon. Clearly, further studies are needed to shed light on these two, not mutually exclusive, hypotheses.

Additional biological information that supports the existence of several populations in the Amazon basin is the difference in the maximum size observed between the Upper Amazon, where specimens of 150 cm SL are common (García et al. 2009a for the Peruvian Amazon and Agudelo et al. 2013 for the Colombian Amazon), and the Upper Madera, where specimens above 130 cm SL are rarely caught (Van Damme et al. 2011a, Carvajal-Vallejos, pers. obs.). This difference is further emphasized by the fact that fishery exploitation, which decreases the maximum size of exploited populations (Rochet and Trenkel 2003), borders on over-exploitation for this species in the Peruvian (García et al. 2009a, b) and Colombian Amazon (Agudelo et al. 2013), whereas it remains relatively weak in the Bolivian Amazon (Van Damme et al. 2011a).

Conservation implications

Acknowledging the existence of different populations will have a substantial impact on the strategies for conservation and management of this species in the Amazon Basin, which is threatened to varying degrees by overfishing, pollution, habitat destruction and dam construction in the five countries (Finer and Jenkins 2012; Castello et al. 2013).

Hydroelectric impoundments in particular appear to be the most immediate threat in the Madera River, where two dams recently were constructed at the Jirau and Santo Antônio rapids in Brazil, and several others are planned (Finer and Jenkins 2012). One of these two dams (Santo Antônio) provides a specific by-pass system for the upstream migration of fishes. Although its effectiveness is still unknown for Dorado, this by-pass system seems very efficient for several species formerly restricted to the downstream portion of the Madera below the Santo Antônio rapids close to Porto Velho (Brazil): recently, fishermen from the Bolivian Amazon have reported the captures of species never seen before (Carvajal-Vallejos, pers. obs). Although the dams may not be preventing the upstream migration of adult *B. rousseauxii*, a major concern remains for the larvae and juveniles. The downstream migration of young stages is likely to be seriously affected by the dams and associated reservoirs, depleting or hampering the recruitment of this species from the Madera (Molina 2011b). Bolivian fishermen are also reporting unusually small sizes for several otherwise big catfish species (Carvajal-Vallejos, pers. obs), suggesting that juveniles, which were drifting downstream to the estuary before, are now unable to pass through the dam and are forced to grow in the Upper Madera. Targeted monitoring of the effects of the dams and the by-pass system on the upstream and downstream movements of the Madera ichthyofauna is needed.

As the Madera contributes nearly one-fifth of the Amazon's water balance and to about half its sediment load (Goulding et al. 2003), it appears reasonable to hypothesize that the Madera might also represent a similar proportion (>20 %) of the Amazon recruitment for *B. rousseauxii*. Moreover, as shown by our results, at least one population seems to be present exclusively in the Madera basin, which means that reduced recruitment from the Madera would translate into a lower return rate of adults to the spawning grounds, which ultimately could lead to a loss of genetic variability and decreased landings. A reduction of the landings could provoke a collapse of the fisheries for Dorado, as well as other migratory species in the Upper Madera River Basin (mainly the Bolivian Amazon and Puerto Maldonado town), having serious effects on the fisheries value chain, people's livelihoods and local food security.

While the hydroelectric projects are at an advanced stage of construction or completed in some cases, very little is known about their effect on this species. It is urgent that more data be quickly generated to complete and better understand the ecology and structure of the Dorado population throughout the basin, and confirm or refute the existence of homing or resident behaviours in the Madera basin.

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Conflict of interest The authors declare that they have no conflict of interest.

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