Research Article

Genetic diversity of Boeseman´s Rainbowfish (*Melanotaenia boesemani*) reared in Indonesian farms compared to endangered natural populations

Media Fitri Isma Nugraha[1, 2,3], Laurent Pouyaud[1], Odang Carman[3], Utut Widyastuti[4], Muhammad Zairin Junior[3], Kadarusman[5] and Jean-Christophe Avarre[1*]

[1]Institut des Sciences de l'Evolution de Montpellier, UMR 226 IRD-CNRS-UM2, Montpellier, France
[2]Research Centre and Development for Ornamental Fish, Indonesian Agency for Marine and Fisheries Research and Development, Depok, Indonesia
[3]Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agriculture University (IPB), Bogor, Indonesia.
[4]Department of Biology, Faculty of Mathematics and Science, Bogor Agriculture University (IPB), Bogor, Indonesia
[5] Politeknik Kelautan dan Perikanan Sorong, KKD-BP Sumberdaya Genetik dan Konservasi, Sorong, Papua Barat, Indonesia
*Corresponding author: jean-christophe.avarre@ird.fr

Abstract
Endemic to two lakes (Ayamaru and Uter) of West Papua (Indonesia), the Boeseman´s Rainbowfish *Melanotaenia boesemani* Allen & Cross, 1980 is a very popular ornamental freshwater fish. As a result, this rainbowfish species faces great threats and is on the red list of endangered species. Therefore, rearing of this species in aquaculture systems appears to be a promising solution to limit capture of wild specimens and prevent its extinction. Although its reproduction cycle has been controlled for more than 30 years, very few farms still raise *M. boesmani* , probably due to the problems reported by the farmers, *such as* decline of production, higher proportion of females per spawning, loss of coloration, lower growth rate and fecundity. Using 12 microsatellites previously developed for this species, comparison of genotypes within six farms around Jakarta indicated that all reared strains originated from Ayamaru Lake. No deficit in heterozygotes was evidenced, suggesting that there was no major inbreeding in these reared populations. Genotype analysis also suggested that *M. boesemani* species is a metapopulation composed of genetically differentiated populations. Altogether, these results indicate that the problems experienced by the farmers are due not to inbreeding depression but to other factors such as inadequate management and/or poor water quality. Yet, increasing aquaculture production is probably the most effective way to alleviate the pressure that *M. boesemani* faces in its natural environment.

Key-words: *Melanotaenia boesemani*, endangered species, aquaculture strains, genetic variability, microsatellites

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Introduction

The Boeseman´s Rainbowfish *Melanotaenia boesemani* Allen & Cross, 1980 is one of the most popular rainbowfish species in the aquarium hobby. When fully matured, males display a very distinct pattern of half-and-half coloration marked by a brilliant blue anterior and bright yellow to orange-red posterior (Fig. 1) [1, 2]. Gerald Allen discovered the species while studying the material collected in 1954-55 by Marinus Boeseman and stored at the National Museum of Natural History in Leiden (Netherlands) [3]. According to several studies [1, 4, 5], the species is only known from Ayamaru Lakes and tributaries and from Uter-Aitinyo Lake (or Uter L.). The two locations are 30 km apart and are separated by rugged karsts (Fig. 2). After the first publication describing *M. boesemani* [3], great interest arose in the potential commercial value of this species. It was introduced to the aquarium hobby in 1983 and has steadily increased in popularity since then [6]. In the mid-1980s, more than 60,000 males were caught and exported monthly from Ayamaru [7]. Such over-exploitation has therefore quickly brought this species to the verge of extinction in its natural habitat [4]. It has been on the red list of endangered species since 2004, and only aquaculture products are now supposed to be exported [8].

Although there is a lack of precise data, very few Indonesian farms, no more than ten, breed this species, all located around Jakarta, and their production does not account for the total exported fish. Boeseman´s Rainbowfish has been domesticated and produced in Indonesian farms since 1983. At present, farmers claim a decrease of both quantity and quality: males are not as colored as in the wild; growth rate and fecundity are slower; and morphological abnormalities frequently occur (unpublished data obtained from the farmers; Fig. 1c). They attribute these observations to loss of genetic variability and possible inbreeding (personal communication).

Twelve nuclear DNA microsatellite markers were recently developed in this species [9] for conservation purposes. We used these microsatellite markers to assess the genetic variability of six different strains of Boeseman's Rainbowfish reared in six Javanese farms. We compared their genetic variability with that of wild populations to determine the geographic origin of the founders and quantify possible loss of variability. Prior to these analyses, the microsatellite markers were further validated by testing their Mendelian inheritance through crossing experiments with five mate pairs of Boeseman's Rainbowfish obtained from a French retailer.

Methods

Fish sampling

A total of 432 *M. boesemani* specimens were analyzed in the present study. Wild fish were captured in 2007 by using gillnets at two distinct locations, respectively in the vicinity of Ayamaru lake (Tiwit tributary, 1°15.463'S and 132°14.939'E, 28 specimens) and at the Uter lake (1°25.957'S and 132°23.258'E, 49 specimens) (Fig. 2). Specimens were captured under local permits obtained by Akademi Perikanan Sorong (APSOR, West Papua). Captive fish were obtained from six farms located around Jakarta. These six farms have been cultivating *M. boesemani* for many years (up to 30 years), do not practice out-crossings with other breeders, and are facing difficulties such as more females in each harvest (only males are suitable for sale), smaller body size, and loss of color brightness. Approximately 30 individuals were collected from each farm. For Mendelian inheritance tests, five breeding pairs were selected from stock purchased from a French ornamental fish retailer (Botanic, Montpellier) and reared in distinct 60L aquariums. Progeny of each parental pair were collected within the two following months, and consisted of 23 to 43 hatchlings, depending on the spawn size. All fish were anaesthetized with 0.1 mL/L Eugenol (in accordance with the EU Directive 2010/63/EU) and a ~1-cm^2 piece of anal fin was collected and stored in absolute ethanol for further DNA extractions. Fish were then allowed to recover from anaesthetic and were released back into either the lakes (at the sampling site) or fish ponds. Experiments on captive animals were conducted at the aquatic experimental facilities of ISEM (PLATAX) (Montpellier) under the laboratory agreement for animal experimentation number A-34-172-24 and the author's personal authorization for animal experimentation number 34-188, both provided by the French government.

DNA extraction and microsatellite amplification

Individual samples were genotyped with 12 nuclear microsatellite markers that were recently developed [9]. DNA was extracted from a small fragment (10 mg) of anal fin clip with the NucleoSpin® 96 Tissue kit (Macherey-Nagel), according to the manufacturer's instructions, using a Janus automated Workstation (Perkin Elmer). Forward primers were end-labeled with fluorescent dyes (5'FAM, 5'HEX, 5'ATO550, 5'ATO565) (Eurofins). Each reaction contained 5 µl of 2x Master mix (Fast-Start PCR kit, Roche), 0.1 µM of forward primer, 0.4 µM of reverse primer, and 0.5 µl of template DNA. Cycling conditions were as follows: initial denaturation at 95°C for 4 min, followed by 30 cycles of 95°C for 20 s, 56°C for 20 s, and 72 °C for 30 s, and a final elongation step of 7 min at 72°C. Amplicon size was analyzed by capillary electrophoresis as previously described [9], in the technical facilities of the labex "Centre Méditerranéen de l'Environnement et de la Biodiversité" (Montpellier). Allele sizing and genotyping were achieved with the Peak Scanner v1.0 and GeneMapper® v5.0 software (Applied Biosystems).

Genetic diversity analysis

Allelic numbers (Na), average observed (Ho) and expected (He) heterozygosities were calculated using the GENETIX 4.05 software [10]. Data were analyzed for possible departures from Hardy-Weinberg equilibrium by estimating the significance of the multilocus inbreeding coefficient (Fis) with 1,000 random allelic permutations of the original datasets. The genetic structure among the investigated populations was also evaluated by calculating pairwise Fst values with 1,000 random permutations. The significance level of *P* value for Fis and Fst was defined as the probability of obtaining absolute values higher than or equal to the observed one under the null hypothesis. The genetic relationships between multilocus genotypes of the six reared strains and the wild populations of *M. boesemani* from Ayamaru and Uter Lakes were also assessed, using a factorial correspondence analysis (FCA) available in Genetix 4.05 software.

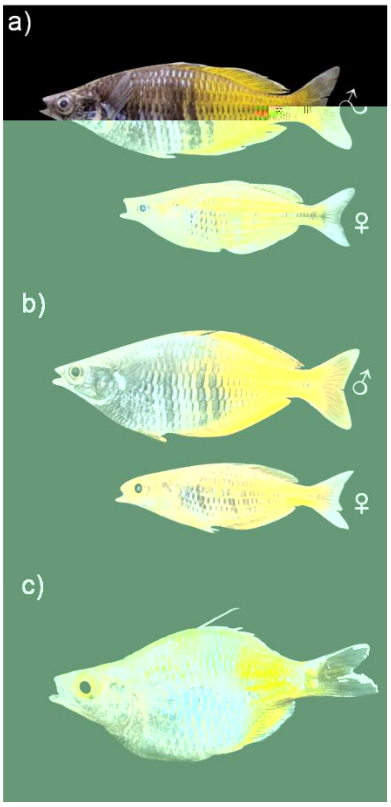

Fig. 1. Coloration patterns of Boeseman´s Rainbowfish *Melanotaenia boesemani.* Panels (a) and (b) show the difference between wild male and female specimens from Ayamaru and Uter Lakes (West Papua, Indonesia), respectively; panel (c) shows a reared specimen from Gusi farm (Jakarta) with a malformation, as frequently observed in the investigated farms.

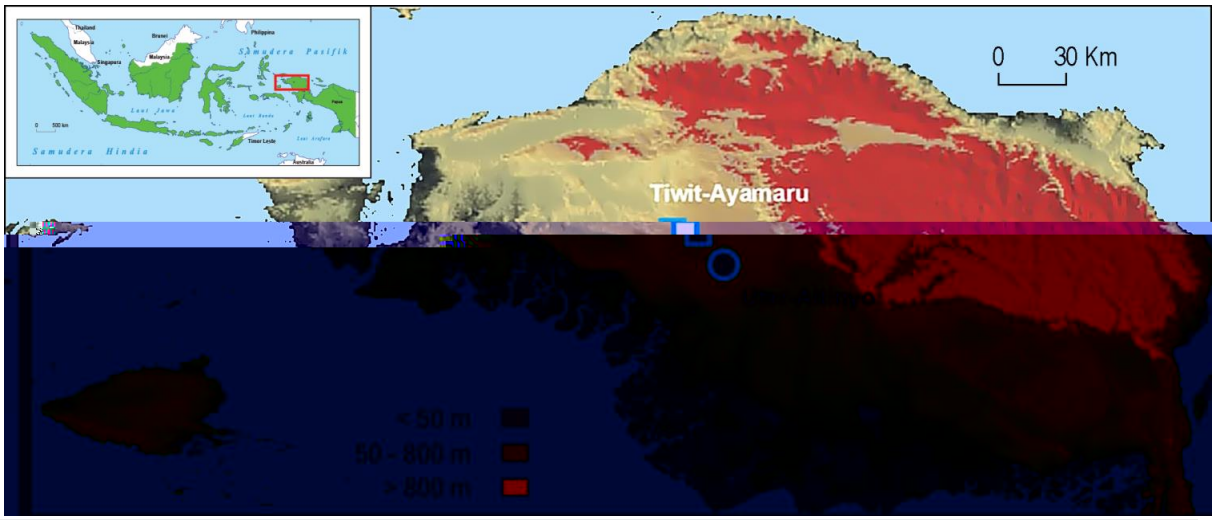

Fig. 2. Geographic localization of Ayamaru and Uter Lakes in West Papua, Indonesia.

Results

Experimental validation of the DNA microsatellite markers

The Mendelian inheritance of each microsatellite marker and the presence of putative null alleles were evaluated through crossing experiments. For this purpose, the genotypes of the progenies were resolved and compared to those of their corresponding parents. Results indicated that all genotypes observed in the offspring matched those expected from the parental ones: there were no heterozygous genotypes different from the predicted ones, genotype frequencies were similar to those expected (Appendix 1), and there was no significant difference between the observed and expected heterozygosities calculated on all loci for each crossing population (Table 1). Therefore, all 12 microsatellite loci seemed subject to Mendelian inheritance and there are no null alleles at these loci.

Table 1. Observed (Ho) and expected (He) heterozygosity calculated on all loci for each crossing population and associated Fis.

	Crossing 1	Crossing 2	Crossing 3	Crossing 4	Crossing 5
Ho ± SD	0.65 ± 0.27	0.69 ± 0.32	0.67 ± 0.30	0.56 + 0.26	0.70 ± 0.38
He ± SD	0.52 ± 0.19	0.49 ± 0.21	0.47 ± 0.19	0.43 ± 0.18	0.49 ± 0.25
Fis	NS	NS	NS	NS	NS

NS: non-significant

Genetic variability and heterozygosity of M. boesemani in aquaculture settings and in natural populations

A total of 151 alleles were detected in the 183 farmed and 77 wild specimens in the 12 microsatellite loci. The total number of alleles per locus for all populations varied from 5 to 22, and all loci were polymorphic in each strain and wild population (Appendix 2). Generally, the domesticated populations showed a lower genetic variability (total number of alleles between 62 and 86) compared to the Ayamaru wild population (107 alleles), but comparable to that observed in the wild population of Uter (82 alleles) (Table 2). Considering only the wild populations, there were 23 private alleles for the Uter Lake population and 48 private alleles for Ayamaru Lake. When all samples were included, there were 17 private alleles for Uter Lake, 16 private alleles for Ayamaru Lake and 21 private alleles for the domesticated populations (*i.e.*, only present in one or more strains and absent in wild populations). There were six alleles shared between Uter Lake and one or more strains, and 32 alleles shared between Ayamaru lake and at least one strain. There were 51 alleles shared between both wild populations and one or more strains, and eight alleles shared between Ayamaru and Uter lakes but absent from any strains (Appendix 2). Likewise, the heterozygosities calculated for the captive populations (Hobs comprised between 0.53 and 0.64) were lower than that calculated for the Ayamaru wild population (0.68) but similar to that of Uter population (0.56) (Table 2). Moreover, the Ho were never significantly different from the He. The multilocus Fis values for all populations (wild and captive) were between -0.010 and 0.061, and none of them was significant except that for the Sukri strain (Fis = 0.061, $P < 0.05$) (Table 2). These results strongly suggest that, with the exception of the Sukri farm, there is no deficit in heterozygotes in any population. Finally, the pairwise Fst values calculated between each population were all significant ($P < 0.05$), except between Warso and Yahya and between Warso and Didi farms, indicating that most of the populations are genetically differentiated from each other (Table 3).

Table 2. Sample size (N), allele number (Na), average observed (Ho) and expected (He) heterozygosity, and multilocus Fis values for each wild population and strain

Population	N	Na	Ho	He	Fis
Uter lake	49	82	0.56	0.55	-0.010
Ayamaru lake	28	107	0.68	0.69	0.013
Farm Gusi	34	79	0.61	0.61	-0.007
Farm Sukri	30	74	0.59	0.63	0.061*
Farm Hasan	30	86	0.64	0.64	-0.001
Farm Yahya	30	75	0.61	0.62	0.010
Farm Didi	29	62	0.53	0.55	0.029
Farm warso	30	71	0.62	0.61	-0.013

* $P < 0.05$

Table 3. Population differentiation based on pairwise Fst estimates

	Ayamaru Lake	Gusi	Sukri	Hasan	Yahya	Didi	Warso
Uter Lake	0.176***	0.228***	0.226***	0.221***	0.249***	0.263***	0.231***
Ayamaru Lake		0.030***	0.028***	0.028***	0.062***	0.075***	

NS: non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Geographic origin of M. boesemani reared in Indonesian farms

A factorial correspondence analysis (FCA) was performed on all multilocus genotypes obtained for the six captive and the two wild populations (Fig. 3). The projection made on axis 1 (5.99%) and axis 2 (2.47%) indicated that the wild population from Uter Lake could be genetically differentiated from that of Ayamaru Lake. Moreover, all the fish collected from the farms (*i.e*. polygon "pooled strains") were partly grouped with those of Ayamaru Lake (*i.e*., polygon "Ayamaru lake"). The third axis of the FCA did not enable us to separate the farm samples from the Ayamaru wild population. Likewise, when the Uter population was removed, the Ayamaru and farm specimens still overlapped. These results suggest that all captive fish originate from the region of Ayamaru Lake.

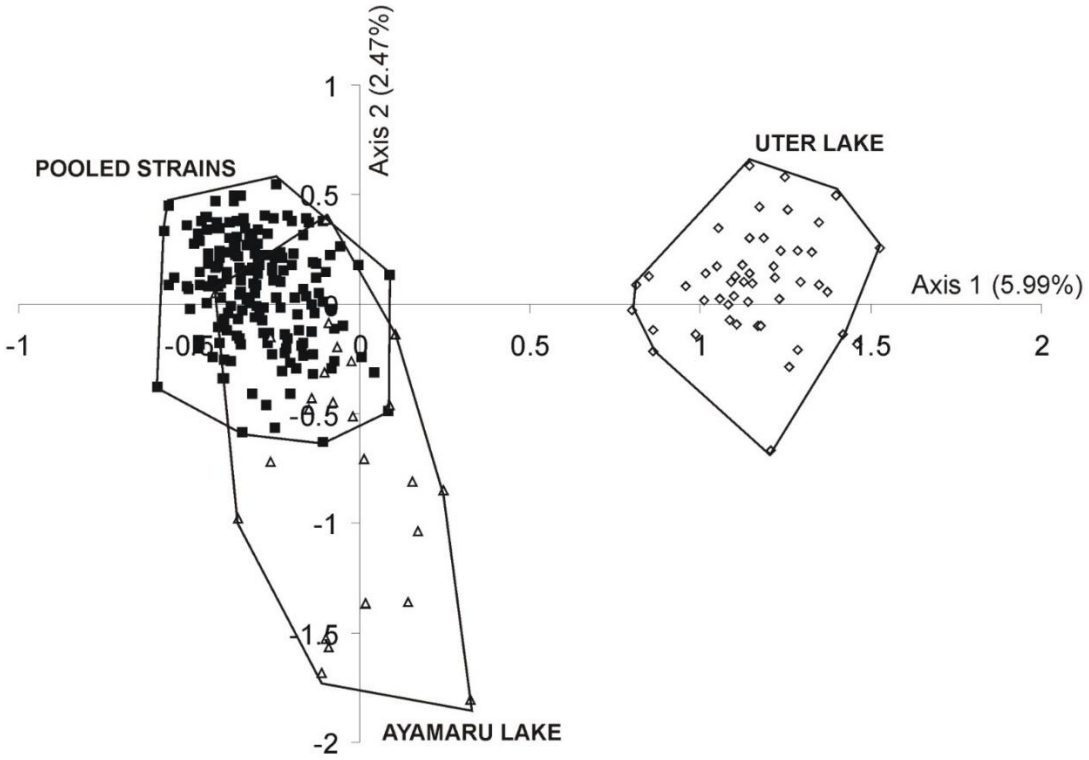

Fig. 3. Factorial Component Analysis based on the multilocus genotypes of Boeseman's Rainbowfish *Melanotaenia boesemani* individuals from the 2 wild populations of Ayamaru (n=28) and Uter (n=49) and the 6 Indonesian farms (n=29-34).

Discussion

The possible presence of null alleles in the 12 microsatellite markers had been checked *in silico* and results suggested that *M. boesemani* species was not affected [9]. However, this needed to be experimentally validated in order to definitely exclude this possibility. The cross-breeding experiments performed in the present study showed that the 12 microsatellite markers follow a Mendelian inheritance, and ruled out the existence of null alleles due to primer misamplifications, as well as genotyping errors due to stuttering or large allele dropout.

Ayamaru and Uter lakes are located in Indonesia in the western part of New Guinea Island. These two lakes are not connected, are 30 km apart, and are separated by rugged karsts. Though they are considered to be the same species, the fish from these two lakes display significant morphological differences: those from Ayamaru lake have a bright blue anterior and bright yellow anterior coloration, whereas those from Uter show a bright blue anterior and a reddish posterior coloration (Fig. 1). Nevertheless, previous analyses based on both the mitochondrial cytochrome oxydase I gene and morphomeristic traits failed to separate these two populations, which are slightly overlapping [11]. Here, the use of 12 microsatellite markers brought clear evidence that they are genetically differentiated (Fig. 3), and emphasizes that population mixtures should be strictly avoided during any upcoming broodstock constitution. From the samples investigated here, many private alleles were evidenced between the two wild populations of Boeseman's Rainbowfish (23 for Uter lake and 48 for Ayamaru lake). Among the 12 markers, Mb_di1, Mb_di3, Mb_di4 and Mb_tetra2 accounted for more than 50% of the private alleles found between the two populations (42 over 71).

Among the investigated farms, Gusi, Hasan and Didi started to exploit Boeseman's Rainbowfish in 1983 and 1986, respectively, soon after the description of *M. boesemani* species by Allen and Cross [3] and the publication of *Rainbowfishes of Australia and Papua New Guinea* [12]. This book greatly increased the popularity of rainbowfishes, especially the newly discovered New Guinea species, including Boeseman's Rainbowfish [2]. In spite of the establishment of these farms, exploitation of this species from its natural environment in Ayamaru has persisted until now. The geography of Uter Lake, surrounded by mountains and characterized by steep and rocky shores, is less favorable to fishing activities than that of Ayamaru, which is more easily accessible. This is probably why all investigated domesticated populations originated from Ayamaru.

The core population of Ayamaru sampled 30 years ago for the cultivated strains was genetically different from that of the wild population sampled in 2007. This was especially evidenced by the 21 private alleles observed in the strains and absent in the wild population of Ayamaru Lake. The presence of these private alleles may suggest a loss of variability that the wild population of Ayamaru has undergone over the last 30 years. However, the fact that heterozygosity and allele number values calculated from the Ayamaru population are comparable with those of other rainbowfish species [9] suggests little or no loss of genetic diversity. Most likely, these private alleles indicate that the genetic diversity of the Ayamaru specimens investigated here are not representative of the whole genetic diversity of Boeseman's Rainbowfish in this area (Ayamaru Lake and tributaries). The wild fish sampled in 2007 were indeed collected in Tiwit River, a tributary of Ayamaru Lake, and could represent a distinct genetic entity from the whole population. In this case, the species could be a metapopulation composed of several population subdivisions according to their geographic distribution in the mosaic of habitats characterizing Ayamaru Lake.

It was recently shown in a wide range of marine fishes that overfishing could result in significant reduction of genetic diversity [13]. Another recent meta-population study using many marine fish species demonstrated that the renewal of a fish population depends much less on the amount of available genitors than on environmental factors [14]. If the data presented here are not sufficient to tackle these questions, they nonetheless highlight the urgent need for a complete and exhaustive sampling campaign to determine the exact genetic structure and diversity of this endangered species, in order to ensure its conservation. This would require collection of fish in many more locations of Ayamaru Lake and its tributaries. Indeed, in addition to overfishing, Ayamaru Lake also experiences important environmental threats (deforestation, urbanization) and episodic droughts. Based on the sequence of a mitochondrial DNA fragment of the native Australian freshwater fish *Rhadinocentrus ornatus*, Mather *et al*. showed that habitat degradation caused by urbanization significantly reduced genetic diversity [15].

Finally, the genetic variability within the six farmed populations was comparable to that of the natural population of Uter Lake. Because no deficit in heterozygotes was evidenced, there was no major inbreeding in these reared populations. Therefore, the problems experienced by the farmers (*i.e.,* decline of production, higher proportion of females per spawning, loss of coloration, lower growth rate and fecundity, morphological abnormalities) are obviously not due to inbreeding depression and are probably caused by other factors such as poor management and/or poor water quality. It is noteworthy that all investigated farms are located in industrial and densely populated peri-urban areas. As Jakarta and its suburbs have no waste water treatment system, the water used for rearing these fish is probably of very poor quality, and may contain many chemical and hormonal pollutants, both of which have been proven to alter major fish traits such as reproduction and growth. Indeed, exposure to low concentrations of endocrine disruptive chemicals (such as estrogens) can impede gonadal function, reduce fertilization success, decrease fecundity, alter mating behavior, and reverse sex of various

aquatic species [16-18]. Such exposure can even cause the collapse of fish populations at trace concentrations [19].

Regarding the coloration pattern, rainbowfish are able to change color according to the turbidity level of their environment [20, 21]. For instance, increased brightness of red colors in environments rich in organic matter (*i.e.,* more turbid) may enhance conspicuousness, allowing individuals to maintain communication in altered visual environments. Thus, the alteration of fish color brightness observed in reared animals may be an adaptation to the aquaculture conditions, where ponds are very shallow and mimic habitats with full-spectrum lighting. However, a better understanding of the visual system of rainbowfish is required to predict how changes in the aquatic light environment affect the physiology and ecology of these fishes and allow farmers to adapt their rearing conditions to maintain bright body colors.

Implications for conservation

The combination of important levels of biodiversity with low human population density (2-6 inhabitants / km^2) led Conservation International in 1997 to declare New Guinea as the only "*Major Tropical Wilderness Area*" remaining in Asia. For illustration, all the species of melanotaeniids from Western New Guinea are endemic to the area. Because many rainbowfish species have restricted distributions and are confined to specific habitats, such as isolated lacustrine environments or small parts of a single river system, they are highly vulnerable to environmental disturbance and over-harvesting. Ayamaru Lake is affected by the development of residential areas and ecotourism. Forest clearance has increased channel obstructions by sediments and led to partial drying of the lake. Meanwhile, simultaneous over-catching of the endemic Boeseman's Rainbowfish drove this species to the verge of extinction [7]. The results presented here (*i.e.,* no significant loss of heterozygotes in the wild populations and no inbreeding depression in the reared strains) indicate that, in spite of the threats that Ayamaru is facing, it is still possible to prevent the extinction of Boeseman's Rainbowfish. This, however, would require increased aquaculture production in order to quickly alleviate the overfishing pressure. This, in turn, would require better management of the quality of waters used for rearing Boeseman's Rainbowfish, which is a general concern in Indonesia.

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Appendix 1. Allele frequencies for each breeding pair (crossing) and its corresponding offspring at the 12 microsatellite loci.

Allele	Crossing and corresponding offspring														
	M1	F1	O1 (n=24)	M2	F2	O2 (n=40)	M3	F3	O3 (n=23)	M4	F4	O4 (n=32)	M5	F5	O5 (n=43)
Mb_di1															
95	-	-	-	-	-	-	0.50	-	0.28	-	-	-	0.50	-	0.29
97	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	0.21
109	-	-	-	0.50	0.50	0.55	-	-	-	-	-	-	-	-	-
113	1.00	0.50	0.67	-	0.50	0.21	-	1.00	0.50	1.00	0.50	0.70	-	0.50	0.24
117	-	0.50	0.33	0.50	-	0.24	0.50	-	0.22	-	0.50	0.30	-	-	-
119	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.26
Mb_di2															
113	-	0.50	0.33	-	-	-	0.50	1.00	-	-	-	-	-	-	-
123	-	-	-	0.50	-	0.35	-	-	-	0.50	-	0.22	-	-	-
127	-	0.50	0.17	-	-	-	-	-	-	-	-	-	-	-	-
129	-	-	-	-	0.50	0.20	-	-	-	0.50	1.00	0.78	-	0.50	0.26
133	-	-	-	0.50	-	0.15	-	-	0.72	-	-	-	-	-	-
135	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	0.23
139	0.50	-	0.33	-	0.50	0.30	0.50	-	0.28	-	-	-	-	-	-
141	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.50	0.51
143	0.50	-	0.17	-	-	-	-	-	-	-	-	-	-	-	-
Mb_di3															
153	-	1.00	0.50	-	-	-	0.50	0.50	0.46	0.50	0.50	0.48	-	-	-
161	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.25
163	0.50	-	0.25	1.00	0.50	0.80	0.50	0.50	0.54	-	-	-	1.00	-	0.50
165	-	-	-	-	0.50	0.20	-	-	-	-	-	-	-	-	-
171	0.50	-	0.25	-	-	-	-	-	-	0.50	0.50	0.52	-	0.50	0.25
Mb_penta1															
160	-	-	-	-	-	-	0.50	1.00	0.65	-	-	-	0.50	-	0.29
180	-	-	-	-	-	-	-	-	-	0.50	0.50	0.59	-	-	-
185	-	-	-	-	1.00	0.50	0.50	-	0.35	-	-	-	-	0.50	0.24
190	0.50	0.50	0.56	-	-	-	-	-	-	0.50	-	0.19	-	-	-
195	0.50	-	0.23	-	-	-	-	-	-	-	-	-	-	-	-
200	-	0.50	0.21	1.00	-	0.50	-	-	-	-	0.50	0.22	0.50	-	0.21
205	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.26
Mb_di4															
112	0.50	-	0.19	0.50	-	0.26	1.00	0.50	0.76	-	0.50	0.28	-	1.00	0.50
114	0.50	0.50	0.52	-	0.50	0.23	-	0.50	0.24	1.00	0.50	0.72	-	-	-
124	-	-	-	0.50	0.50	0.51	-	-	-	-	-	-	-	-	-
140	-	0.50	0.29	-	-	-	-	-	-	-	-	-	-	-	-

164	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	0.50
Mb_tetra1															
143	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	0.74	-	1.00	0.50	0.50	0.50	0.47
147	-	-	-	-	-	-	0.50	-	0.26	0.50	-	0.23	0.50	0.50	0.53
157	-	-	-	-	-	-	-	-	-	0.50	-	0.27	-	-	-
Mb_tri1															
113	1.00	0.50	0.71	0.50	1.00	0.75	1.00	0.50	0.76	1.00	1.00	1.00	1.00	1.00	1.00
116	-	0.50	0.29	-	-	-	-	-	-	-	-	-	-	-	-
119	-	-	-	0.50	-	0.25	-	0.50	0.24	-	-	-	-	-	-
Mb_tri2															
138	0.50	-	0.27	-	1.00	0.50	-	-	-	0.50	0.50	0.39	-	1.00	0.50
144	-	-	-	0.50	-	0.22	-	1.00	0.50	0.50	0.50	0.61	1.00	-	0.50
147	0.50	1.00	0.73	-	-	-	0.50	-	0.43	-	-	-	-	-	-
150	-	-	-	0.50	-	0.28	0.50	-	0.07	-	-	-	-	-	-
Mb_di5															
83	-	-	-	-	-	-	0.50	-	0.28	0.50	-	0.30	0.50	0.50	0.53
89	-	-	-	0.50	-	0.26	-	-	-	-	-	-	0.50	-	0.26
91	0.50	-	0.19	-	0.50	0.24	-	0.50	0.24	-	0.50	0.27	-	0.50	0.21
95	-	-	-	-	-	-	-	0.50	0.26	-	0.50	0.23	-	-	-
97	-	0.50	0.27	0.50	-	0.24	-	-	-	0.50	-	0.20	-	-	-
105	0.50	0.50	0.54	-	0.50	0.26	0.50	-	0.22	-	-	-	-	-	-
Mb_tetra2															
180	-	-	-	-	0.50	0.29	-	-	-	-	-	-	0.50	-	0.28
188	-	-	-	-	-	-	-	0.50	0.20	-	-	-	-	-	-
192	-	-	-	-	-	-	-	-	-	-	-	-	0.50	-	0.22
204	-	0.50	0.25	-	0.50	0.21	1.00	-	0.50	0.50	1.00	0.77	-	1.00	0.50
208	0.50	-	0.31	-	-		-	-	-	0.50	-	0.23	-	-	-
212	0.50	0.50	0.44	1.00	-	0.50	-	-	-	-	-	-	-	-	-
256	-	-	-	-	-	-	-	0.50	0.30	-	-	-	-	-	-
Mb_tri3															
133	0.50	0.50	0.56	1.00	0.50	0.76	1.00	1.00	1.00	0.50	1.00	0.72	1.00	1.00	1.00
142	0.50	0.50	0.44	-	0.50	0.24	-	-	-	0.50	-	0.28	-	-	-
Mb_tri4															
99	0.50	0.50	0.35	1.00	0.50	0.73	0.50	0.50	0.50	0.50	-	0.23	0.50	-	0.116
102	0.50	-	0.35	-	0.50	0.28	-	0.50	0.28	0.50	1.00	0.77	-	0.50	0.186
111	-	0.50	0.29	-	-	-	0.50	-	0.22	-	-	-	0.50	0.50	0.698

M: male; F: female; O: offspring

Appendix 2. Number of alleles per locus for each population

Locus	Uter Lake n=49	Ayamaru Lake n=28	Gusi farm n=34	Sukri farm n=30	Hasan farm n=30	Yahya farm n=30	Didi farm n=29	Warso farm n=30	Total number of alleles
Mb_di1	9	14	8	8	10	9	9	8	19
Mb_di2	13	14	9	8	10	9	9	9	17
Mb_di3	10	8	7	5	7	7	5	6	18
Mb_penta1	6	8	8	7	7	7	6	6	10
Mb_di4	10	15	10	8	11	12	7	13	22
Mb_tetra1	6	7	3	4	6	5	2	3	10
Mb_tri1	4	4	4	3	3	2	2	2	6
Mb_tri2	3	4	4	4	4	5	3	4	6
Mb_di5	6	8	8	9	8	7	6	7	12
Mb_tetra2	10	18	10	11	12	6	6	7	21
Mb_tri3	3	3	3	3	4	2	3	2	5
Mb_tri4	2	4	5	4	4	4	4	4	5

Appendix 3. Allelic frequency for each population

Locus	Uter Lake	Ayamaru lake	Gusi farm	Sukri farm	Hasan farm	Yahya farm	Didi farm	Warso farm
Mb_di1								
87	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
89	0.020	0.036	0.000	0.000	0.000	0.000	0.000	0.000
95	0.357	0.125	0.206	0.183	0.117	0.067	0.017	0.050
97	0.061	0.018	0.044	0.083	0.017	0.000	0.000	0.000
101	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
103	0.000	0.000	0.147	0.050	0.100	0.033	0.000	0.017
105	0.000	0.036	0.000	0.000	0.017	0.000	0.052	0.000
107	0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.000
109	0.378	0.018	0.088	0.067	0.067	0.150	0.103	0.233
111	0.031	0.000	0.000	0.000	0.000	0.033	0.017	0.050
113	0.071	0.250	0.235	0.450	0.433	0.500	0.448	0.367
115	0.010	0.036	0.029	0.000	0.000	0.050	0.069	0.000
117	0.061	0.304	0.221	0.117	0.100	0.050	0.121	0.083
119	0.000	0.018	0.029	0.033	0.117	0.100	0.138	0.133
121	0.000	0.000	0.000	0.017	0.017	0.017	0.000	0.067
125	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
127	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
137	0.000	0.000	0.000	0.000	0.017	0.000	0.035	0.000
139	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
Mb_di2								
113	0.000	0.089	0.029	0.033	0.167	0.033	0.121	0.033
117	0.083	0.000	0.029	0.083	0.067	0.000	0.017	0.000
119	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
123	0.000	0.054	0.059	0.000	0.050	0.000	0.017	0.017
125	0.031	0.018	0.000	0.000	0.000	0.000	0.000	0.000
127	0.021	0.036	0.044	0.083	0.017	0.000	0.000	0.000
129	0.260	0.036	0.029	0.150	0.067	0.083	0.103	0.067
131	0.042	0.054	0.000	0.000	0.017	0.100	0.000	0.133
133	0.010	0.071	0.147	0.100	0.067	0.083	0.052	0.033
135	0.031	0.268	0.279	0.250	0.200	0.300	0.172	0.217
137	0.333	0.071	0.000	0.000	0.167	0.167	0.121	0.217
139	0.115	0.161	0.279	0.267	0.183	0.167	0.345	0.250
141	0.021	0.054	0.103	0.033	0.000	0.033	0.052	0.033
143	0.021	0.036	0.000	0.000	0.000	0.000	0.000	0.000
145	0.000	0.036	0.000	0.000	0.000	0.033	0.000	0.000
147	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
149	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mb-di3								
145	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
153	0.160	0.107	0.061	0.167	0.133	0.100	0.086	0.117

155	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
157	0.000	0.000	0.015	0.033	0.050	0.017	0.000	0.033
159	0.000	0.107	0.000	0.000	0.000	0.017	0.000	0.000
161	0.000	0.179	0.015	0.000	0.017	0.033	0.035	0.017
163	0.000	0.446	0.788	0.650	0.650	0.550	0.603	0.433
165	0.021	0.071	0.030	0.000	0.033	0.033	0.017	0.067
167	0.000	0.018	0.000	0.033	0.033	0.000	0.000	0.000
169	0.011	0.054	0.000	0.000	0.000	0.000	0.000	0.000
171	0.000	0.000	0.076	0.117	0.083	0.250	0.259	0.333
173	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
175	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.000
179	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
181	0.521	0.000	0.000	0.000	0.000	0.000	0.000	0.000
183	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.000
185	0.096	0.000	0.000	0.000	0.000	0.000	0.000	0.000
187	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mb_Penta 1								
160	0.644	0.268	0.368	0.167	0.133	0.017	0.018	0.000
165	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
170	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000
175	0.000	0.000	0.147	0.067	0.083	0.067	0.036	0.069
180	0.000	0.071	0.074	0.100	0.167	0.283	0.286	0.138
185	0.011	0.214	0.044	0.083	0.217	0.217	0.232	0.259
190	0.078	0.232	0.191	0.267	0.200	0.017	0.036	0.138
195	0.167	0.125	0.029	0.033	0.133	0.067	0.000	0.017
200	0.033	0.018	0.132	0.283	0.067	0.333	0.393	0.379
205	0.067	0.000	0.015	0.000	0.000	0.000	0.000	0.000
Mb_di4								
98	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000
100	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
106	0.020	0.000	0.029	0.000	0.000	0.000	0.000	0.000
110	0.000	0.000	0.074	0.000	0.000	0.000	0.000	0.017
112	0.296	0.268	0.324	0.483	0.150	0.433	0.328	0.283
114	0.378	0.143	0.147	0.083	0.283	0.117	0.103	0.150
116	0.051	0.036	0.000	0.000	0.000	0.017	0.000	0.017
118	0.031	0.089	0.044	0.033	0.017	0.017	0.000	0.117
122	0.010	0.000	0.000	0.000	0.017	0.017	0.000	0.000
124	0.143	0.143	0.132	0.217	0.283	0.200	0.276	0.217
126	0.031	0.036	0.015	0.017	0.000	0.050	0.172	0.083
128	0.031	0.054	0.000	0.000	0.017	0.000	0.000	0.000
130	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000
132	0.000	0.018	0.000	0.000	0.017	0.067	0.069	0.033
134	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.017
138	0.000	0.018	0.000	0.017	0.000	0.000	0.000	0.000

140	0.000	0.000	0.162	0.033	0.033	0.017	0.000	0.017
142	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000
144	0.000	0.018	0.000	0.000	0.067	0.000	0.017	0.000
148	0.000	0.018	0.000	0.000	0.100	0.033	0.000	0.017
152	0.000	0.036	0.000	0.000	0.000	0.017	0.000	0.017
164	0.000	0.000	0.059	0.117	0.017	0.017	0.035	0.017
Mb_Tetra 1								
131	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000
135	0.010	0.036	0.000	0.000	0.017	0.000	0.000	0.000
137	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.000
139	0.184	0.036	0.000	0.000	0.000	0.000	0.000	0.000
143	0.735	0.607	0.779	0.717	0.741			

Mb_Tetra2

156	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000
160	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000
168	0.000	0.000	0.044	0.017	0.033	0.000	0.000	0.052
172	0.010	0.054	0.000	0.000	0.000	0.000	0.000	0.000
176	0.010	0.036	0.000	0.000	0.000	0.000	0.000	0.000
180	0.020	0.196	0.338	0.207	0.200	0.133	0.155	0.241
184	0.214	0.054	0.118	0.017	0.017	0.000	0.000	0.000
188	0.133	0.089	0.088	0.086	0.050	0.000	0.000	0.000
192	0.388	0.161	0.015	0.052	0.017	0.000	0.000	0.000
196	0.092	0.071	0.044	0.017	0.017	0.067	0.000	0.000
200	0.071	0.018	0.029	0.035	0.050	0.033	0.017	0.017
204	0.010	0.071	0.162	0.310	0.300	0.283	0.379	0.293
208	0.000	0.071	0.000	0.121	0.217	0.383	0.259	0.276
212	0.000	0.036	0.132	0.121	0.033	0.100	0.172	0.035
220	0.000	0.036	0.000	0.000	0.017	0.000	0.000	0.000
224	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
228	0.000	0.018	0.000	0.017				