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Breeding development in captivity of theAmazon killifish (*Anablepsoides* sp) from the Pastaza River basin with *Panagrellus redivivus* as live food.

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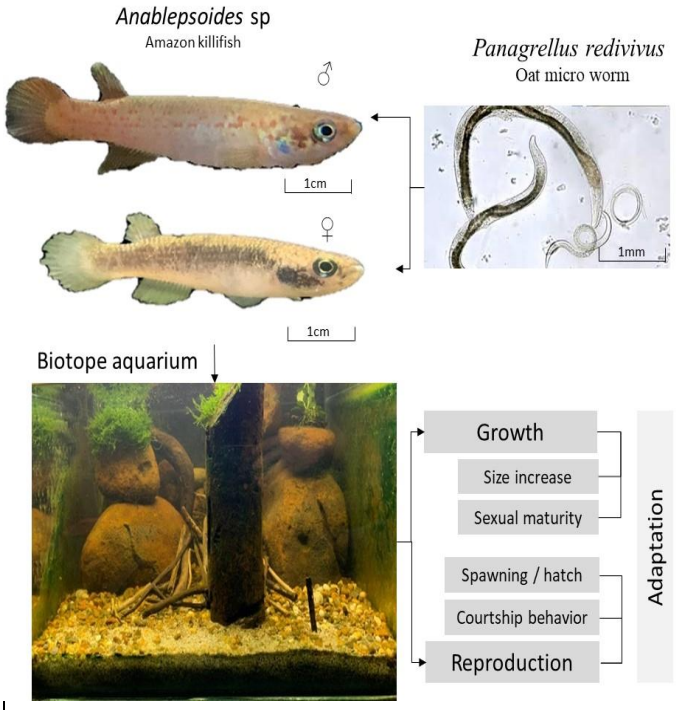
Graphical Abstract



Abstract

In this study we analyzed the breeding development of the local Amazon killifish from Pastaza basin highlands, genus *Anablepsoides sp*. in Ecuador. This species inhabits the foothills freshwater ecosystems, however, information about their populations is still unknown. Because of their bright colors, *Anablepsoides* sp harbours potential for ornamental aquaculture. The goal of this study was to analyze the breeding development of *Anablepsoides* sp with oat micro worms (*Panagrellus redivivus*) as a live food source.

Five individuals were caught in a stream from the Puyo River basin near to the Union Base community. Fish were maintained in a biotope aquarium for 18 weeks at 23.5°C, pH 7.3, and were fed exclusively with micro worms. These conditions were checked continuously throughout the experiment. Furthermore, we took biometric measurements and we examined whether individuals reached sexual maturity. Breeding behavior, if present, was systematized through an ethogram.

Our results show that the Amazon killifish adapted to artificial biotope conditions. This was reflected with an increase in size from SL 30,4±5,5 and TL 34±6,36 to SL 34,80±5,26 and TL 39,60±7,13. Males are bigger reaching greater sizes (SL=44mm and TL=52mm) than females (SL=34mm and TL=39mm). In terms of courtship, a single male engaged in female persecution. Two females exhibited aggression by persecuting other females. A single female had a spawning event were two fry hatched.

Overall, our results suggest that *P. redivivus* is an adequate nutrient source for *Anablepsoides* sp because it enabled fish adaptation to captivity, growth and breeding.

Introduction

Anablepsoides sp. (Cyprinodontiformes: Rivulidae) inhabit small lacustrine aquatic environments, with a depth from 1 to 50 centimeters at eastern Andean foothills in Ecuador. The specie's comfort ranks consider temperature between 20 and 24 °C, pH 4,1 to 6,5, and total hardness (TH) less than one. The killifish can be support the wild inclemency for a wide hard variable conditions as precipitation, altitude, and light (Silva, 1991).

Taxonomic and demographic information is scarce; actually only occurs 3 species records in Ecuador, *A. limoncochae*, *A. montícola*, and *A. urophthalmus*, (Barriga, 2012). There's also new species possibility, due to the low ichthyofauna research in the country. Moreover, the null information on captivity adaptation issues as reproduction, feeding and health. The genus *Anablepsoides* is used in other world places as an ornamental fish for its striking colors and how possible biological control agents as mosquitoes' larvae and pupae predator (Garcia et al., 2019). In relation with the reproduction issues, actually is known that this animal has a continuous throughout annual cycle.

The goal of this study was to analyze the breeding development of *Anablepsoides* sp with oat micro worms (*Panagrellus redivivus*) as a live food source. An optimal fish growth could contribute to facilitate the adaptation to the commercial food and their breed in captivity would have a high potential to increase income for small farmers of the Amazon region.

Materials and Methods

Catch area description. The Puyo River is an Amazon tributary located in the Ecuadorian Andean foothills, between 1500 to 900 masl. Studies of the water quality of this lotic ecosystem suggest that it conserves acceptable parameters for recreational use and the development of local flora and fauna (Rodríguez-Badillo, 2013), however, the basin is affected by changes in land use and pollution triggered by the urbanization process. Five wild individuals of *Anablepsoides sp*. were captured in a stream next to the Unión Base community, with the follow physical-chemical characteristics: pH 6,1; O_2 7,17 mg/l; temperature 21.30 °C and a total solid dissolved 6 ppm.

Biotope maintenance and feeding. Five individuals were kept in captivity in a 60 liters aquarium, in which be done a perfectly imitation of their natural habitat with elements of their environment, such as rocks, sand, wood stalks and plants, thus the specimens have all the elements for optimal development. The container had a stable water temperature of 23,5±1 °C, 7,3 pH and a <25 ppm total solids dissolved. The animals were maintained with oat micro worms live food source for 18 weeks. *Panagrellus redivivus*, have a high nutritional value with averages values as 47,3%, protein, 23,5% lipids and 23,2% carbohydrates (Luna Figueroa, 2009).

Behavioral follow-up. For the behavior recording, was made an ethogram, as observational tool, according to Ewing (1975) scheme. Ethological components of *Anablepsoides* sp were distinguished following activities description as: Rest (R); Swimming (S); Small movements (SM); Follow-up (FU); Away (A); No escape by mating (NEM); Copulation / spawning (CS); Parental care (PC); Feeding (F). Each observation was realized once per week, after the six week, when the fish shows sexual differentiation as indicator breed maturity (Schalk, Montaña, & Libson, 2014)..

Results and Discussion

Growth performance. *Anablepsoides sp.* was adapted to an artificial habitat, thanks to the biotope type that allowed to reduce the stress of captivity, as well as the use of live food that helped in the absence of an interruption of development, in this way is possible observe the increase in size, and a sexual dimorphism (Infante-Rivero, 2018). In general males are bigger than females.

The table 1, shows the initial Standard Length (SL_i) when individuals were extracted from nature, all female were juveniles while the unique male was an adult. After 18 weeks, the killifish fed with *P. redivivus* shows an average increase of 4,40 mm evidencing the efficiency of live food in size increase and over sexual development, reaching an average SL_f of 34,80 mm resulting in an adult reproductive stage.

Table 1, *Anablepsoides* sp Standard Length (SL) growth in 18 weeks captivity

Indv	Sex	SL_i (mm)	SL_f (mm)	Increase (mm)	Growth (%)	Growth rate (mm/week)
1	F	30	34	4	13,3	0,22
2	F	27	31	4	14,8	0,22
3	F	28	33	5	17,8	0,27
4	M^A	40	44	4	10	0,22
5	F	27	32	5	18,5	0,27

The table 2 indicates that the initial Total Length is 34±6,36 and the final is 39,60±7,13. With 5,60 mm difference, due that the male's tail is more bigger and colorful than the females or hermaphrodites ones. Resulting evident that the male use his tail to draw attention at the moment of courtship and copulation.

Table 1, *Anablepsoides* sp Total Length (TL) growth in 18 weeks captivity

Indv	Sex	TL_i (mm)	TL_f (mm)	Increase (mm)	Growth (%)	Growth rate (mm/week)
1	F	34	39	5	14,7	0,27
2	F	30	35	5	16,6	0,27
3	F	31	37	6	19,3	0,33
4	M	45	52	7	15.5	0,38
5	F	30	35	5	16.6	0,27

Behavior observations. Table 3 shows that *Anablepsoides* sp is a diurnal species, with a marked male / female / hermaphrodite differences of activity, that implies a greater energetic loss in the male owed is vehement in chasing all the females. The hermaphrodites present a male similar behavior in which they follow the other females, but only they calm down before the courtship of the male and are predisposed to the copulation and spawning of eggs. Finally, it was evidenced that they do not present parental care of the fry, being these totally independent from the beginning of their life (da Silva Goçalves, Souza, & Volcan, 2011).

Table 3, *Anablepsoides* sp ethogram in group captivity

Behavioral elements	**Observation period**					
	Diurnal			**Nocturnal**		
	♂	♀	⚥	♂	♀	⚥
Rest (R) The statics of the fish, the slow movement of the fins						
Swimming (S) Displacement of the body with the help of its fins as propulsion						
Small movements (SM) Subtle movements of the body with the help of its fins						
Follow-up (FU) Pursuit by courtship						
Away (A) Flight before persecution						
No escape by mating (NEM) Consent to persecution and courtship						
Copulation of eggs, spawning (CS) Moment when female copulates and lays eggs for subsequent male fertilization						
Parental care (PC) Parental care of juveniles exists						
Feeding (F) Action of chasing *Panagrellus redivivus* for food						

Conclusions

It was demonstrated that the use of *P. redivivus* as a food supplement is efficient for the reproductive development of *Anablepsoides sp.* fish, since this food proves that its high nutritional value is very useful for the adaptation of animals to captivity together with an alternative such as the biotope aquarium, it was achieved the reproduction of fish that have an ornamental value, disease vector control and also allows the conservation of vulnerable and poorly studied species that predominate in sensitive habitats.

With this study is possible to make a contribution for a productive point of view that requires more scientific information on aspects such as nutrition, reproduction and growth in controlled conditions.

The reproductive development and the spawning achieved mark the beginning of future research in Amazonian ornamental fish with economic importance, showing the viability of reproducing organisms in aquatic Amazonian biotopes conditioned with equivalence to their autochthonous ecosystem. The results obtained could be used for the rivulids conservation at the Neotropic.

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