Breeding and domesticating *Brycon siebenthalae* females for reproduction

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ABSTRACT. *Brycon siebenthalae* (yamú) (Characidae) have quite an untamable "nature", with intense, enduring responses to stress. Three groups of females were induced to spawn in order to study the effects of manipulation during breeding. Groups one and two were formed by four year-old specimens bred in earth ponds. Females from group one and two were subjected to monthly and anual "domestication" practices, respectively, from the time they were six months old. The third group of females was brought from the natural environment. It was observed that females bred and monthly tamed had better induction responses, relative fecundity and post-spawn survival. The physiological responses to different stress situations during the reproductive procedure did not significantly affect ($p>0.05$) fertility and embryonic and larval survival among groups.

Key words: *Brycon siebenthalae,* domestication, reproduction, yamú.

RESUMO. Criação e domesticação de fêmeas de *Brycon siebenthalae* destinadas a reprodução. *Brycon siebenthalae* (yamú) (Characidae) tem uma natureza indomável, apresentando respostas ao estresse intensas e duradouras. Três grupos de fêmeas foram tratados com hormônios gonadotróficos para induzir a maturação final e desova, buscando avaliar o efeito da manipulação das fêmeas durante o período de maturação gonadal. O primeiro e segundo grupo eram formados por peixes de quatro anos de idade criados em viveiros de terra. As fêmeas do primeiro grupo foram submetidas ao manejo mensal de "domesticação" a partir do sexto mês de idade. O segundo grupo foi submetido a esse manejo com freqüência anual. O terceiro grupo de fêmeas foi capturado do ambiente natural. As fêmeas submetidas ao manejo mensal apresentaram melhores resultados referente a resposta ao tratamento hormonal, a fecundiade relativa e a sobrevivência após o manejo da reprodução. Os distintos procedimentos de manejo das fêmeas durante a maturação gonadal não afetaram ($p>0,05$) a taxa de fertilização e a sobrevivência dos ovos e larvas provenientes dos diferentes grupos.

Palavras-chave: *Brycon siebenthalae,* domesticação, reprodução, yamú.

Introduction

It is generally recognized that man has not completely domesticated species in aquaculture like in other livestock activities. It is very important for the production of any species that it tolerates manipulation as the starting point of normal reproduction practices, as well as of new tendencies of genetic improvement and industrial explotation. Domestication can be understood as a set of periodic manipulation activities of fish with views to reduce altered behavioral and homeostatic responses (Schreck *et al*., 1997). It is expected that, after some time, the domestication practices will decrease stress; in other words, they will reduce the intensity and duration of primary hormonal hyper-secretions of catecholamines and cortisol (Mazeaud *et al*., 1977; Donaldson, 1981; Sumpter, 1997). The cascade of metabolic events produced as secondary response to stress is very variable and depends on multiple factors, all pointing to the reestablishment of the animals' normal behavior, with significant expense of energy. Hormonal functions, gonadal development stage and nutritional conditions in mature specimens will add up to modulate the intensity and duration of physiologic responses to stress (Wendelaar-Bonga, 1997).

Brycon siebenthalae (yamú) is a species with great potentialities for fishfarming in continental warm waters, specially for its growth speed and feed conversion capacity (Arias, 2001). However, its

particularly nervous "nature" generates great stress before any stimulus, with intense, enduring physiological responses (Arias, 2002). To evaluate the manipulation effects during reproductive induction, different indicators of reproductive performance were measured in yamú females.

Material and methods

Three groups of females were used: groups one and two (G1 and G2), formed by four year-old specimens, obtained from induced breeding, kept in earth ponds and fed with 3% of the biomass/day, six days/week. Food offered was commercial feed with 30% crude protein and 3,000kcal /kg gross energy. Stocking density was 300g of live weight/m^2. Six month-old fingerlings from G1 were monthly submited to taming procedures consisting of capture, manipulation (manual domination) and later liberation at the pond. Fingerlings from G2 were annually captured but not manipulated. Females of group three (G3) were obtained from natural environment and induced 15 days after captured.

Experiments were carried out in the Fishfarming Station of the Aquaculture Institute of Los Llanos University (4º 05' N y 73º 37' O), during April 2001. Five females of each group were selected for each experiment from their external reproductive maturity characteristics (protuberant abdomen and reddish genital papilla) according to Woynarovich and Horvath (1983). They were then selected by measurements of the ovocytes's diameter (minimum average 1,100µm) and the percentage distribution of final phases of nuclear oocyte migration (50% as minimum average value of migrating nuclei), from oocytes obtained through cannulation, according to Harvey and Caroslfeld (1993). Three selected females of each group in each occasion were simultaneously induced to ovulate with Carp Pituitary Extract (previous dose of 0.25mg / kg, 1st dose of 0.5mg / kg after 24 hours and 2nd dose of 5mg / kg after 12 hours), according to Pardo-Carrasco *et al*. (1998). Environmental conditions during the inductive procedures were: temperature 26.3 ± 0.2°C, pH 6.1 ± 0.3 and dissolved oxygen 6.3 ± 0.2mg / L. Spawn were obtained by abdominal pressure and dry fertilized with semen of 80% minimum espermatic motility. For each spawn were registered the total weight and the quantity of oocytes per gram. Oocytes diameters of those samples were measured and evaluated. Percentages of fertility and embryonic survival were measured six and ten hours after fertilization, respectively. Larvae produced in each spawn were counted twelve hours after hatching. Female survival after induction was registered eight days after spawn. The results are reported as average ± standard error. Compared averages among groups were done through ANOVA and Tukey-Kramer test in all cases, with significant $P<0.05$.

Results

Results obtained for each of the reproductive performance indicators for the three studied groups of females are presented in the Table 1. In G1, 92% of specimens monthly manipulated responded to induction. In G2, 89% of females annually captured ovulated successfully, 33% of G3 females coming from natural environments responded to induction. Similar behavior was registered in post-induction survival: 83%, 78% and 17%, respectively.

Females from group G1 presented mean weight of spawns and oocytes diameters smaller than those of the other two groups. While absolute fecundity showed significant differences ($P<0.05$). Fertility percentages and embryonic survival were not significantly different. Although manipulated females (G1 and G2) produced larger number of larvae per spawn than wild females (G3), there were no differences among the groups.

Table 1. Indicators of reproductive acting of three groups of females of *Brycon siebenthalae* with different manipulation.

Groups [1]	No. [2]	Weight (Kg)	Weight spawns (g)	Number oocyte / (g)	Absolute fecundity	Relative fecundity [3]	Oocyte diameters (µm)	Fertility (%)	Embryonic survival (%)	Number of larvae spawn	Post-induction survival (%)
G1	11/12	2.19 [a]	169.5 [a]	1409 [a]	238,248 [a]	107,560 [a]	1,263 [a]	63 [a]	56 [a]	76,091 [a]	83
		±0.04	±8.4	±15	±11,012	±3,188	±21	±6	±8	±19,193	
G2	8/9	2.62 [b]	238.2 [b]	1212 [b]	289,237 [b]	108,131 [a]	1,389 [b]	77 [a]	69 [a]	131,250 [a]	78
		±0.11	±11.9	±19	±17,316	± 3,119	±20	±5	±5	±21,687	
G3	2/6	2.07 [a]	178.5 [ab]	1252 [b]	223,168 [ab]	110,198 [a]	1,494 [b]	43 [a]	43 [a]	60,750 [a]	17
		±0.03	±8.5	±37	±4,038	±633	±84	±28	±42	±59,250	

1. G1: monthly domestication, G2: yearly capture, G3: natural environment. 2. Number of females that responded positively / number of subjected females to treatment of hormonal induction. 3. Absolute fecundity / Weight of female. Among groups, different letters in line indicate significant differences ($p < 0.05$).

Discussion

Domestication, as an adaptation procedure for fishes to handling routine practices may result, after some time, in less altered behavioral and homeostatic responses (Schreck *et al*., 1997; Schreck, 2000) and, later on, after successive generations in confinement, in stable and normal answers to manipulation and reproductive induction, as reported for *Ictalurus punctatus* (Plumb *et al*., 1975) and *Oncorhynchus mykiss* (Ayles and Baker, 1983).

Decrease of stress during manipulation means the reduction in intensity and duration of catecholamines

and cortisol secretions, considered as physiologic primary responses of fish to any disturbing agent (Donaldson, 1981; Schreck, 1981; Cech *et al*., 1996; Sumpter, 1997; Pottinger and Carrick, 1999). This reduction of initial hormonal hyper-secretions as consequence of higher tolerance to domestication stimuli may end up generating less metabolic changes as secondary responses to stress (Pickering, 1981; Balm, 1997). Secondary responses to stress, such as increase of blood glucose and hepatic glycogenesis (Pickering, 1981; Vijayan and Moon, 1992; Barton, 2000), changes in hematology values (Mazeaud *et al*., 1977; Carneiro, 2001), blood electrolytic and osmo-regulation alterations (Eddy, 1981; Camichael *et al*., 1984; Cech *et al*., 1996; McDonald and Milligan; 1997) and depression in the immune system (Balm, 1997; Carneiro, 2001), vary and depend on multiple factors. Some of this factors may be the species, origin, age, sex and singular physiological conditions of the animals, among others (Mylonas *et al*., 1998; Pottinger and Carrick, 1999). In reproductive active specimens, the hormonal activity, gonadal development stage and nutritional conditions also have influence (Lam, 1985; Pankhurts and Van der Kraak, 1997; Wendelaar-Bonga, 1997), as well as the stressor type and severity (Donaldson, 1981; Pickering, 1981; Sumpter, 1997; Barton, 2000).

Both the response to hormone inductor and survival of post-induction breeders were larger in the group of females subject to monthly manipulation practices (G1).

During breeding, these females could have acquired more tolerance to different stress stimuli and, therefore, showed less intense and less durable physiologic responses, reflecting better responses to inductive reproduction as discussed by Pickering (1981) and Morgan and Iwama (1997). More precisely, the decrease of negative effect of cortisol on gonadotropic hormones and other hormones involved in the oocytes growth and maturation, which is a consequence of domestication, did not affect the hormonal activity as discussed by Wendelaar-Bonga (1997), Sumpter (1997) and Pottinger and Carrick (1999). Or, as proposed by Schreck *et al*. (2001), the females would present a "progeny-protecting system", as a mechanism through which they would use an alternating metabolic way for the excessive cortisol produced by periodic stress, avoiding negative effects on the reproductive period.

The inverse relationships registered between oocytes diameters and number of oocytes per gram in the groups and the differences in diameter and quantity of eggs per gram among the groups, as well as the relative fecundity, seemed not to have been affected by stress responses during induction procedures in none of the groups. Contreras-Sánchez *et al.* (1998) found similar results in *Oncorhynchus mykiss* submitted to moderate stress during vitellogenesis. The results obtained in this study can be attributed to other factors such as: stress due to confinement, affecting the quality and size of oocytes during maturation (Foo and Lam, 1993; Campbell *et al*. (1994); Sumpter *et al*. (1994); Pankhurts and Van der Kraak, 1997), and/or the environmental quality of contention ponds (Schreck, 1981; Wendelaar-Bonga, 1997) and/or the nutritional conditions of females (Watanabe, 1990; Izquierdo *et al*., 2001), specially the accumulation of lipides in vitellogenesis (Wiegand, 1996). On the other hand, wild females capable of overcoming stress during the procedure have responded to induction and produced eggs of bigger diameter. This may support the possibility of little influence of physiological responses to stress on oocytes during induction procedures and the advantage of natural nutrition regimes of wild females (Vásquez, 1994).

The embryonic and larval survival had values that were not significantly different among groups and were similar to those found by Contreras-Sánchez *et al*. (1998). However, it is possible that physiological responses to stress might have affected ontogenesis and larval development in a different way, as much as the nutritional conditions of females (Lam, 1985; Foo and Lam, 1993; Campbell *et al*., 1994), and/or the incubation of eggs and manipulation of larvae, also affecting reproductive products (Laine and Rajasilta, 1999; Schreck, 2000).

There were observed several responses, of different intensity and duration, of the different stress stimuli generated to yamú, which were similar to those registered by Ruzzante (1994) and Schrek *et al*. (1997) to different marine fish species. Behavioral responses as the result of domestication activities, such as escape, struggle and aggression, were visible responses to stress that showed that females from G1 were better adapted to manipulation at the time of induced reproduction than females from the other two groups. Domesticated females from G1 exhibited less evasive and resistent behaviors to manipulation and were, in general, more tolerant to handling than those from the other groups (Ruzzante, 1994).

Domestication, as known in other animal species, is not possible in fish (Barnabé *et al*., 1996). We can, therefore, expect that animals subject to manipulation activities, such as those mentioned in this study, may not acquire tolerance to periodically produced stress and therefore may not respond to induction treatments. In spite of that, the "domesticated" females in this study showed better results than the others. Some necropsies and observation carried out in the females that died after induction suggests that the death was caused by osmoregulatory disfunction, one of the main causes of death from acute stress (McDonald e Milligan, 1997).

Conclusion

In terms of inductor's responces, number of occytes per weight of female and post-induction survival, it was observed that the group of females "domesticated" monthly had better responses than the groups of females with annual manipulation and of wild females. Physiological responses to different stress factors during the reproductive procedure did not affect fertility, embryonic survival or larval survival.

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