Effect of food restriction in spawning of yamú females *Brycon siebenthalae* (Osteichthyes, Characidae)

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ABSTRACT. Under natural conditions, many fish species, such as freshwater tropical reofilics, voluntarily restrict their food supply during the period before reproduction. Mature females fed with commercial food were divided in two groups four months before their reproduction period. One group was fed with 3% biomass/day of food, while for the other group food was restricted 50% of that amount. During two consecutive reproductive cycles females from both groups were induced to spawn, and the reproductive performance and visceral fat index were compared as well. Females from restricted treatment showed better results after hormonal injection and produced larger eggs after spawning, although the visceral fat index was significantly smaller ($P<0.05$). The spawn weight, fertility and embryonic survivals were not different among groups during the two years.

Key words: *Brycon siebenthalae,* food restriction, visceral fat index, reproduction.

RESUMO. Efeito da restrição alimentar sobre o desempenho reprodutivo das fêmeas de yamu *Brycon siebenthalae* (Osteichthyes, Characidae). Algumas espécies de peixes, tais como os reofílicos tropicais, apresentam uma restrição alimentar voluntária durante o período pré-reprodutivo, quando se encontram no ambiente natural. Fêmeas maduras alimentadas com ração comercial foram divididas em dois grupos quatro meses antes do período reprodutivo. Um grupo foi alimentado com o equivalente a 3% da biomassa/dia, enquanto o outro recebeu a metade desta quantia. Fêmeas originárias dos dois grupos foram induzidas à desova durante dois períodos reprodutivos simultâneos, quando foi comparada a performance reprodutiva e o índice de gordura visceral. Fêmeas provenientes do tratamento com restrição alimentar mostraram melhores resultados após o tratamento de indução hormonal, produzindo óvulos maiores na desova, enquanto que o índice de gordura visceral foi menor ($P<0,05$). A fecundidade absoluta foi menor no grupo de fêmeas restritas no segundo ciclo reprodutivo. O peso da desova, a taxa de fertilidade e de sobrevivência dos embriões foi semelhante entre os dois grupos.

Palavras-chave: *Brycon siebenthalae,* restrição alimentar, índice de gordura visceral, reprodução.

Introduction

Induced reproduction of confined reofilic fish is fundamental in providing seeds for commercial cultivations. Artificial as well as natural breeding depends in great measure on nutritional state of breeders, and in case of captive animals its subordinated to quality of portions and feeding practices implemented.

In natural environments, many species fast during the period that precedes its reproduction (Vazzoler, 1996). During this period it uses fat reserves to cover its metabolic needs (Lowe-McConnell, 1999) and in case of females, additional energy is required for the eggs (Sumpter *et al.*, 1991). Fasting, in addition to the migratory activity that many fish species realease, drives them to reach maximum reproduction capacity (Wallace and Selman, 1981; Potts and Wootton, 1984; Townshend and Wootton, 1984).

Based on previous observations, studies on food restriction of fish destined to breeding have been carried out. Answers have been diversed and contradictory. Some have registered similarities or minor differences between restricted and non restricted animals (Townshend and Wootton, 1984; Carvalho, 2001). Others have shown differences in fecundity and/or size of eggs and the relationship between them (Reimers *et al.,* 1993; Hopkins and Unwin, 1997). And a few have listed the eventual differences of performance with the amount of visceral fat. Regarding yamú, a native species of the Orinoco River basin, its known that it realease fasting and reproductive migration (Arias, 1995). In this study, females of this species were induced to spawn after a 50% food restriction during four months before their reproduction, during two consecutive breeding cycles, relating its performance with visceral fat index comparing them with non restricted females.

Material and methods

During April and May of years 1999 and 2000 at Fishery Station of Aquaculture Institute of Universidad de Los Llanos, a total of 89 yamú females were induced to spawn with carp pituitary extract (previous dose 0.25 mg/kg 24 hours interval, 1st dose 0.5 mg/kg 12 hours interval, 2nd dose 5 mg/kg) according to Pardo-Carrasco *et al.* (1998).

During four months before induction, a group of breeders (females with mean weight of 1.49 ± 0.1 kg for 1999 and 1.76 ± 0.3 kg for 2000), that had been kept at a 300 g/m^2 and fed daily at a rate of 3% the corporal weight, six days a week with a commercial ration of 30% crude protein and 3,000 kcal/kg of gross energy, was aleatorily separated in two groups. Maintaining the density, ration and type of diet in experimental group (R), diet was restricted to 50% (it was fed three days a week). Control group (C) continued to receive diet six days a week.

During reproductive cycle, females were pre-selected from each group per its external characteristics of reproductive maturity according to Woynarovich and Horvath (1983). Selected by measurement of oocyte's diameters (minimum media 1,100 μm) and percentage of final phases of ovocitary nuclear migration (minimum media 50% of migrating nuclei), obtained through cannulation (Harvey and Carolsfeld, 1993). Environmental conditions during reproductive procedures were as follows: temperature 26.3 ± 0.2ºC, pH 6.1 ± 0.3 and dissolved oxygen 6.3 ± 0.2 mg/L. Ovulations occurred six hours and 40 minutes ± 18 minutes at 26.7ºC after last inducing dose. Eggs were obtained by stripping and fertilized in dry with semen of 80% minimum mass motility. Every spawn was weighted, the quantity of eggs per gram was determined and samples of oocytes were collected to evaluated its diameters. Six and 10 hours after fecundation fertility percentages and embryonic survival were measured, respectively. Six females per group per year were sacrified in the reproductive cycle to estimate the gonadossomatic index (GSI) and the visceral fat index (VFI), calculated through percentage relationships of ovary weight and visceral fat with the total weight, respectively. Results were analysed by t-test at a significance level of $P < 0.05$.

Results

Table 1 shows the results of reproductive performance obtained in the two cycles for the two groups. A no significant ($P<0.05$) weight loss was registered in experimental group in both years during the four months restriction. Response to inductor was larger in experimental group (85%) for both years versus 73% and 63% in control group for 1999 and 2000, respectively. On the first year, there were no significant differences ($P<0.05$) between groups in none of the utilized indicators with the exception of VFI which was significantly smaller in experimental group. On the second year direct performance indicators, number of eggs per gram, absolute fecundity and VFI were significantly smaller for experimental group.

In Figure 1, pre and post ovacitary induction diameters of restricted and non restricted females for the two years were graphed. In all cases, due to inductor's effect, an increase in diameters of oocytes were registered. Comparison among groups show significant differences between the post induction oocytes diameter, being oocytes of restricted females ($P < 0.05$) bigger for both years.

Table 1. Reproductive performance of *Brycon siebenthalae* females in the food restricted and not restricted groups.

Year	Group[1]	[Δ]Response (%)	Weight (Kg)	Weight spawns (g)	Nº Eggs /g	Absolute Fecundity	Fertility (%)	Embryos Survival (%)	GSI	VFI
1999	R n* =20	85	1.46^{a} ± 0.11	108.3^{a} ± 11.1	1458^{a} ± 52	178,433^{a} ± 18,386	64.0^{a} ± 7.0	63.2^{a} ± 8.3	7.6^{a} ± 0.3	1.95^{a} ± 0.06
	C n* = 22	73	1.49^{a} ± 0.12	102.7^{a} ± 10.1	1479^{a} ± 46	169,654^{a} ± 11,395	64.4^{a} ± 7.0	60.9^{a} ± 6.9	8.0^{a} ± 0.3	2.24^{b} ± 0.12
2000	R n* =27	85	1.66^{a} ± 0.08	89^{a} ± 12.6	1744^{a} ± 42	153,641^{a} ± 21,440	76.2^{a} ± 5.3	65.9^{a} ± 5.8	8.5^{a} ± 0.2	2.03^{a} ± 0.07
	C n* =30	63	1.81^{a} ± 0.07	136.2^{b} ± 14.1	1597^{a} ± 66	223,748^{b} ± 28,346	70.6^{a} ± 6.1	60.2^{a} ± 6.0	9.3^{a} ± 0.1	2.48^{b} ± 0.03

[1]R= Restricted experimental group; C = Not restricted control group; n* = total number of induced females; [Δ]Response % = Percentage of positive spawn of the induced females; Among lines, different letters indicate significant differences ($P < 0.05$).

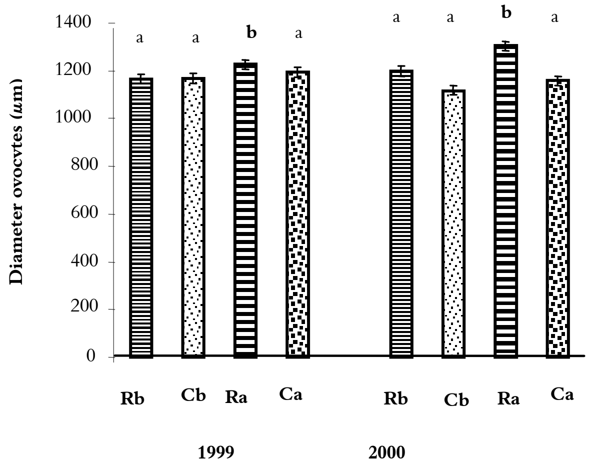

Figure 1. Effect of food restriction on the diameter ovocytes (μm) in yamú females during two reproductive periods. (Rb = Restricted before induction. Cb = Not restricted before induction. Ra = Restricted after induction. Ca = Not restricted after induction). Different letters on the columns indicate significant differences ($P < 0.05$)

Discussion

The first effect of food restriction during the four months of experiments was no significant decrease of corporal weight. Knox *et al.* (1988) in *Salmo gairdneri*, Karlsen *et al.* (1995) in *Gadus morhua* and Carvalho (2001) in *Brycon cephalus* registered similar behaviors.

Kadri *et al.* (1995), Karlsen *et al.* (1995) and Duston and Saunders (1999) discussed the weight loss during the restriction and pointed out that such loss could be due to greater energetic expense of vitellogenesis. The little difference in weight loss reported in restricted females of different species has been attributed to compensatory type physiologic mechanisms (Souza *et al.*, 1997). Farbridge *et al.* (1992) and Zamal and Ollevier (1995) found increase of water content in tissues as compensation for fat reduction during food restriction of *Clarias gariepinus* and *Salmo gairdneri*, respectively.

Moderate (50%) and short (four months) restriction during pre-reproductive cycle did not affect normal growth of yamú's ovaries. It was deduced from the comparison of gonadosomatic indexes between groups of both years which did not show significant differences. Ridelman *et al.* (1984) in *Salmo gairdneri* and Coward and Bromage (1999) in *Tilapia zillii* did not find differences in GSI of restricted and non restricted fishes. In *Salmo gairdneri* (Ridelman *et al.*, 1984) and in *Salmo salar* (Duston and Saunders, 1999), found that moderate pre-reproductive restriction did not affect gonadal development of females of these species.

Townshend and Wootton (1984) in *Cichlasoma nigrofasciatum,* Jobling *et al.* (1993) in *Salvelinus alpinus* and Carvalho (2001) in *Brycon cephalus*, also reported that moderate but long restriction did not affect gonadal development. On the other hand, species of salmonids restricted with different intensity before vitellogenesis had their gonadal maturity affected (Bagenal, 1969; Thorpe *et al.*, 1990; Reimers *et al.*, 1993; Duston and Saunders, 1999). Contradictions in the results of food restriction in fish presented by bibliography can be due to several factors such as: species, origin, age and physiological, hormonal and environmental conditions (Foster and Moon, 1991; Vijayan and Moon, 1992; Wendelaar-Bonga, 1997; Pottinger and Carrick, 1999; Schreck, 2000). Additionally, in case of breeders, gonadal development and broodstock nutrition must be considered (Wallace and Selman, 1981; Townshend and Wootton, 1984; Lam, 1985; Kadri *et al.*, 1995; Izquierdo *et al.*, 2001). Response to inductor was significantly bigger in both years for restricted females. Pardo-Carrasco *et al.* (1998) for the same species in normal conditions have registered induction responses 78% smaller than those found in this study. This indicates that food restriction does not affect response to inductor and rather improves it. Karlsen *et al.* (1995) in *Gadus morhua* and Ali and Wootton (1999) in *Gasterosteus aculeatus* did not find that pre-reproductive food restriction affects response to inductor in those species.

In the fist reproductive cycle, the weight of spawn and the number of eggs obtained in both groups were similar. In the second cycle, a significant decrease on weight of spawn of restricted females was observed. Burton (1994) concluded that similar results in flounder *Pleuronectes americanus,* were product by nutritional pre-vitellogenesis state more than pre-reproductive food supply. In this same order, Kadri *et al.* (1995) assured that, in *Salmo salar,* during reproductive migration natural anorexia depends on specific storage of corporal reserves and therefore, the effect of pre-reproductive restriction in confinement is not significant. The number of eggs per gram for the second cycle increased in both groups, being larger, but not statistically significant, in experimental group. As a consequence of the last two indicators, a significantly larger absolute fecundity was calculated for the control group in the second cycle. Townshend and Wootton (1984) found a significant decrease in fecundity of restricted females during vitellogenesis. Similar results were found by Billard and De Fremont (1980) and Knox *et al.* (1988) in *Salmo gairdneri* and Karlsen *et al.* (1995) in *Gadus morhua*. Ali and

Wootton (1999) did not find that pre-reproductive restriction affected the fecundity in *Gasterosteus aculeatus*.

In other species, a direct relationship between food restriction and fecundity and an inverse relationship between food restriction and size of eggs has been reported (Bagenal, 1969; Billard and De Fremont, 1980; Townshend and Wootton, 1984). In this work, it was found that food restriction diminished fecundity but did not affect diameter of oocytes in the second cycle, particularly. Coward and Bromage (1999) in *Tilapia zillii* found that restricted and not restricted animals during 18 months had similar egg sizes but had diminished fecundity similar to that reported in *Salmo gairdneri* (Knox *et al.*, 1988). Ridelman *et al.* (1984) restricting up to 50% during 40 days previous to spawn in *Salmo gairdneri* and Ali and Wootton (1999) in *Gasterosteus aculeatus* females restricted for 56 days before spawn, did not find differences in fecundity and diameter of oocytes between groups of these two species. This effect describes the variety of responses, besides those already mentioned, depending on the intensity, form, duration and time in which food restriction is applied.

Transfer of visceral fat to gonads during vitellogenesis was experimentally proven in *Brycon* species (Zaniboni-Filho, 1985; Romagosa, 1998; Carvalho, 2001; Zaiden, 2000). Similar process occurs in natural environments to *B. cephalus* (Zaniboni-Filho, 1985) and to *B. siebenthalae* (Arias, 1995). Differences in increased oocyte diameter in post-induction of restricted and non restricted yamú females could be explained by the smaller amount of fat in the abdominal cavity found in experimental group of females that could have moved greater visceral fat reserves towards ovaries than those of control group. The diminution of VFI during vitellogenesis process as a result of visceral fat transfer to ovaries was registered (Hunter and Leong, 1981; Oliveira *et al.*, 1997) and as effect of food restriction (Zamal and Ollevier, 1995; Souza *et al.*, 1997). Estimated VFI was significantly lower in the experimental group than in the control group in the two years, and would indicate a higher increase of abdominal cavity space due to a larger fat loss in restricted females. These could have permitted that oocytes of restricted females would have acquired a larger size during post induction latency.

Similarity in data of fertility and embryonic survival of groups for the two cycles, permit stating that food restriction as practiced did not have influence in these two indirect indicators of reproductive performance. Fertility and embryonic survival rates similar to those found are reported for confined *Brycon* species (Pardo-Carrasco *et al.*, 1998; Romagosa, 1998; Pardo-Carrasco, 2001).

Conclusion

In conclusion, 50% food restriction four months previous to induced reproduction of yamú females is a food supply strategy that does not diminish reproduction performance, improves response to inductor and produces bigger eggs post induction.

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