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Ratio « number of males on number of females » for the mass production of *Sarotherodon melanotheron*'s fry in concrete tanks

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Abstract

The appropriate proportion of male and female parents that give a highest number of juveniles is a major concern in tilapia culture *Sarotherodon melanotheron* due to the oral incubation by males. A study of the ratio "number of males to number of females" on fingerlings production has been performed in order to determine the optimal ratio for *S. melanotheron*. Five ratios were tested : R1 (1 male to 2 females), R2 (1 male to 3 females), R3 (2 males to 2 females), R4 (2 males to 4 females) and R5 (2 males to 6 females). The experimental device consists of five concrete tanks (1m x 1m x 60cm) filled to 2/3, about 0.17m^3 of water. The fish are fed three times a day with a diet containing 35% crude protein. Once a week, the fertilized eggs are collected and incubated in a tank. The hatching rate and larval growth are followed. The experiment was duplicated and lasted 6 weeks to harvest eggs and 40 days to track the growth of larvae. The results show that the number of egg laying is significantly different from a ratio to each other ($p < 0.05$) and increases with the number of males and females. The highest number of egg laying is obtained with R5 ratio "2 males to 6 females" that seems best for the mass production of juveniles of *S. melanotheron*. These results provide a basis for the intensive production of *S. melanotheron* for the aquaculture development in lagoon.

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Introduction

Lagoons in southern Benin offer enormous potential for the development of fish farming (Niyonkuru & Laleye, 2012; Chikou *et al*., 2013; Fagnon *et al*., 2013). However, the inadequacy to the brackish waters of the two species, *Oreochromis niloticus* and *Clarias gariepinus*, always used in fish farming, is an obstacle to the development of lagoon fish farming (Codjia, 1987). Thus, researchers are more and more inclined towards the domestication of native species adapted to brackish waters (Legendre, 1986; Chikou 1992; Toko *et al*., 2009, Chikou *et al*., 2011). In this regard, the potentials of *Sarotherodon melanotheron* heavily fished in the lagoons of southern Benin are well known. But, there is still not enough juveniles of this specie for fish farmers increasingly in demand. The nursery center of Tohonou (Benin), created for this purpose in 1994, could not provide a solution to this problem of lack of fry *S. melanotheron*. The main reason was the low profitability of the production due to small quantities of fingerlings produced in ponds.

In fact, if the ratio «male/female» is 1 to 3 in *O. niloticus* (oral incubation of eggs by female) (Mélard, 1986), this is not the case in *S. melanotheron* for what oral incubation is performed by the male. A male can't efficiently incubate the eggs laid by three females regularly. It's therefore necessary to search for the appropriate ratio «number of males to number of females» that gives the highest number possible to fry. This is what justifies the present study aimed to determine the appropriate proportion of male and female parents of *S. melanotheron* that would give per unit time a higher number of eggs and juveniles.

Materiel and methods

The experimental device

The experimental setup is divided into two categories.

(1)- For parents, it consists of 10 basins 1m² surface and a depth of 60cm each. The basins are partially covered by screens to protect the device against the sun and too intense warming of the waters. It also creates the necessary calm to the parents for breeding. Each tank has a water tap and a central pipe 20cm long PVC functioning as a monk (drain device). It is supplied with water from the well of the University of Abomey Calavi. Each basin is filled to 2/3, about 0.17m³ of water, with a constant renewal of 2 to 3 liters of water per minute.

(2)- For egg incubation and larval rearing, the device consists of a pool of 4m² surface and 1m deep. Within this basin are arranged incubation tanks where small baskets are placed to receive the fertilized eggs corresponding to the ratios (treatments). The basin contains water on a column of 20cm on average so as not to drown the little baskets that contain eggs. The volume of water inside is 0.86cm³. It has a central monk PVC and is fed by water from the well of the university with a renewal telling 2 to 3 liters of water per minute. An air diffuser helps maintain a relatively high level of oxygen for the survival of fertilized eggs. The basin is covered by a tray woven palm branch against the sun, rain and other weather.

The living material and ratios tested

Males and females of *S. melanotheron* come from the aquaculture station of the Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences at the University of Abomey-Calavi (LHA/FSA/UAC). They come from lake Nokoue and are kept in the resort for their multiplication. They have an average size of 11.6 ± 1.9cm and an average weight of 27.1 ± 13.4g. Five (5) ratios were tested in duplicate and appear as shown in Table 1. A total of 50 breeding including 16 males and 34 females are divided in the 10 basins. If there are two males in a basin, a marking amputation of the upper part of the caudal fin is made to recognize them (marked male & unmarked male). The objective was to determine the male that incubated eggs and identify if this is the same male who incubated each time. This will determine the effectiveness of each male.

Charging and livestock tracking

Before putting the fish in the basins, all fish were measured and weighed. Those must be marked were. Length measurements (Total and Standard length) followed by weighing (Total weight) were doing.

Sexing done on this specie was easier because males are easily distinguished from females. The cover of the mature female is transparent and red gills below gives a purple appearance.

In the male, the lid and part of the sub-cap have a metallic golden color. Every morning, the pond bottom is cleaned with a scoop to remove the remains of uneaten food and fish waste. The water flow is adjusted as needed.

Water quality control

The physicochemical parameters of the water were measured in all basins. This is the temperature (°C), dissolved oxygen (mg/L), pH, TDS (mg/L) and conductivity (µS/cm). These parameters were measured three times per day (7:45, 12:45 and 17:45) and three times per week (Monday, Wednesday and Friday).

Fish feeding

The fish are fed to the feed for fish (granules) made in the Laboratory of Hydrobiology and Aquaculture of the Faculty of Agricultural Sciences (LHA/FSA) with a crude protein content of 35%. The fish are fed manually every day at 8:00, 13:00 and 18:00. The feed is distributed at 5% of the fish biomass during the experiment.

Control of eggs production

The technique involves draining the pool water by avoiding disturbance to fish that are caught by basin. Each fish is then subjected to a systematic control by gently opening its mouth. Females are also controlled for whether, in some situations, female *S. melanotheron* also incubate the eggs. If there are eggs, they are collected in a small bowl containing the basin water and immediately counted. They were then incubated in small baskets installed in pools where the water is kept clean at the same temperature as the water in the parent basins. A sample of eggs is taken and weighed to determine the average weight of eggs. On graph paper, the diameter of the eggs is determined to achieve the ovarian structure in female *S. melanotheron*. The male (marked or unmarked) which incubated eggs is noted. This control is done once a week, on Mondays between 7:00 and 8:00 am. The total number of laying per basin is noted and total fecundity of females was assessed.

Hatching and larval rearing

The eggs incubated are monitored daily to detect the exact incubation period. At hatching, the number of hatched eggs was counted to estimate the hatching rate. The larvae are examined and size at hatching is made on graph paper and their weight using a sensitive scale (Type AHAUS, 0.001 g). Hatched larvae are fed with Coppens food (45% crude protein) at 20% of their biomass for 40 days. Control of growth is done every 5 days for larvae within the total length (Lt cm) and total weight (Pt g).

Data processing

To address the data growth in larvae, different mathematical formulas were used.

- Total production and total weight gain noted GT (g) = Bf - Bi;
- Specific growth rate observed SGR (%/day) = 100 x (LnPmf - lnPmi) / dt;
- Mortality noted M (%) = 100 x (Ni - Nf) / Ni;
- Survival rate denoted S (%) = 100 - M (%) or 100 x Nf / Ni;

[Pf = average final weight (g) and Pi = average initial weight (g), dt = number of days of feeding, Ni = initial number of fish, Nf = final number of fish, Bi = initial biomass (g), Bf = final biomass (g), GT = Total production, Pt = total weight (g), SGR = specific growth rate and Lt = total length of fish (cm).

Statistical analysis

Tables, curves and various graphics were made with Excel (2007). The data obtained were processed statistically using Statview (version 5.1) software. Different means were compared using Student's t tests or ANOVA with a factor. The effect of different Ratios on the spawning was tested using analysis of variance test (ANOVA 1). Larval mortalities were compared with the Chi-square test (χ^2).

Results and discussion

Tables 2 and 3 present the average values of physicochemical parameters of the water. The average values of physico-chemical parameters of water are 28.7 ± 0.1°C for temperature, 7.2 ± 0.2 mg/L for dissolved oxygen, 5.8 ± 0.1 for pH, 375.1 ± 17.5 µS/cm for the conductivity and 182.8 ± 7.6 mg/L for TDS.

Table 1. The Ratios « number of males on number of females » tested.

R1 : (1 male to 2 females) (N = 3)	R1' : (1 male to 2 females) (N = 3)
R2 : (1 male to 3 females) (N = 4)	R2' : (1 male to 3 females) (N = 4)
R3 : (2 males to 2 females) (N = 4)	R3' : (2 males to 2 females) (N = 4)
R4 : (2 males to 4 females) (N = 6)	R4' : (2 males to 4 females) (N = 6)
R5 : (2 males to 6 females) (N = 8)	R5' : (2 males to 6 females) (N = 8)

Table 2. Average values of physico-chemical parameters of water in the basins (*CV = Coefficient of Variation).*

Ponds		T°C	O_2 (mg/l)	pH	Conductivity (µs/cm)	TDS (mg/l)
R1	Average	28.7	7.4	5.7	369.7	178.1
	Standard deviation	0.9	1.6	0.7	127.6	62.5
	CV %	3.0	21.6	11.6	34.5	35.1
R2	Average	28.7	7.3	5.8	403.4	193.2
	Standard deviation	0.9	1.6	0.8	129.5	65.4
	CV %	3.1	22.3	13.8	32.1	33.9
R3	Average	28.6	7.3	5.7	386.5	187.3
	Standard deviation	1.0	1.5	0.8	137.6	63.7
	CV %	3.3	21.1	13.1	35.6	34.0
R4	Average	28.9	7.4	5.7	384.3	192.1
	Standard deviation	0.7	1.4	0.8	111.4	58.2
	CV %	2.5	18.9	14.6	29.0	30.3
R5	Average	28.6	7.5	5.9	370.9	178.8
	Standard deviation	0.9	1.3	0.7	143.8	70.2
	CV %	3.1	17.6	12.6	38.8	39.2
R1'	Average	28.6	7.2	5.9	350.8	177.1
	Standard deviation	0.7	1.4	0.8	150.3	60.0
	CV %	2.6	19.1	13.7	42.8	33.9
R2'	Average	28.7	7.1	5.8	351.1	171.4
	Standard deviation	0.9	1.5	0.9	140.2	67.2
	CV %	3.2	20.5	15.0	39.9	39.2
R3'	Average	28.7	7.0	5.9	394.8	189.5
	Standard deviation	0.9	1.5	0.9	122.2	60.7
	CV %	3.3	21.2	15.3	31.0	32.0
R4'	Average	28.6	6.9	5.9	363.2	175.2
	Standard deviation	1.1	1.4	0.8	137.3	64.8
	CV %	3.7	20.4	14.1	37.8	37.0
R5'	Average	28.7	7.1	5.9	376.6	185.5
	Standard deviation	0.9	1.5	0.8	114.0	60.5
	CV %	3.0	20.9	13.5	30.3	32.6

The differences observed during the days and weeks are not significantly different ($p > 0.05$) between the Sex Ratios. In general, the physico-chemical characteristics are within recommended intervals for breeding tilapias. For *S. melanotheron*, thermal preferendum is between 22 and 32°C (Chikou 1992; Ouattara *et al.*, 2005). Some reductions in performance appear below 2.3 mg/L for dissolved oxygen and pH values below 6 (Ouattara, 2005). The average pH of 5.8 found seems relatively low, but did not induce a significant effect on livestock and hence spawning females. In addition, this study was conducted entirely outside the natural environment (brackish water) of *S. melanotheron*.

It thus demonstrates the possibility to do in freshwater all phases (breeding and rearing) of the breeding of this estuarine tilapia.

Table 3. Average physico-chemical parameters of the water used for the incubation of eggs of *S. melanotheron*.

	T°C	O2 (mg/L)	pH	Cond (µS/cm)	TDS (mg/L)
Average	29.0	8.1	5.7	361.9	177.2
Standard deviation	1.2	1.7	0.5	141.2	67.5
CV %	4.0	20.3	9.4	39.0	38.1

CV = Coefficient of Variation.

Table 4. Results of egg laying during the experiment (S = week).

Treatments	S1	S2	S3	S4	S5	S6	S7
1 male to 2 females							
R1	PP	PP	PP	72	PP	PP	PP
R1'	PP	PP	PP	PP	PP	PP	PP
1 male to 3 females							
R2	PP	PP	PP	PP	PP	PP	PP
R2'	PP	81	PP	PP	296	PP	264
2 males to 2 females							
R3	PP	PP	PP	PP	PP	PP	77
R'3	PP	124	PP	98	PP	PP	PP
2 males to 4 females							
R4	135	83	PP	PP	PP	254	306
R4'	PP	PP	249	PP	300	PP	PP
2 malesto 6 females							
R5	99	162	61	206	125	85	106
R5'	PP	PP	136	PP	110	PP	114

PP: No Ponte. The figures indicate the number of eggs obtained spawning.

Table 5. Number of eegs laying and total number of eggs collected per treatment.

Treatments	Number of egg layings	Number of egg layings	Number of eggs by laying
R1 + R1'	1	72	72
R2 + R2'	3	641	213
R3 + R3'	3	299	100
R4 + R4'	8	1327	166
R5 + R5'	11	1204	109
Total number	26	3543	660

Tables 4 and 5 show the results obtained during experience according to the egg laying. It was found that no female has incubated fertilized eggs.

This confirms the observations of several authors who reported that only males incubate the eggs in *S. melanotheron* (Chikou, 1992; Wuemenou 1988; Lacroix, 2004; Levêque *et al*, 2004; Ouattara *et al*., 2005). The number of eggs laying is different between the treatments and increases with the number of females and males. The highest number of laying is obtained with the ratio 5 (2 males to 6 females) witch present small eggs (Table 6). We found that the R1 (1 male for 2 females) gave only one single spawning throughout the experiment. We could think of competition between the two females, which is not favorable to regular spawning and continued incubation for the male. Moreover, if the two females should lay normally, the only male does not incubate all the eggs series. For the R2 where we have 3 females, there were three clutches for all the breeding period (7 weeks of observation).

Table 6. Weight and average diameter of eggs in females of *S. melanotheron (*see Fig 1 to the general distribution of the eggs diameters).

Parameters	1 male to 3 females (R2)	2 males to 2 females (R3)	2 males to 4 females (R4)	2 males to 6 females (R5)	2 males to 6 females (R5')
Average weight of eggs (Wa) (mg)	32.5	49.3	17.6	24.6	51.7
Average diameter of eggs (mm)	2.93±0.11	3.1±0.11	3.02±0.24	2.77±0.89	3.11±0.11

Table 7. Incubation of eggs by males marked and unmarked.

Controls	S1	S2	S3	S4	S5	S6	S7
2 males to females							
A3	-	-	-	-	-	-	Marked
B3	PP	No Marked	PP	No Marked	PP	PP	PP
2 mâles pour 4 femelles							
A4	Marked	Marked	PP	PP	PP	Marked/No marked	Marked/No marked
B4	PP	PP	No marked	PP	Marked	PP	PP
2 mâles pour 6 femelles							
A5	Marked	Marked	No marked	Marked/No marked	Marked	No marked	Marked
B5	PP	PP	Marked	PP	Marked	PP	Marked

Table 8. Absolute fecundity (Fa = total number of eggs) of spawning *S. melanotheron*

Fecundity	R1	R2	R3	R4	R5
Fa	72	213	100	166	109
Ecart - type	-	116	23.54	91.41	40.71

It is understandable that male of *S. melanotheron* can incubate once a fortnight. This confirmation is reinforced that we had laying in S2, S5 and S7 for the same basin R2.

According to the incubation of eggs by males, by observing the marked males (Table 7 and), we can see the same situation for the sex-ratio 3 (2 males to 2 females) where egg laying occurred in S2 and S4 in the B3 basin. This observation can also be made at the R5 where egg laying occurred in S3, S5 and S7. However, it is possible to see the same male incubated two successive weeks, occurs after a one week rest. This is the case of sex ratios 4 and 5 (2 males to 4 females and 2 males to 6 females).

These observations are similar to those found by Legendre (1987) cited by Wuemenou (1988) indicates that an incubation period of 21 days on average (ie 3 weeks). They allow us to say that the sex ratio 5 (or 2 males to 6 females) allows for maximum spawning in 7 or 11 weeks for both basins combined (treatment and repetition). This gives an average of 5-6 clutches for 7 weeks. But considering only the R5 basin, it can be at least one egg per week. That is very important for weekly fry production.

Table 9. Results of hatching.

N°	Number of eggs incubated	Number of hatched eggs	Number of unhatched eggs	Duration of hatching	Hatchability %
1	135	135	0	Sd	100.0
2	99	95	4	1	96.0
3	83	82	1	2	98.8
4	162	162	0	Sd	100.0
5	81	79	2	5	97.5
6	124	121	3	2	97.6
7	61	61	0	3	100.0
8	249	244	5	5	98.0
9	136	135	1	1	99.3
10	72	72	0	4	100.0
11	149	148	1	Sd	99.3
12	57	57	0	2	100.0
13	98	98	0	4	100.0
14	125	124	1	1	99.2
15	296	296	0	Sd	100.0
16	300	298	2	3	99.3
17	110	109	1	2	99.1
18	146	145	1	5	99.3
19	108	108	0	1	100.0
20	85	82	3	1	96.5
21	77	76	1	3	98.7
22	185	185	0	5	100.0
23	121	120	1	2	99.2
24	106	106	0	Sd	100.0
25	264	262	2		99.2
26	114	110	4	Sd	96.5
Total	3543	3510	33		99.0

Sd = same day.

Table 10. Results of rearing larvae of *S. melanotheron*.

Parameters	Basket 1	Basket 2	Basket 3	Basket 4
Initial average weight (mg)	21	20	17	23
Final Average weight (mg)	1490	1200	1030	1150
Number of days	40	40	40	40
Growth Rate (%/day)	10.66	10.24	10.26	9.78
Survival Rate (%)	79.31	81.03	100	98.15

In Table 8 relating to the female fecundity (total number of eggs per clutch), the treatments 5 and 4 (2 males 6 females and 2 males to four females) are those that have given the highest total number of eggs (eggs respectively in 1204 and 1327 eggs).

On the other hand, the highest number of eggs per spawning (fertility) was observed in treatment 2 (2 males for 2 females). A discussion of this situation is not plausible due to the weights of females, which may influence fertility. They are not very homogeneous from a treatment to another.

The hatchability of eggs of *S. melanotheron* during the experiment (Table 9) is in all cases very high and ranges from 96% to 100%. During larval rearing, survival rates obtained are higher (79.3 to 100%) and can be explained by the good development conditions established in basins that contain baskets. These survival rates obtained are comparable to survival rates (89.4 ± 0.0) obtained by Ouattara *et al.* (2005) in *S. melanotheron* reared in basins in Côte d'Ivoire.
The specific growth rate (SGR %) is high enough for all the larvae (Table 10). It varies from 9.78 to 10.66 % /day.

The daily individual growth ranged from 0.005 ± 0.00 g/day to 0.12 ± 0.00 g /day for 40 days of culture. Increasing the daily growth of the larvae can be explained by the fact that over time, these larvae grow and consume more dry food.

At the end of breeding, the average weight of juvenile *S. melanotheron* ranged from 1,03 g to 1,49 g.

These are interesting results and it would be useful if time permitted to continue to grow and see after how long juveniles from 10 to 15g salable fish farmers with brackish water ponds would be reached.

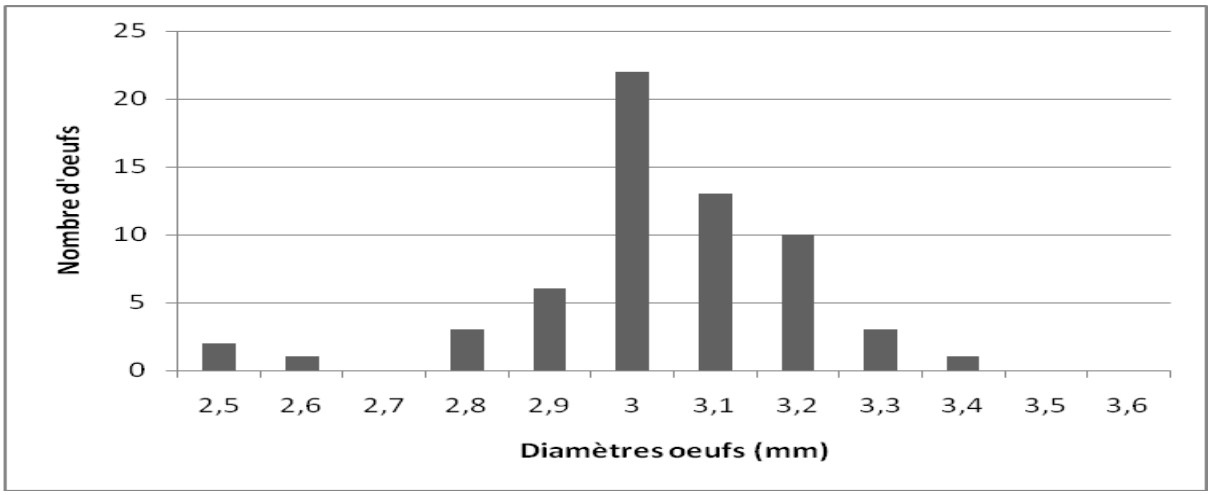

Fig. 1. Distribution of the diameters of eggs in a female *Sarotherodon melanotheron* (female size Lt = 11.6 cm and weight = 22.4 g Pt).

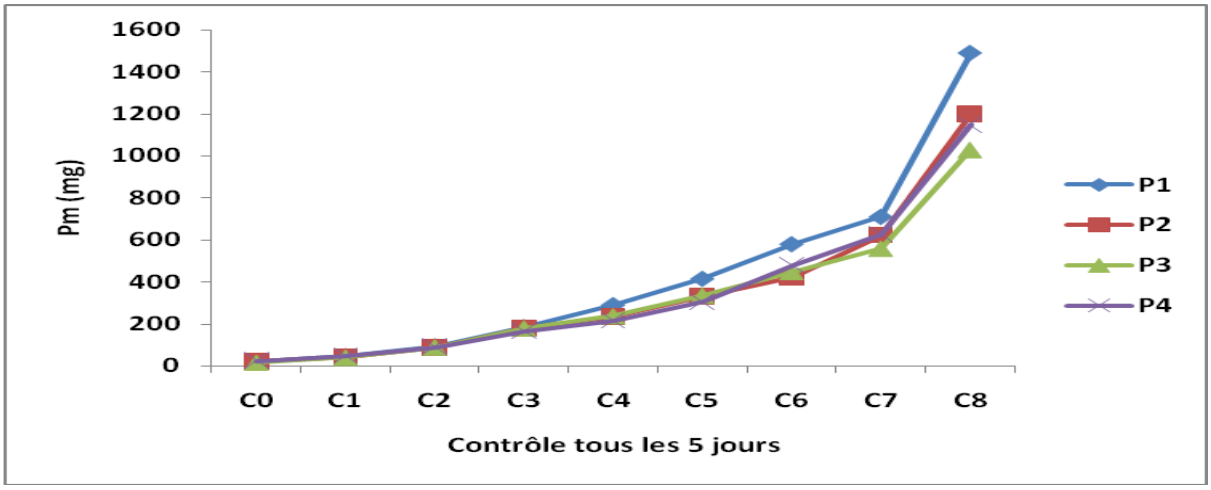

Fig. 2. Evolution of daily weight gains of larvae of *S. melanotheron* high off-soil basins in baskets (C=weekly monitoring).

Conclusion

The results provide a basis for the intensive production of juvenile *S. melanotheron* in ponds for the promotion and enhancement of fish of lagoon ecosystems. The sex ratio R5 of "2 males 6 females" may be retained because it helps to have at least one egg laying per week. Animal performance obtained larvae *S melanotheron* are good in terms of experience. The study of the evolution curves of average weight and daily growth show that the larval and juvenile production of *S melanotheron* are possible and desirable in ponds. However, we suggest

continuing the experiment using intermediate combinations to detect more clearly the best ratio male to female.

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