Studies and Observations on the Spawning of *Oreochromis Niloticus* Species Reared at SCDP Nucet - Dambovita

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Abstract
At the Fish Culture Research and Development Station Nucet, in 2011 were achieved studies and observations on the reproduction, sexual organs and seminal products of *Oreochromis niloticus* tropical species. A batch of 140 breeders of 1 year old Nile tilapia (T_1) was stoked both in a pond as in Ewos tanks in two variants of density (V_I) – 8 fish/tank, (V_{II}) – 12 fish/tank. In a third variant were used 2 years old Nile tilapia breeders (T2), (V_{III}) – 4 fish/tank. The male/female ratio in all variants from tanks was 1:3. Independently, into another tank were stoked 20 males. Into the pond, spawning was achieved naturally, and on the tank variants, the eggs were gathered from the mouth of females and incubated in different incubation systems. On both rearing systems, under the climatic conditions from Nucet (south of Romania) were achieved 3 generations of Nile tilapia fry. On the female breeders, were determined: the gonad-somatic ratio, theoretical and adjective prolificacy (no. of eggs/g of ovary), and for males were achieved spermatozoa motility tests and determination of spermatozoa number per unit of volume.

Keywords: animal products, earthen ponds, Ewos tanks, flow-through, *Oreochromis niloticus*, spawning.

1. Introduction

The *Oreochromis niloticus* (Nile tilapia), (Pisces: Cichlidae), is a species with grate economical importance and significant role in the tropical aquatic ecosystems. The diet is plankton eater and omnivorous, fertilization is external, with incubation of eggs only on the female's mouth (maternal incubation). The species is suitable for extensive and intensive rearing. The optimum temperature in rearing systems ranges from 25 to 31 ^{0}C [1]. At water temperatures of 16^{0}C, tilapia stop feeding, under 20 ^{0}C do not spawn, while sever mortality occurs at 12 ^{0}C [2,3]. That why, it is not recommended introduction of species in traditional systems of countries with environmental conditions outside of its limits of tolerance.
It is the most popular species of bony fish in Africa [4]. This is attributed to many positive qualities including tolerance to poor water quality, wide range of food, plasticity in growth, firm flesh and good taste [5] and the ability to efficiently convert organic and domestic wastes into high quality protein [6]. The size and age varies at first maturity. Under favorable natural conditions, Nile tilapia reaches sexual maturity at 20 – 30 cm (150 – 250 g), but also can be mature on a much smaller size (phenomenon indicates a crowded or stressful environment). [6] and [7] found that cultured Nile tilapia reach first maturity at about 30 g, which was also confirmed at Nucet.
In the paper are presented results of growth, spawning, studies of sexual organs and seminal products completed by hydro-chemical data that characterized the environmental conditions from Nucet, situated in the southern area of Romania.

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2. Materials and methods

A lot of 140 exemplars of 1 year Nile tilapia obtained in Nucet by naturally directed spawning

were stoked at 21.06.2011, in two different growth areas:
- in natural environment (0.5 ha pond) – for testing the growth potential under unfed condition and to confirm the achieving natural spawning on extensive stoking in polyculture;
- in 1 m^3 Ewos tanks (flow-through system) – were stock Nile tilapia for accomplish some studies of feeding ethology (fed diet) and spawning in captivity and gonadal and seminal product studies.
When sex differentiation of 1 year Nile tilapia (T_1 45.6 g/ex) was possible, 100 fish (78♂:12♀) were stocked in a pond of 0.5 ha. The pond was stocked shortly before with *Polyodon spathula* fry and cyprinid breeders (koi carp and grass carp) for there ecological role in natural mobilization of pond bottom and that control the submerged vegetation.
At the same time, in a Ewos tank were stocked 40 exemplars of Nile tilapia T_1 (34.4 g/ex.) to the increasing body weight (for a month) allowed us to determine sex. The operation of sex determination was under the influence of a narcotic (MS 222 Sandoz). With one year breeders (T_1) were established two experimental variants: V_I – 8 fish/tank (6 ♀ and 2 ♂); V_{II} – 12 fish/tank (9 ♀ and 3 ♂); and a third variant with two years breeders (T_2): V_{III} – 4 fish/tank (3 ♀ and 1 ♂). In all experimental variants the optimal ratio (♀: ♂) was 3:1 [8].
In a Ewos tank, another 20 males T_1 were stoked separated.
In the pond, Nile tilapia was fed exclusively by natural foods and in Ewos tanks by protean fodder for tilapia (Aller Tilapia XS), 3 mm granulation and a protean content of 33 %, delivered manually three times per day (at 9^{00}, 12^{00}, 16^{00}).
The biological material being easy to follow in Ewos tanks, provided us some information on feeding and reproductive behavior and allowing in the same time to achieve some studies on the reproductive organs (gonads and seminal products).
The relative prolificacy (number of eggs incubated by a female) was determine by sampling the embryonated eggs from the mouth of T_1 and T_2 females, setting then the theoretical prolificacy (number of eggs/g of ova) and the gonad somatic ratio (ova weight × 100/body weight).
The sperm was taken by gently pressure of abdomen of males with apparent "bridal clothes". Macroscopic assessments were made on the appearance of sperm, the consistency and color. The microscopic studies (×20 and ×40) have watched the motility and viability of spermatozoa, also making the appreciation on number of spermatozoa/mm^3 by counting them in Goreaev counting room by means of Potain dropper, using distilled water as dilution liquid.
The morphological aspect of spermatozoa was revealed by Mai-Grunwald – Giemsa coloration.
To obtain Nile tilapia fry we used artificial incubation that made by moving eggs from female mouths in different hatching devices: Zug-Wais and incubator for hatching poliodon eggs (by the model used in the countries of former USSR).
Chemical analyses of water were conducted in laboratory of Fish Culture Research and Development Station Nucet by analytical method.

3. Results and discussion

At the end of a rearing season (100 days, June-September), under the climatic and ecological conditions from SCDP Nucet – south Romania, the extensive rearing in pond (natural diet) of a 1 year Nile tilapia batch led to achieving superior growth performances comparing to Nile tilapia reared in captivity (tanks) and feed by artificial diet. The average body weight of Nile tilapia from pond was ranged from 392 to 578 g comparing to average weight of fish reared in tanks (92 – 289 g).
The breeders batch of *O. niloticus* species (T_1 and T_2) last through the winter in aquariums at 20 ^{0}C had a sustained spawning activity in July and August, after about a month from stoking them in natural environment and tanks. From the stoking of breeders into the new environment to the onset of spawn, water temperature gradually increased to a maximum of 26 – 28 ^{0}C, but eggs were attained also at 20 and 22 ^{0}C (September).
The physico-chemical analysis of water indicates some differences between experimental variants but within the normal limits (Table 1).

Table 1. The main chemical parameters of technological water

Crt. No.	Parameter	U.M.	Critical /ideal values EL-SAYED, 2004 [7]	Variant I T_1 (8 fish/tank) 2 ♂ : 6 ♀	Variant II T_1(12fish/tank) 3 ♂ : 9 ♀	Variant III T_2 (4 fish/tank) 1 ♂ : 3 ♀
1	pH	U pH	4 -11	8.0 – 8.2	7.8 – 8.2	8.0 – 8.4
2	Alkalinity	ml HCl/l		2.0 –3.2	2.2 –2.9	1.7 – 2.5
3	Total Hardness	(^{o}D)		3.80 – 5.96	3.92 – 5.82	3.05 – 4.22
4	Dissolved Oxygen	mg O_2/l	<2/>6	3.5 – 14.1	3.8 – 6.2	4.2 – 10.6
5	CCO-Mn	mg/l $KMnO_4$		14.6 – 30.2	9.3 – 24.3	17.4 – 29.5
8	AmoniaNH^+_4	mg/l	>15/<3	1.29 – 2.91	1.43 –2.67	1.18 – 2.99
9	Nitrite (NO-2)	µg /l	>5/<1 mg/l	0.038 – 121.02	0.019 – 134.64	0.022 – 0.169
10	Nitrate (NO-3)	µg /l	>500/<20 mg/l	0.06 – 193.1	0.02 – 128.31	0.02 – 0.263
11	P of PO_4	mg/l		remains	0.02	0.01 – 0.037

During spawning time the color of Nile tilapia males and sometimes sexual dimorphism facilitate the gender differentiation. Male color its head, pectoral and caudal fins purple (Figure 1a), and female remains with a bland color, but is obviously rounded belly (Figure 1b).

a b

Figure 1. Nile tilapia breeders: a – male; b – female

Gender differentiation is performed under narcosis by highlighting the anal-urinary-genital openings with methylene blue solution.

Other external differences between sexes are based on the fact that males have two orifices under its belly, in which, one is the anus and the other the urogenital aperture. The female has three; the anus, the genital and the urinary apertures (Figure 2 – sketch after [9] and original photo).

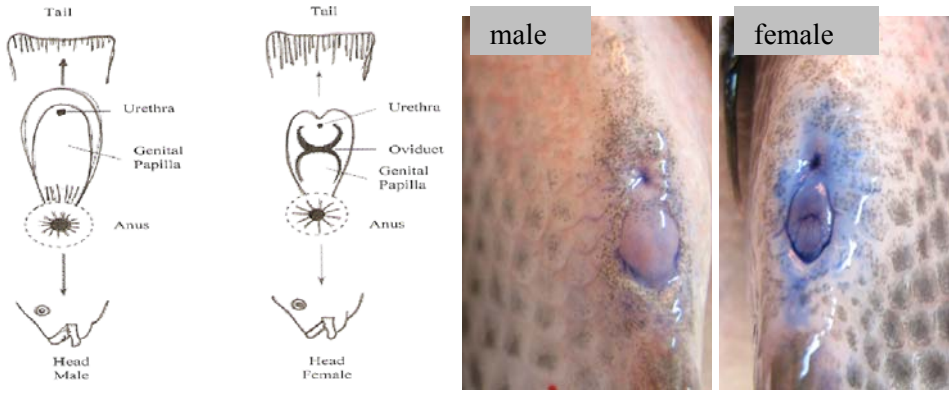
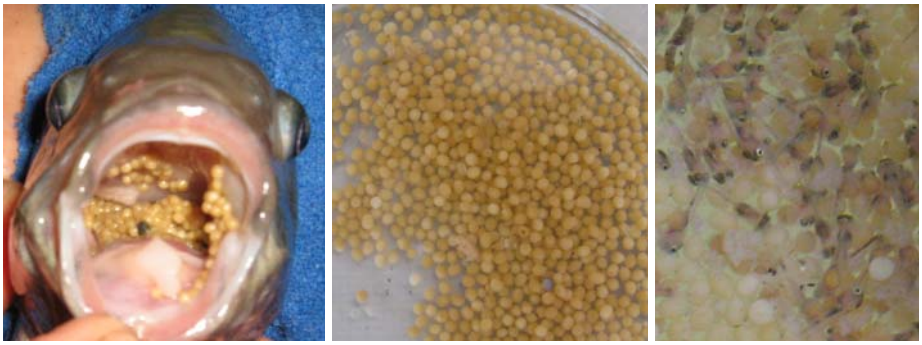


Figure 2. Highlighting openings: anal, genital and urogenital on Nile tilapia

During spawning process Nile tilapia females repeated releases a clutch of 20 – 50 eggs [10]; then the dominant male passes over eggs releasing a milky cloud of sperm. The female returns immediately and take the fertilized eggs in mouth and sometimes hitting with mouth the ventral part of other males that creep strongly and repeatedly in front of it. After "finishing" spawning, phenomenon known by the end of long agitation (24 – 48 hours) and lack of interest for food,

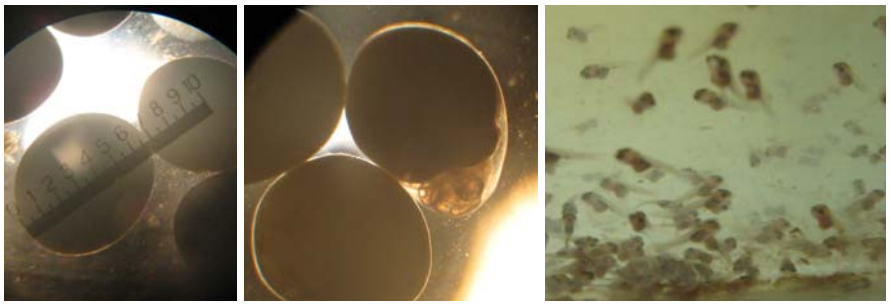
female with eggs in mouth withdraws isolated into the water mass. For the operation of collecting eggs from oral cavity of females (Figure 3) they are capture by one and immerse in a narcotic solution. After collection eggs are incubated. Good results were achieved at incubation in Zug-Weiss device.



a b c

Figure 3. Female with eggs in the mouth (a); eggs and larvae collected from female's mouth (b, c)

To achieve a maximum of fry/female embryonated eggs is taken from the oral cavity after 5 – 6 days from fertilization (after hatching), or at 24 – 48 hours after hatch. Eggs in the last stages of embryonic development or fresh hatched larvae are stocked directly in aquariums.



a b c

Figure 4. Embryonated eggs (a), larvae and fry (b, c) of Nile tilapia

The number of eggs per female of Nile tilapia in a rearing season (100 days), depends on age, temperature evolution and body weight of female, as follow:

- in the weight category 75 – 116 g (T_1), relative prolificacy at 26 – 28 ^{0}C ranged from 370 to 1562. In autumn at 20 ^{0}C the number of eggs/female was just of 48 – 233 although female body weight growth;
- in the weight category 470 – 534 g (T_2) the number of eggs/female at 26 – 28 ^{0}C was just of 978 – 1890 well bellow expectations comparing to the results compared to performances from literature (3500 eggs by a female of 550 g [1]). The lust clutch of a T_2 female from the vegetative season (at 22 ^{0}C, in September) was of 714 embryonated eggs.

Relatively small number of embryonated eggs/female of 2 years Nile tilapia (T_2) suppose to be caused by the appearance of some cysts most likely appeared by locking the resorption of not eliminated eggs at the follicular level determined by the temperature decrease (summer-late autumn interval).

Theoretical prolificacy varies with female size. On the T_1 females (weight category 80 – 130 g) theoretical prolificacy is of 162 – 213 eggs/g of ova and on the T_2 females (weight category 470 - 534) is of 98 – 127 eggs/g of ova.

The gonad-somatic ratio was accomplished by sacrificing T_1 and T_2 females (beginning of October, at the water temperature of 15 ^{0}C). The gonad-somatic ratio varied with fish size, age and rearing environment. At the T_1 females reared in pond, the gonad-somatic ratio was of 3.59 % and for those from tanks 2.32 %. At the T_2 females reared exclusively in captivity the gonad-somatic ratio was 3.18 % slightly below the level of T_1 females reared in pond.

Incubation in 2009 of Nile tilapia eggs at 27 – 29 ^{0}C led to obtaining a law percentage of females in the batch of breeders (only 3.4 %). In 2011 females percentage was 19.2 % because they have retained for breeding batch formation only the fingerlings from last clutch (beginning of September 22 – 20 ^{0}C).

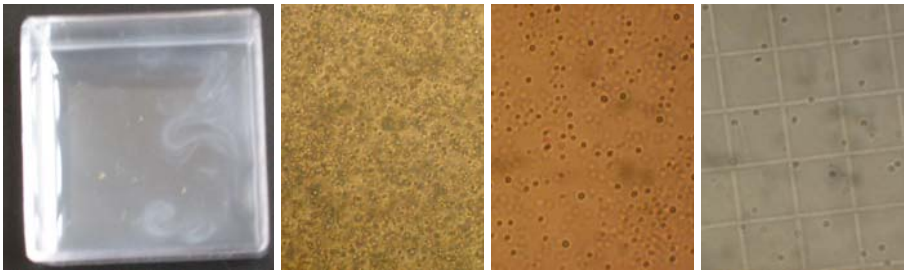
In Figure 5 are presented the female and male sexual organs of Nile tilapia.

a b

Figure 5. Sexual organs of Nile tilapia: a – sexual organ of female; b – sexual organ of male.

Males have been appreciated in semen quality by macro and microscopic analysis. Macroscopic appearance – the semen looks watery, translucent, slightly disturbed by a milky cloud (smoke aspect) (Figure 6a). Examination of sperm motility was done under a microscope, shortly after sampling, putting on a glass slide a drop of semen then adds a drop of water. Thus was made the distinction of eddy and rotator swirling of spermatozoa (Figure 6b).

The spermatozoa concentration of semen was determine at unit volume by Potain pipette and Goreaev counting camera. It was found a number of 665 mil. of spermatozoa/mm^3.



a b

Figure 6. Examination of Nile tilapia sperm: a – semen; b – spermatozoa motility

The morphological aspect of spermatozoa has been revealed by Mai-Grunwald-Giemsa straining. Visualization of preparations was made with 21 f field glass and 90 × object glass (Figure 7). Dyeing the preparation clearly reveals only two parts of spermatozoa: head and tail (the flagella).

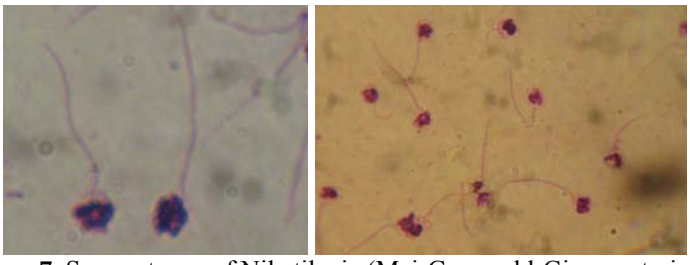


Figure 7. Spermatozoa of Nile tilapia (Mai-Grunwald-Giemsa straining)

4. Conclusions:

Rearing of Nile tilapia in ponds from southern Romania can be done successfully if stoking of ponds is made in May at over 20 ^{0}C and harvest at 14 ^{0}C.

Under climatic conditions from southern Romania, by using the biological material of Nile

tilapia acquired at Nucet and passed over winter in aquariums can be achieve 3 or 4 generations of fry in a rearing season (June – September).

Incubation of eggs and larvae of Nile tilapia exposed to high water temperatures (29 ^{0}C) lead to getting the majority of male offspring and incubation and expose of larvae to lower temperatures (20 ^{0}C) lead to increase the female offspring percentage.

To increase the spawning timing and shortening of clutch is necessary to separate the fry from females.

References

1. Melard, Ch., Les bases biologiques de l'elevages intensif du tilapia du Nil; Cahiers d'ethologie appliquee, thesis, Univ. De Liege, Belgique, 1986.

2. Balarin, J.D. and Haller, R.D., The intensive culture of tilapia in tanks, raceways and cages. In: Muir, J.F. and Roberts, R.J. (eds) Recent Advances in Aquaculture. Croom Helm, London and Canberra, and Westview Press, Boulder, Colorado, 1982, pp. 267-355.

3. Chervinski, J., Environmental physiology of tilapias. In: Pullin, R.V.S. and Lowe-McConnell, R.H. (eds) The Biology and Culture of Tilapias. ICLARM Conference Proceedings No. 7, ICLARM, Manila, Philippines, 1982, pp. 119-128.

4. Abdel Tawwab, M., A.A. Mousa, M. Hand Ahmed and S.F.M. Sakr, The use of calcium pre-exposure as a protective agent against environmental copper toxicity for Juvenile Nile tilapia, Oreochromis niloticus (L.). Aquaculture, 2007, 264, pp. 236-246.

5. Fryer G. and T.D. Iles, The Cichlid Fishes of the Great Lake of Africa: Their biology and evolution. Oliver and Boyd, Edinburg, Scotland, 1972, pp. 641.

6. De Graaf, G.J., Galemoni, F. and Huisman, E.A., Reproductive biology of pond reared Nile tilapia, Oreochromis niloticus L. Aquacult. Res., 1999, 30, 25-33.

7. Abdel Fattah M. El-Sayed, Tilapia culture. CABI Publishing, Wallingford, UK, 2004, pp. 400

8. Costache, M., Oprea, D., Radu, D., Bucur, C., Testing the Reproductive Potenţial of Nile Tilapia (*Oreochromis niloticus*) under Eco Technological Conditions from Nucet – Buletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 2011, 68 (1-2), pp. 118-124.

9. Beveridge, M.C.M. and McAndrew, B.J. (eds), Tilapias: Biology and Exploitation. Kluwer Academic Publishers, Dordrecht/Boston/London, 2000, pp. 505.

10. Rothbard, S.M., Observation on the reproductive behaviour of *Tilapia zillii* and several *Sarotherodon spp.* under aquarium conditions. Bamidgeh, 1979, 31: p. 35-43.