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Reproduction of Neon Tetra (*Paracheriodon Innesi)* Under Controlled Conditions

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Abstract: **Studies on reproduction of Neon tetra were carried under controlled conditions. Neon tetra is a species popular for keeping in aquaria. It was established that spawners of this species produce viable gametes during a few (5–6) spawning periods only; later, although they get involved in reproduction viable issue cannot be obtained from them. From the breeding perspective fish of that species should be reproduced again shortly after the completed spawning and that time should be from 5 to 15 days. Excessively long keeping the fish between spawning periods results in a significant deterioration in quality of gametes expressed by the decreased number in obtained issue. It was shown that before spawning spawners should be kept in water at 22ºC. The negative effect of keeping the reproducers in water at 25ºC accumulated with time.**

Keywords: **Neon tetra, controlled reproduction, hatch, Aquarists.**

I. INTRODUCTION

Aquaculture is not only breeding fish for consumption but also breeding ornamental species (Tlusty 2002, Cek, Gokce 2005, Chelappa Et Al. 2005). Breeding of aquarium fish develops particularly dynamically in Asia although recently extensive development has also been observed in some European countries, e.g. Czech Republic. Characidae living mainly in the tropical waters of South America are one of the groups of ornamental fish commonly bread worldwide. The best-known representatives of that group are Neon tetras. That group also includes other tetras, This is a small fish in captivity reaching ca. 3-6 cm in total length. In the natural environment it is most numerous in the La Plata basin. The species was imported to Europe in 1922. Considering its size, it is a relatively fertile fish. During a single spawning act that is extended over time and usually takes from 2 to 4 hours, the female lays even over 2000 grains of eggs (KUJAWA 2000). The larvae hatch in most cases within 24 hours from fertilization of the eggs. That species is found in two colors: natural and albino. The natural (wild) form has red and blue, shiny body. A horizontal blue belt runs posterior side of the body It has a light-blue back over a silver-white abdomen. The genus is characterized by an iridescent blue horizontal stripe along each side of the fish from its nose to the base of the adipose fin, and an iridescent red stripe that begins at the middle of the body and extends posteriorly to the base of the caudal fin.

The eye iris is silvery-gray and the pupil is black. In case of albino form the body is yellowish-orange and the odd fins are intensely red. A white horizontal belt runs along the body. The eye pupil is red and the iris is white. Very limited number of reproductive acts during which the fish produce viable gametes is one of the characteristics of small body size fish belonging to the Characidae family. In case of some species such as cardinal tetra (*Paracheirodon axelrodi*) or neon tetra (*Paracheirodon innesi*) the number of such acts can be just two or three. The quality of gametes and number of offspring obtained can also be influenced by other factors such as feeding, water temperature, light conditions or length of interval between consecutive reproductive acts (BROOKS et al. 1997, TARGOŃSKA 2007).

Determining the influence of the temperature of water used for keeping the spawners on the number of offspring obtained was the primary goal of this study. Checking the number of reproductive acts during which Neon tetra produce viable gametes and the length of intervals between individual spawning acts on the number of hatched fish obtained was the secondary goal.

II. MATERIAL AND METHODS

Around 1000 larvae of Neon tetra were obtained from one of the private aquarium breeders in Mumbai, Maharashtra. The larvae were initially reared in 1 dm^3 tanks at the concentration of 100 larvae per 1 dm^3 and after 4 weeks in four tanks of 50 dm^3, working in a closed system (KUJAWA et al. 2000). During the initial rearing taking 4 months the fish were fed 3 times per day with aquarium feeds: flakes by Tropical as well as frozen Chironomus and trout granulate. The temperature during initial rearing was within 22–23°C. After reaching sexual maturity by the fish the mass spawning was conducted. For that purpose in each of twenty 10 dm^3 spawning tanks five pairs of spawners were placed. Spawn grilles were placed on the bottom of each tank (TARGOŃSKA 2007). Water temperature was increased to 25–26oC. Generally, spawning occurred after a few days, most frequently between day 2 and day 4 as of placing the fish in the tanks. After 24 hours from spawning the hatch was counted (hatching occurred 18–22 hours after spawning) on the basis of five return samples, according to the methodology described by TARGOŃSKA (2007) for rosy barb (Puntius conchonius). After the first (controlled) spawning the fish that reproduced were split into three groups and three separate experiments were conducted.

Experiment 1. The first experiment investigated the influence of consecutive spawning acts on the number of obtained eggs. For that purpose 10 reproductive pairs (1 male and 1 female) were used. Every 15 days they were moved to spawning tanks. The time between individual spawning acts was 11–12 days. After each completed spawning the fish were caught and 24 hours after spawn the number of fry was counted.

Experiment 2. In the second experiment the investigation concerned the duration of the most appropriate period between spawning from breeding perspective. For that purpose 10 reproductive pairs that completed the first spawning were moved every 5 days to spawning tanks. After completed spawn the procedure was the same as in case of experiment 1.

Experiment 3. The third experiment investigated the influence of the temperature (22 and 25°C) of water in which fish were kept before spawning and possible effect of accumulation. For that purpose two sets of fish (10 reproductive pairs each) were used. Three times, at 15 days intervals they were moved to the spawning tanks. After completed spawn the procedure was the same as in case of experiment 1.

Physicochemical parameters of water used for Neon tetra spawning were constant. For that purpose tap water was mixed with water obtained from the process of reversed osmosis. As a result water with carbon hardness below 2°n and total hardness at 3°n was obtained. The temperature of water in spawning tanks was 25–26°C. On tank bottom spawn grilles with mesh size of 5 mm were placed. The tanks were aerated. The spawning tanks were placed in dark, as Neon tetra spawn is sensitive to light. Three times a day the tanks were checked whether spawning took place. After completed spawning the fish were caught. The hatch was counted 24 hours after completed spawning on the basis of 5 return samples (TARGOŃSKA 2007).

The results obtained were analyzed statistically. The differences in the number of hatch between groups in individual experiments were processed by variance analysis and Tuckey's post-hoc test at significance level of 5%. The correlation between the hatch number and the consecutive spawning as well as intervals between individual spawning acts were subjected to regression analysis. The correlation between the hatch number obtained and the consecutive spawning acts for fish maintained at different temperatures was also determined.

III. RESULTS

Indifferent of the type of experiment conducted the percentage of ovulating females was high and ranging from 90% to 100%. In analysing the hatch numbers from consecutive Neon tetra spawning acts it was determined that for the reproductive acts 1 to 5 the numbers of obtained fry were at similar level of around 1000 individuals (Figure 1). A significant decrease of the value investigated was found during further reproduction acts. From spawning 8 and two later ones no single hatched individual was obtained. (Figure 2) presents the numbers per hatch obtained from spawning acts performed at fixed time intervals, i.e. after 5, 10, 15, 20, 25 and 30 days from the controlled spawning. It was established that the interval between

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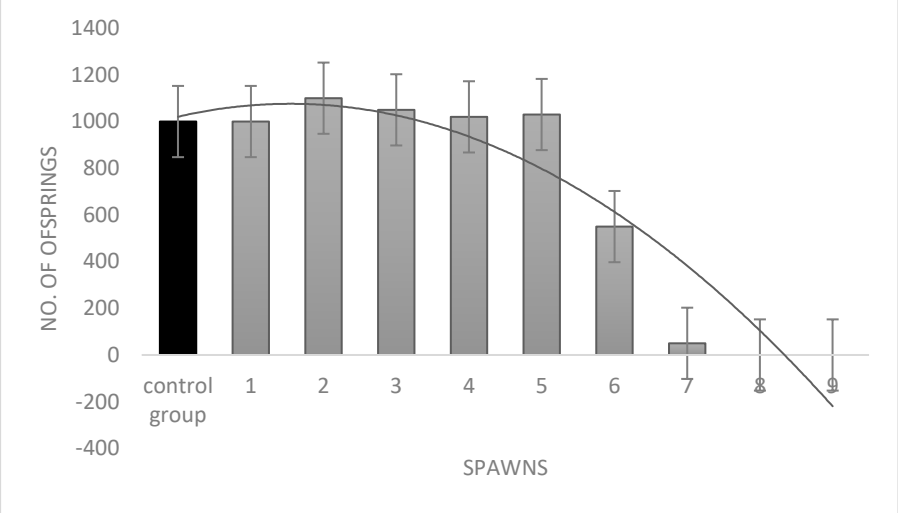
individual reproductive acts in case of Neon tetra should be from 5 and 15 days. Longer than 15 days keeping of the fish before consecutive spawning resulted in a significant decrease of the hatch numbers obtained, for example, when comparing the number of larvae obtained in case of 10 and 30 days interval between spawning acts an almost 60-fold decrease in the number of larvae obtained was recorded.



$$y = -14.462x^2 + 44.32x + 1002.3$$

$$R^2 = 0.82$$

Fig.1. The influence of the number of spawning acts completed on the average hatch numbers obtained from one pair of the Neon tetra spawners. The data marked by the same letter index within individual spawning acts did not differ statistically.

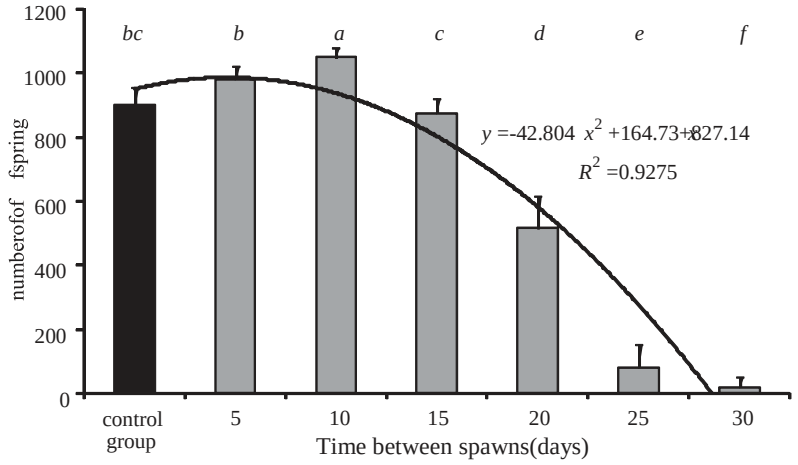


Fig. 2. Influence of the length of interval between spawning acts on the average hatch number obtained from one spawning pair of Neon tetra. The data marked by the same letter index within individual spawning acts did not differ statistically.

It was also established that the level of temperature is significant for the numbers of hatch obtained (Figure 3). Longer keeping of fish at the higher of the tested temperatures (25°C) had a significant influence on decreasing the numbers of offspring obtained as compared to the results obtained when the fish before spawning were kept in water at 22°C.

Accumulation of the negative effect of the temperature influence on the results of reproduction in case of longer keeping the spawners at 25°C was also determined. During the experiments conducted no cases of spawners death were recorded.

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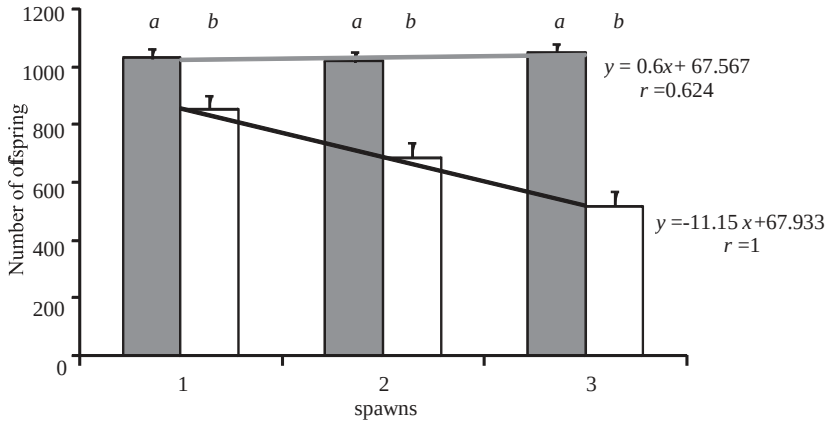

Fig. 3. Influence of the temperature of water used for keeping the Neon tetra spawners on the average number of hatch obtained from a single spawning pair. The data marked by the same letter index within individual spawning acts did not differ statistically

IV. DISCUSSION

In modern aquaculture obtaining high quality gametes, which allows both obtaining the required number of larvae for initial rearing and planning of production, is one of the most important problems to be solved. It is also highly important from economic perspective. This applies not only to fish reared for consumption but also other fish including the decorative and aquarium species.

Numerous factors influence the effects of reproduction, in this study understood as spawning and obtaining a specific number of larvae from the hatch. They include, among others, the fish diet and environmental conditions. According to BROOKS et al. (1997) the differences between species in the influence of environmental conditions on effectiveness of fish reproduction and quality of gametes produced by them were determined. In some cases the environmental conditions to which spawners are subjected can also influence such characteristics of the offspring as the survival rate, growth rate and resistance to stress (BROOKS et al. 1997, TARGOŃSKA 2007).

The fact that viable offspring of Neon tetra can be obtained during a few initial reproductive acts only (counting from the first, controlled spawning) and later, although the fish performed the reproduction and laid spawn obtaining viable offspring is not possible should be considered very interesting from both research and application perspective. On the basis of the data obtained from the presented experiments it can be concluded that during further reproductive acts spawn was laid but hatched larvae were not obtained because developing spawn died during the initial hours of incubation. That observation is confirmed by numerous observations presented by aquarium owners and it is a characteristic of Characidae as well as some Cichlidae fish.

The genus Xenotilapia Cichlidae living in Lake Tanganyika can effectively reproduce just once in the lifetime. Sometimes, under aquarium conditions, another spawning can be induced by placing a group of young females in the tank (PRUSIŃSKA – unpublished data). The reasons for that situation have not been explained sufficiently so far. During these studies not a single viable hatch was obtained from spawning 8 on. Considering the number of spawning pairs and the data published by KUJAWA (2000) on fertility of that species it should be estimated that not a single larvae hatched from around 20 000 grains of spawn. Dying of spawn in this case could be caused by so-called internal factors only as similar environmental conditions were maintained during all the experiments. Internal factors such as quality of the gametes and fitness of the spawners are considered very important for survival of embryos and the development of early ontogenesis although so far few studies have been published on that subject (BUNN et al. 2000).

The influence of some factors (positive or negative) on the effect of fish reproduction can accumulate over time. That was confirmed, among others, by TARGOŃSKA (2007) during studies on the influence of feeding of reproducers on the effects of reproduction of Symphysodon discus fish. The accumulation of negative influence of high water temperature in which spawners were kept was also found in case of the studied species of Neon tetra. Extended keeping of fish in water at 25oC caused a decrease in the number of offspring. The influence of water temperature on reproductive capacity has been described for many fish species (KRAAK, PANKHURST 1996). A similar relation to that in the current study between the

temperature at which the spawners were kept before reproduction and survival of the hatch was observed in case of Menidia audens, when the fish were kept under controlled conditions (HUBBS, BRYAN 1974).

The results of studies obtained indicate that keeping spawners of Neon tetra in excessively high temperature and excessively long intervals between consecutive spawning actions can have a significant influence on effects of reproduction. It is known that during selection of reproducers to the spawning stock the age and number of spawning actions completed should also be considered. The results presented in this paper close the information gap concerning biotechnology of aquarium fish reproduction, which is quite significant even for species highly popular in aquaculture.

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