

First Experimental Induced Breeding of the Largemouth Bass *Micropterus salmoides* Lacépède, 1802 (Centrarchidae) in Algeria

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ABSTRACT

In North Africa, this experimental work represents the first artificial induced spawning of *Micropterus salmoides* on artificial gravel nests. During February 2012, gillnet fishes were collected from 3 dams located in the west of Algeria. 24 adults including 10 males and 14 females were selected, and transferred into two under cover concrete rectangular raceways (R1 and R2) supplied with suitable water quality. Males and females were kept separated, and supplied with *ad libitum* natural food. The experiments started at 14°C water temperature, and were carried out on 3 batches of fishes. In R1, the batch B1 consisted of 3 males and 5 females. In R2, the batch B2 included 6 males and 8 females, and B3 of a couple of fishes. 52 days later, when the water temperature reached 23°C, the fishes began to exhibit courtship behaviours. As a control group, fishes of B1 were not given any hormonal treatment. Fishes of B2 were given a single injection of 4500 IU of HCG/Kg of body weight. The couple of fishes in B3 were given a single injection of a solution of 4500 IU of HCG/Kg of body weight mixed with the extract of 1g of pituitary gland of *Cyprinus carpio*. A 1m² artificial spawning gravel nest was immersed at each corner of the raceways. No spawning occurred in B1 despite courtship behaviour. For B2, spawning occurred on the bottom of R2 and on one artificial nest (average 2000 eggs/m²). B3 showed better spawning (average 3500 eggs/m²). The artificial nests were transferred into nursery raceways under laboratory conditions. The global rate of hatching was 80%. Monitoring of the fry growth was carried out until the 24th day post-hatching.

KEYWORDS: *Micropterus salmoides*; Human Chorionic Gonadotrophin; Pituitary gland extract; Artificial spawning; Artificial nest; Algeria.

1. INTRODUCTION

Micropterus salmoides is an indigenous species to the United States [1,2]. In fact, there are two recognized subspecies of largemouth bass, the northern largemouth bass *Micropterus salmoides salmoides* native to the Midwestern United State, and the Florida largemouth bass *M. salmoides floridanus* LeSueur [3,4]. Several worldwide introductions have expanded the distribution of *M. salmoides* to freshwater bodies in Africa, Europe, South America and Asia [4,5,6]. It is considered as an alien invasive predator [7] and has a large food spectrum including invertebrates, amphibians and mainly fish.

During 1985, started in Algeria a national program of aquaculture with the introduction, in the northern freshwater ecosystems, of fingerlings of *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Sander lucioperca* and the largemouth bass *Micropterus salmoides* imported from Hungary [8,9]. Since that period of restocking, the unique records of successful attempts of artificial controlled spawning were made on *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis* and *S. lucioperca* [10,11,12,13]. In the north-west of Africa, no work has been done on *M. salmoides* ([13,14], that reproduces naturally in the local freshwater ecosystems. The target of this experimental work was to master the technical aspects of an artificial spawning induction by an intramuscular injection of Hormones and to follow up the growth of the larval and fingerling stages.

2. MATERIAL AND METHODS

Samplings

During February 2012, 24 wild adults *Micropterus salmoides*, including 10 males (Average weight 1,200 kg ; Mean Total Length (TL) = 34,1cm) and 14 females (Average weight 1,500 kg ; Mean TL = 42,5 cm), were selected from various gillnet captures in 3 dams in the western Algerian area (**Fig.1**), with 4 specimens from Beni-Bahdel Dam (Tlemcen district), 16 specimens from Bou-Hanifia Dam (Mascara district), and 4 specimens from Sidi-M'hamed-Ben-Ali Dam (Sidi-Belabbes district).

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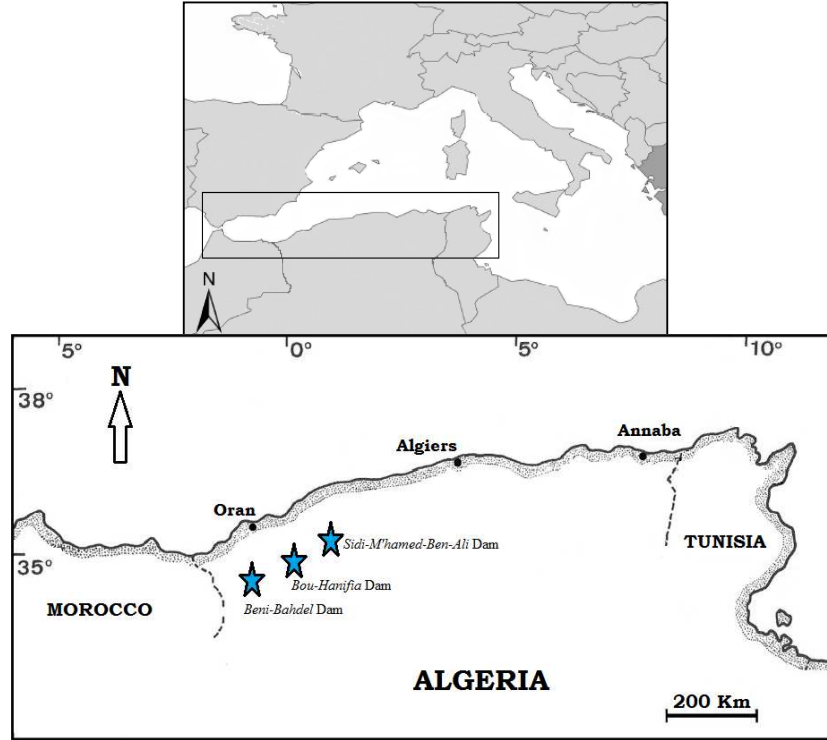


Figure 1: Sampling sites of *Micropterus salmoides*.

Immediately after capture, fishes were submitted to a prophylactic bath in a solution of 1ppm Methylene blue during 5 minutes. In order to avoid acute toxicity, the fishes were anesthetized in a induction bath of Phenoxyethanol (1ml/10L) during 5 to 10 minutes or in a 80 ppm bath of clove oil and ethanol [15,16,17,18]. The individuals were measured, weighted, and tagged on the dorsal fin in order to recognize and control each individual during the experimental protocols. Fishes were then transferred to an experimental aquaculture station in fresh oxygenated water (Mean Temperature 12°C) within appropriate tanks for fish transportation.

Acclimation

During 52 days (08th February to 30th March 2012), males and females were held in two separate under cover concrete raceways R1 and R2 (7.20 x 3.80 x 1.30 m) supplied with optimal water quality. The water supply was sufficient to insure a daily renewal of the entire volume of the raceways. The water temperature and dissolved oxygen were daily controlled. Initially, the experiment started with a mean water temperature of 14°C.

Although that the recommended stocking ratio is of 3 males to 2 females [4], and regarding to the limited number of the captures, 3 batches were randomly separated. In R1, the first batch B1, as a control group, included 3 males and 5 females. In R2, the batch B2 consisted of 6 males and 8 females, and the batch B3 consisted of a couple. In both raceways, males and females were kept separated by a wire mesh device. During the entire experiments, feeding was *ad libitum* and the fishes were supplied with fingerlings of *Luciobarbus setivimensis*, *Liza ramada*, *Alburnus alburnus*, *Cyprinus carpio*, *Gambusia affinis affinis* and *Oreochromis niloticus* collected from the local natural reservoirs.

Protocols for inducing the artificial spawning

In R1, as a control group B1, the fishes were not given any hormonal treatment but were kept in similar conditions for water quality and food supply as in R2. Fishes in R2 were carefully recovered one by one from the raceway, and anesthetized as described before. According to their weight [19], the fishes of B2 were given a single hormonal injection of 4500 IU of HCG/Kg of body weight. The fishes of B3 were given a single injection of a solution of 4500 IU of HCG/Kg of body weight mixed with the extract

of 1g of pituitary gland of *C. carpio*. Immediately after full recovery of the fishes from the anaesthesia, the separating devices were removed from R1 and R2 in order to enable direct contact between the fishes. However in R2, the batches B2 and B3 remained separated by the wire device.

M. salmoides is known to spawn in areas of shallow water [20]. With a surface area of 1m², an artificial nest was immersed at the each corner of the raceways at a depth of 35 to 45 cm. The nests were made with rectangular plastic crates and filled with small gravel. Previously to immersion, the crates and the gravel were disinfected in a solution of 1% Methylene blue, and then rinsed thoroughly with water.

3. RESULTS

At the end of Match, when the water temperature reached 23°C, the fishes began to exhibit constant courtship behaviour (i.e. nipping, nudging, inverted swimming etc.) [3,20]. The males remained close to the nests. The sexual dimorphism in *M. salmoides* is still debatable. Regarding to the size of mature specimens, females are larger than males [1,20,21]. The sexual dimorphism can also be reflected by differences in color [3]. The males from B1, B2 and B3 submitted to smooth abdominal stripping released soft milt. The females showed a rounded abdomen (**Fig.2A**). Indeed, individuals greater than 35 cm in TL can be sexed by looking at the scaleless area surrounding and immediately adjacent to the urogenital opening. In the male, this area is nearly circular in shape while in the female it is elliptical or pear-shaped [4] with a reddish genital opening (**Fig.2B**).

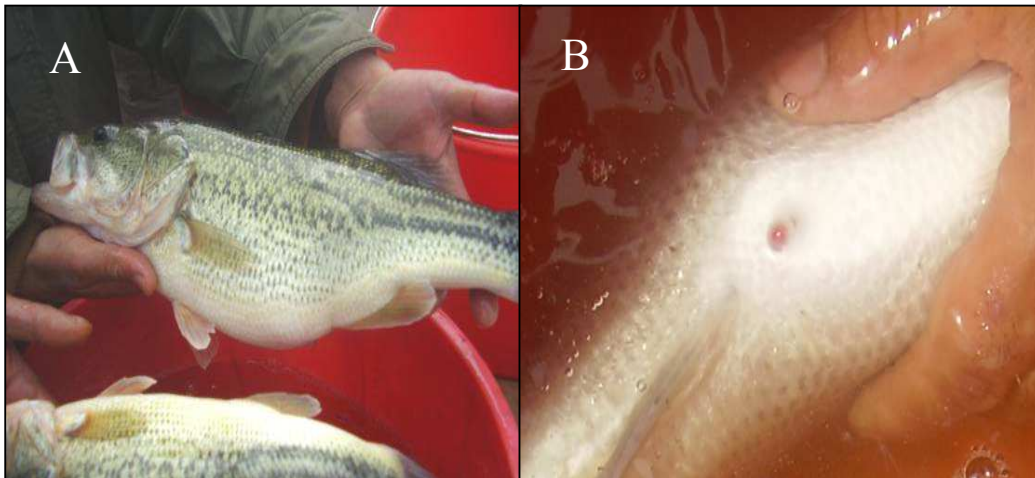


Figure 2: Mature female *M. salmoides* with round abdomen (**A**) and reddish genital opening (**B**).

Spawning

No spawning occurred in R1 despite the courtship behaviour of the males. In R2, 24 hours following the hormonal injection, amongst the 4 immersed artificial nests only 2 were covered with eggs. The first one was from B2 treated with HCG (average 2000 eggs/m²), and the second one from B3 injected with HCG + Pituitary extract (average 3500 eggs/m²). For a mean weight of 1.5 kg of a female *M. salmoides*, the mean spawning was 3700 eggs/kg of fish. The two empty nests from B2 were removed as the females spawned on the concrete bottom of R2.

Hatching and larval care

In R2, the eggs covering the concrete bottom were closely guarded by the males. The artificial nests covered with eggs were transferred into two nursery raceways for better monitoring of the subsequent developing stages under laboratory conditions. With 1 mm in diameter, the eggs were yellow to orange. The incubation period lasted 3 days at 22°C mean water temperature. The hatching from the artificial nests reached a global rate of 80%. Concerning the spawning on the concrete bottom in R2, the hatching rate was lower (17%) owing to the development of *Saprolegnia*. To monitor the fry growth, 92 individuals were transferred into 2 aquariums (75 x 45 x 40 cm). The remaining larvae were transferred into small stocking ponds.

The feeding of the larval stages was *ad libitum* and started from the 4th Day-Post-Hatching (DPH). The diet consisted of phytoplankton, egg yolk, then progressively with live *Artemia* and small larval aquatic insects. Starting from the 6th DPH, in order to insure a good growth with a suitable conversion rate [22], the larvae were reared at a low density (01 larva per Litre), optimal water conditions (24 °C Mean temperature; 12 mg/l dissolved oxygen), and were fed with abundant supply of *Daphnia sp.* Under laboratory conditions, the post-hatching results are as follows (**Tab.1**; **Fig.3**).

Table 1: Monitoring of the larval stages of *M. salmoides* reared under laboratory conditions.

Day Post Hatching	Mean Total Length	Visible characteristics	Supplied Food
1 st	5 mm	Hatching of transparent larva; Mouth not opened; Swim bladder not visible; Abdominal cavity filled with yolk sac that gradually decreases in volume until the 3 rd DPH.	-----
3 rd	6 mm	Well developed eyes; Important decrease in volume of yolk sac; appearance of a primitive digestive tract.	-----
4 th	6 mm	Mouth opened; Disappearance of yolk sac; Primitive intestine present; Eyes very active; Hunting preys behaviour.	Phytoplankton + egg yolk
7 th	7 mm	Mouth visible; Digestive tract very apparent; Pigmented eyes.	
11 th	9 mm	Black spots at the head and the dorsal area; Dorsal and caudal fins present; Food visible at the level of the digestive tract.	Cladocera + <i>Artemia sp.</i> + larvae of aquatic insects + artificial food
17 th	12 mm	Mouth well developed with prominent lower jaw; Lateral line visible; Pectoral fins visible.	
21 st	19 mm	All fins well formed; accentuated pigmentation at the back and lateral sides.	
24 th	45 mm	Fishes bearing intersected caudal fin; anal fin and dorsal fin with a well developed flexible part and spiny part less developed; Ventral fin hardly distinguished; pectoral fins; Lateral line marked with black spots from the caudal fin to the posterior area of the eye; dorsal side of the fish with less accentuated pigmentation; Lower jaw quite visible; Genital areas not visible.	Small shellfish + fingerlings

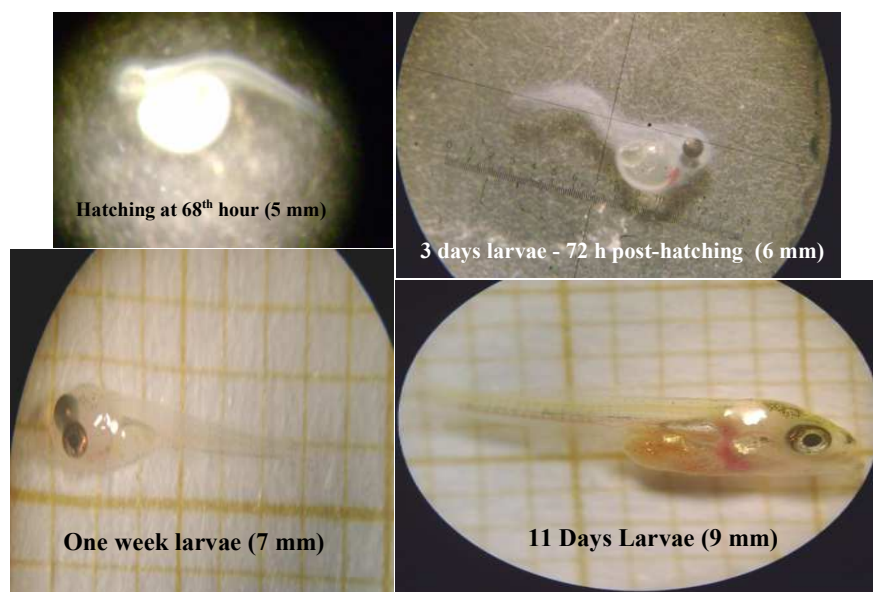


Figure 3: Length growth of fry *Micropterus salmoides*.

During the fry rearing, cases of mortality were observed owing to growing cannibalism (**Fig.4**). As a preventive measure, artificial fish food, with high level of proteins (45%), was supplied until the fishes refused it. Small fingerlings of wild fishes were then supplied from the 24th to the 45th day.



Figure 4: *Micropterus salmoides* larval cannibalism.

During the larval stage, the TL growth displayed a gradual increase until the 17th day, and then remarkably rose up from the 21st day till the 45th day (**Fig.5**) where fingerlings reached 45 mm (**Fig.6**).

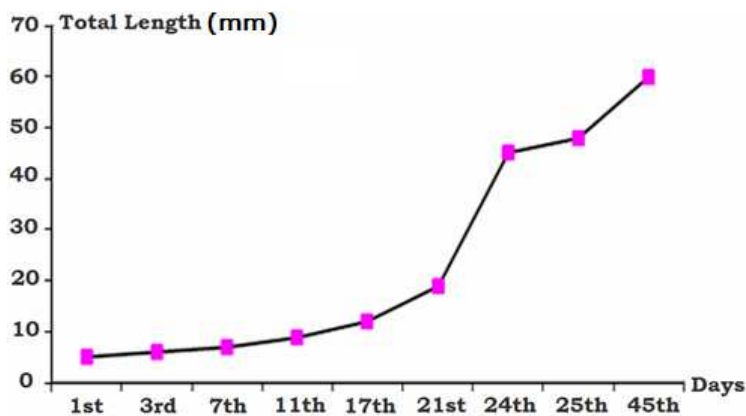


Figure 5: Total length growth of *M. salmoides* under laboratory conditions (N = 30; Mean Temp. 24 °C; 12 mg/l dissolved oxygen).



Figure 6: *Micropterus salmoides* 24th DPH (45 mm).

4. DISCUSSION

Artificial spawning

Hypophysation is the way that gives the most positive results for inducing artificial spawning. The injection of hormones, or extracts of pituitary gland, stimulates the process of final oocytes maturation and provokes the ovulation. Artificial spawning of marine and freshwater fishes, through intramuscular injections of gonadotropin and/or hypophyseal extracts, is well documented [10,11,12,13,19,23,24,25,26,27,28,29,30,31,32,33,34].

Hypophysation by the intra-peritoneal way can also be practised by injecting, through the abdominal wall, an appropriate volume of hormones in order to induce the maturation of oocytes and artificial spawning. Regarding previous intra-peritoneal experiments [11,12], if the injection is not made extremely carefully, the needle can provoke the perforation of the intestinal tube enabling the diffusion of the intestinal content into the abdominal cavity, and the mixing of the intestinal content with the injected volume of hormones. The intra-peritoneal injection can also induce the loss of the injected hormone into the intestinal tube. Such events had compromised the success of artificial spawning and even the death of fishes as it was observed with the silver carp *Hypophthalmichthys molitrix* and *Sander lucioperca* [10,11]. On *Sander lucioperca* and *Clarias gariepinus*, the use of pituitary extracts associated with mammal Gonado Stimulating Hormones (GSH), HCG, and LH-RH are practical to induce artificial spawning, and to provide eggs of good quality [29,35,36]. Fresh or preserved pituitary glands from mature fish of the same species (homoplastic) or from other related species (heteroplastic), and the HCG have been successfully used in most species [24,29]. The HCG stimulates directly the gamete maturation in the gonads [26,37]. It has been used to induce artificial spawning of *M.salmoides* [3,4,37,38,39].

Rottmann et al.[40] indicate that the external environmental factors (photoperiod, water temperature, nutrition, and spawning grounds) may vary considerably among species. This fact is related to the endocrine affinity defining fishes, depending on their original climatic and geographical distribution, as being warm or cold species according respectively to their physiological demand for living and reproducing in warm or cold water temperature. According to Spengler [3] and Lam [41], these external factors have a major impact on the process of maturation of oocytes (to stage 4), ovulation, and spawning. Indeed, Spengler [3] indicated that the water temperature is one of the main external factors in the reproduction of *M.salmoides*, and that in females, the maturation of oocytes is mostly induced by the increase of temperature but not photoperiod. Stuber et al. [42] reported that the natural reproduction of *M.salmoides* occurs with the rapid rise of water temperature above 15.5°C whereas Lorenzoni et al.[1] indicated that the reproduction of this fish occurs when the water temperature is close to 20°C or above. In captivity conditions, manipulation of the environmental factors such as water temperature, photoperiod and spawning substrate is crucial during the steps of artificial reproduction of fishes. According to Harvey and Hoar [24], in the warm climates, once can consider that a high water temperature and a long photoperiod are the primary factors of an accelerated gonadal maturation.

In cold countries such as Canada, *M.salmoides* males can reach sexual maturity by ages three to four, and females at four to five [43]. In Italy, *M.salmoides* mature individuals are able to initiate their reproductive period in late winter and early spring, and the sexual maturity is reached at age 2 years (22cm) for males, and 3 years (30 cm) for females [1]. In warmer temperate climate such as in Algeria, females *M.salmoides* can possibly mature much faster but this has to be confirmed by relevant investigation on the seasonal gonad development. Because of the climatic conditions, the spawning season of *M.salmoides* occurs during April and May in the USA [37] and in Italy [1]. In Algeria, the warm temperate climate conditions are favourable for an earlier artificial spawning of *M.salmoides* as the water temperature in ponds usually reaches 20°C to 25°C during the day starting from the beginning of March. However, during the night the air temperature can drop to 15°C or less, and would compromise any expectations of artificial spawning in open air with no cover conditions [10,11,12,13].

The fish behaviour can also boost the spawning steps [20,40]. It is possible to observe the fishes spawning when mature females are in the presence of other active females in spawning grounds, or when females are in presence of active females who already had received a hormonal treatment [12,13,36]. This previous element has not been considered in this work in order to fulfil the control group concept with B1. Despite the courtship display of the males, females of B1 did not spawn, probably because they did not reach the final steps of the maturation of the oocytes (stage 4), prior to the ovulation stage (i.e. spawning). In R2, for a mean weight of 1.5 kg of a female *M.salmoides*, the estimated mean spawning (3700 eggs/kg of fish) is within the range of the natural spawning (5000 to 15000 eggs) reported in the literature [44].

Hatching, larval care and development

Hatching occurred 70 hours at 22°C whereas Hill and Cichar [45] indicated it at about 45.5 hr post-fertilization at 22.2°C. With the increase in age and body size, the voracious young *M. salmoides* changes its diet, with juveniles normally feeding on small crustaceans, before switching to insects and then finally to fish prey as adults [2]. If *M. salmoides* is reported to begin piscivorous feeding at approximately 100 mm TL [46], it is a fish-eater when reaching 5 cm in total length (present study). According to Brown et al. [43], in Lake Washington (USA), the feeding and diet of young *M. salmoides* included fishes when fry reached 61-80 mm. Other authors [45,43] indicated that if *M. salmoides* swallow prey whole, the ratio between gape and prey size is critical in determining when the fish become piscivorous. In the consulted literature, the growth of fry is not available and the available data concerned individuals > 40 mm or over [21,45,46,47, 48,49]. During the larval breeding of the present study, the recorded mortalities were mainly due to predation. The cannibalistic feeding is a predominant feature in *M. salmoides* [6].

4. Conclusion

Despite the fact that this investigation was carried out on a limited number of individuals, this first experimental controlled reproduction of *M. salmoides* in Algeria was successfully carried out through the use of hormonal induction with HCG, and HCG mixed with pituitary extract. It is evident that the use of HCG alone to induce an artificial spawning of *M. salmoides* does not remain reliable whereas successful results are reported in the literature for Cyprinids, *Sander lucioperca* and *Clarias gariepinus*. This work confirms the successful use of artificial gravel nests that led to a high rate of hatching. The low density rearing of the larvae and the fingerlings, under a high level of dissolved oxygen and a sufficient natural food supply, led to satisfactory growth performances of the fry. Further experiments of induced reproduction on a larger number of *M. salmoides* would enable to refine the protocols of artificial spawning of *M. salmoides* in order to insure valuable restocking of the various dams, lakes and water reservoirs of the country.

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