Artificial reproduction of royal carp (*Cyprinus carpio*)

Abdelkader Rouabah, Nora Mimoune, Djamel Khelef and Rachid Kaidi*

Abstract

The aim of this study was to establish an experimental protocol for the artificial reproduction of royal carp (*Cyprinus carpio*) with the hormonal induction of spawning. For this purpose, we conducted this study at an aquaculture farm in Khemis Meliana (Ain Defla, Algeria). The study included six royal carp broodstock, 4 females and 2 males. Hormone injection was performed in the dorsal muscle under the fin. The induction of carp was done successively in females by GnRH at a dose of 3 mg/kg and in males by hCG at a dose of 500 IU per kg weight. Fertilisation was done artificially by the dry method. After incubation of eggs, a binocular magnifying glass was used to check the condition of eggs and the development of the embryos over time. The results obtained show that the artificial reproduction of this species was possible, with success of ovulation and fertilisation, and hatching and growth of the larvae. Stripping was done after 24 h of hormonal stimulation at a temperature of 21°C. Disaggregation of eggs was performed efficiently with whole milk. The total number of eggs was 552,000 eggs with a weight of 1104 g. The average latency time was 22 hours. The hatching rate after 3 days of incubation on artificial spawning grounds at 21±1°C was 441,600 larvae (80%). Larvae food follow-up started from the 3rd day post-hatching with a combination natural food (rotifers) and artificial food. At the end of this experiment, we can conclude that it is possible to improve reproduction through the proper use of hormonal stimulation techniques and by improving feeding and abiotic factors that are dominant in fish farming.

Key words: *royal carp; GnRH; hCG; artificial reproduction; food*

Introduction

Aquaculture is a vast and varied activity, composed of the farming of sea fish, freshwater fish, shellfish, molluscs, and algae, and is becoming increasingly important in the food development sector. It is considered a major sector in the production of food with high animal protein content, and has become

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important in filling the deficit in fishery products on all continents (Laffoley et al., 2019). In the last decade, it has experienced considerable important growth worldwide, and it is increasingly considered to be an integral part of the means used to ensure food security and global economic development (FAO, 2012).

There are several methods of breeding farmed fish. Their choice depends on the reproductive biology of the species, local environmental conditions and available facilities. These methods can be classified into three categories: natural, semi-natural and artificial reproduction (FAO, 2012; Tkacheva et al., 2020).

The royal carp (*Cyprinus carpio*), is a valuable commercial fish with a growth rate acceptable to the market size under intensive culture. It is also an excellent fish for farming, because of its economic character compared to other production and its high market value. It can constitute a fish community in reservoirs and is widely used in rearing due to its broad ecological tolerance to a wide range of temperatures, dissolved oxygen levels, and its short trophic chain (Tessema et al., 2020). *Cyprinus carpio* is an introduced species in Algeria with the first introduction dating back to 1860. Since then, more than 86 actions to restock lakes and reservoirs in Algeria have been recorded as part of the national programme for the introduction of exotic species (Kara, 2012).

According to Legendre et al. (1996), the seasonal character of fish reproduction is one of the major barriers that hinder supply on the international market, and the control of artificial reproduction thus became an obligatory step towards the optimisation of aquacultural production. The aim of this study was to examine artificial reproduction in the royal carp (*Cyprinus carpio*) with the hormonal induction of the spawning.

Material and methods

Study site

Our experimental protocol on royal carp artificial reproduction, and the embryonic and larval follow-up, was carried out at an aquaculture farm, located in Ain Sultane (Khemis Miliana, Ain Defla, Algeria, figure 1). This farm was opened in 2019 with the objective of reproducing noble species (Pikeperch, *Clarias gariepinus*, *Oreochromis nilotica*, Chinese and Koi carp) using intensive techniques to accelerate their growth, with the simultaneous integration of aquaculture with agriculture. This experimental site is located more than

Figure 1. Ain Sultane aquaculture farm

10 km from the city of Ain Sultane, and the farm covers an area of 20 hectares. It is located in the plain of Cheif, and the land is irrigated by water pumped at 50 L per second.

The farm is equipped with 10 large geomembrane tanks: each with a diameter of 15 m, depth of 1.20 m and volume of 218 m^3. There are six small tanks indoors, each with a volume of 4 m^3. All tanks are equipped with a drainage system. The hatchery has an of 200 m^2, it is thermos-regulated and consists of 12 tanks each of 9 m^3 and equipped with a drainage system. The water is very rich in organic matter and trace elements and is then used to irrigate fruit trees, thus increasing productivity.

Broodstock

As biological material, 2 males of royal Carp (*Cyprinus carpio*) weighing 4 and 5 kg and 4 females with a body weight of 5 kg were used for reproduction.

Pond preparation

All broodstock ponds were thoroughly cleaned to remove all vegetation and waste on the walls and floor. The broodstock were introduced into the ponds carefully, taking biomass into consideration.

Physico-chemical parameters of the water

Water properties were regularly measured using a multi-parameter tool: temperature, O_2, pH, salinity, NO_2, NO_3, NH_3, NH_4.

Spawning ground creation

Artificial spawning grounds were made with welded mesh, mosquito netting, wire, and ropes. They were then disinfected with sodium hypochlorite (NaOCl), washed with water and exposed to the sun for drying and disinfection (Figure 2).

The pond was emptied of spawners by means of a draining system and when the water level was low, we fished them with nets.

Figure 2. Spawning ground preparation and cleaning

Experimental design

Selection and sexing of broodstock

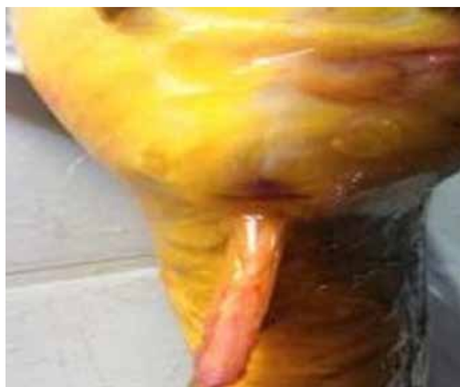
Selection

Broodstock selection consisted of capturing adult fish before the natural spawning period or before their migration to the spawning grounds, while avoiding stressing them as they are very vulnerable to injury when caught in nets or during transport.

The selection was based on certain gender-dependent criteria. Mature females were selected on the basis of belly roundness, indicating the proper development of ovaries, and the genital papilla was swollen, protruding, red or pink. For males, larger ones were selected since their testes are well developed and full of semen. This was confirmed by the release of a few drops of semen when pressing on the flanks. The dorsal surface of the pectoral fins was roughened; there was the presence of rough patch at the base of the pelvic fin and the head; and these males are more active and move more than others.

Males and females can easily be distinguished due to pronounced sexual dimorphism (Figure 3). It is necessary to take care that they are neither sick, wounded, nor parasitised, and only the

Male genital papilla



Female genital papilla

Figure 3. Genital papilla

best subjects should be selected. It is also important to take into consideration that wild spawners are not easy to capture because they become nervous and jumpy while refusing to feed.

Sexing

Examination of the distinguishing characteristics of sexual dimorphism in carp *Cyprinus carpio* was done manually to separate males from females. In females, sexual dimorphism was very marked by the significant difference of size. The females have a more dilated abdomen than males and the presence of genital papilla which slightly emerges of the size of a hazelnut.

Sedation of spawners

Before each manipulation, the broodstock were anesthetised with a solution of clove oil (*Eugenia cariophylata*) which is a natural anaesthetic. Many hatcheries and research institutions use clove oil in balneation (sedation) to immobilise fish (for their handling, sorting, marking, artificial breeding without stressing them) using one drop per litre of water or 0.5 mL per 10 litres of water (Javahery et al., 2012).

Weighing and marking

After sedation of the spawners and before injection, they were marked for easy identification at any time**.** The broodstock were weighed and measured with an ichthyometer (length, width, and weight). Once sexed, marked and weighed, the selected fish were carefully placed in the broodstock pools so that they could be maintained in good health during all the experiment steps.

The marking was made on the caudal fin, with different colours to facilitate the identification of the spawners; alternatively, we used $AgNO_3$ which macerates the skin and allows for the marking of spawners (Figure 4).

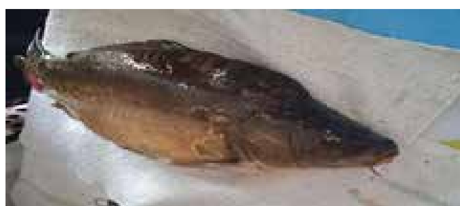


Figure 4. Broodstock marking

Preparation of injections

The hormones used were GnRH and hCG. First, the broodstock was

weighed with a precision scale in order to determine the total volume of the hormone solution needed.

For females, we used 3 mg GnRH diluted in 0.5 mL saline solution per 1 kg of live weight (Billard, 1997) and for males, we used 500 units of hCG per 1 kg of weight. GnRH was ground in a porcelain mortar to a fine powder, with the addition of saline solution measured in a 5 mL graduated syringe. After the solution was well mixed, it was aspirated with another 5 mL graduated syringe. For hCG, we mixed a solution containing 5000 IU per mL (1 mL solution bought with hCG and 9 mL saline solution) with the powder and aspirated with 2 graduated syringes of 5 mL.

Hormonal induction

The hormonal solution (GnRH for females and hCG for males) was injected into spawners close to the dorsal fin in the direction of the head of the animal slowly by tilting the syringe at 45° and by entering the first third of the needle (0.5 cm). The injection zone was gently massaged at the injection zone to avoid the return of the hormonal solution (Figure 5).

The genital papilla of the female was sutured in the form of X with a surgical needle to avoid the unwanted release of the eggs into the water. The spawners (four female and two males) were placed in a pond and a happa was installed on which the spawners will spawn.

Latency

The latency time depends on temperature. It is of the order of 18 to 24h or 360° h. The female spawners release chemical substances (pheromones) at the time of ovulation that stimulate the males to do the nuptial dance and at this moment the females are ready for fertilization. This is the same for males.

Stripping

In females, the tail and fins and especially the area of the genital papilla were well dried. Before stripping, we removed the thread that sutured the genital papilla and then proceeded with stripping by exerting an abdominal pressure from top to bottom that allowed us to recover the eggs (*in vitro*) in a plastic container that was previously dried and tared so that the eggs could be weighed and counted (Figure 6).

The males were also stripped according to the same protocol as the females and their sperm were recovered at the level of the genital opening with a 5 mL syringe, after cleaning the genital papilla of the fish and after having taken care to eliminate the first drops of sperm because they are often soiled by intestinal contents, water or blood, which in contact with water may prematurely

Figure 5. Hormonal induction of the royal carp

Figure 6. Collection of eggs by stripping

activate fertilisation. A creamy, whitish or yellowish milt, without any trace of blood is considered good quality (Gilles et al., 2001).

Artificial fertilisation

The technique described by Billard (1995) was used. The eggs recovered from the female were used and mixed with the milt of the two males with a disinfecting spoon for 3 to 4 minutes at dryness. The male gametes are then activated by a fertilising solution called Woynarovich which contains 10 L water, 40 g salt and 30 g urea. Only pond water was used for fertilisation (Figure 7).

Disaggregation

Before putting the eggs in the Zug bottles, 5 g tannic acid was added to 10 L water, and 1 L of this solution was added to 10 L of fertilized eggs. After about 20 seconds, the water was changed 2 to 3 times and the eggs were quickly placed in the Zug bottles. Whole milk (3.2% milk fat) was added for 15 min, and this was repeated three times until deagglutination. This was followed by

Incorporation of spermatozoa on eggs

Mixing of gametes

Activation of gametes by the fertilising solution

Change of the fertilising solution

Figure 7. Artificial fertilisation

rinsing with water to eliminate dead cells (Figure 8).

Incubation and embryo monitoring

After fertilisation, the egg starts cell division and continues its development until hatching. Incubation is strongly regulated by temperature and continuous oxygen supply (Figure 9). The duration requires 60 to 70 degree-days or 3 to 4 days at a temperature of 20-22°C (Billard, 1995).

During incubation, the cell divides into the morula, blastula, gastrula, thus forming an embryo with a head, a tail and two eyes with closure of the blastopore. The embryo then transforms into a larva before hatching (Woynarovich, 1980).

At this stage, a light microscope (40 x) was used to check the state of embryonic development of the eggs as a function of time and to identify the different stages of embryonic development. Egg development is a rapid process that goes from egg swelling to embryonic morphogenesis (Figure 10).

Egg hatching

The larvae hatch from the shell due to the action of the frontal enzyme. At this time, the larvae have no mouth, intestine, swim bladder, or cloaca, but only the yolk sac that provides it all the necessary nutritional elements for its development.

Larvae begin to actively search and take food on the 3rd day post- hatching if the temperature is around 22°C (Roule, 1932). Thus, the larvae become post-larvae.

Figure 8. Disaggregation with whole milk

Figure 9. Incubation of eggs in the spawning grounds

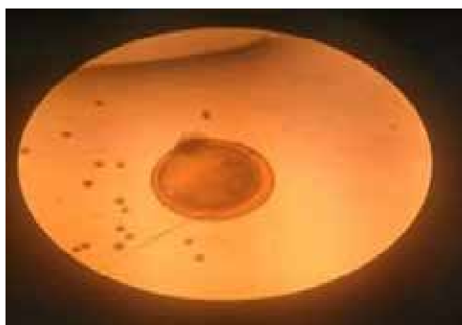


Figure 10. Egg (magnification 40 x)

Larval monitoring

During the first two days post-hatching, the larvae swim vertically to fill the swim bladder and keep the swimming horizontal, and feed through their vitelline sac. On day 1 post-hatching, a microscope is used to observe the general shape of the larvae. After day 5 post-hatching, larvae were fed *Saccharomyces cerevisiae*, phytoplankton, rotifers and *Daphnia nauplii* using a plankton net. The tank was fertilised to accelerate the planktonic bloom. Figure 11 showed the administration of yolk of eggs to feed the larvae.

Zooplankton culture

Zooplankton is the preferred food for most fish larvae during their early life stages, because regardless of its nutritional value and very high digestibility, this live food is easily detected and captured by the larvae, due to its small size, swimming movements in the water column. In this study, we produced zooplankton from cow manure.

We placed a quantity of cow manure into a bag. This organic matter increases the natural food (phytoplankton and zooplankton) and a quantity of lime ($CaCO_3$) according to the pH in order to increase the pH of the media and ultimately to obtain high concentrations of chlorophyll. Finally, 500 g NPK fertilizer was added, containing nitrogen, phosphorus, and potassium, as the main mineral substances used by the phytoplankton. The bag should be well closed and small holes made for the penetration of water and the progressive decomposition of organic matter and its leaching into the water. It is necessary to take care not to provoke eutrophication of the water. The purpose of this operation is to obtain the phytoplankton necessary to feed the zooplankton.

In a second step, daphnids were collected with a plankton net and put in the phytoplankton tank after 6 to 12 days to obtain zooplankton. The dominant zooplankton groups found in cow manure are rotifers, cladocerans, and copepods.

Results

In vitro fertilisation and incubation of eggs with the use of artificial reproduction techniques that involve the use of natural or synthetic hormones promoting the final maturation allows us to obtain a substantial amount of data.

Reproduction

We used 6 broodstock: 2 males and 4 healthy females with the following characteristics (Table 1).

Figure 11. Feeding with egg yolk

Table 1. Results of the measurements of the spawners

	Females	Male 1	Male 2
Average length cm	62	56	56
Average width cm	53	/	/
Average weight kg	5.7	4.25	4.6

Males were injected with hCG (500 IU/1 kg weight)
Male 1: 2200 IU hCG (4.2 mL hCG solution) at 17:00h
Male 2: 4600 IU hCG (4.6 mL hCG solution) at 17:15h.

The females were injected at 16:30h with GnRH. One GnRH ball corresponds to 3 mg/1kg weight so each female needed 6 balls of GnRH x 3 mg=18 mg.

Latency

Temperature fluctuated between 19 and 21°C during the day and the spawners require about 360°C/h of temperature to complete gametogenesis (ovulation). In this particular case, 24 hours of latency time was required.

Fertilisation

Female belly volume became more swollen just before stripping. After the harvest of eggs by stripping and weighing the eggs, the total mass of eggs was 1104 g. For the two males, we took just the quantity of 5 mL milt. To determine the weight and number of eggs, a 1 g sample was taken and counted, with 500 eggs in 1 gram. Therefore, the total number of eggs = number of eggs in 1 g × total weight of eggs = 500 × 1104 = 552,000 eggs.

Fertilisation rate

A random sample of 100 eggs was taken from the spawning grounds on day 1 of incubation. The fertilisation rate (according to Guenachi and Dilmi, 2002) = fertilized eggs ×100 / total number of eggs = 80 × 100 /100 = 80%.

Number of fertilised eggs

The number of fertilised eggs = total number of eggs× fertilisation rate /100 = 552000 **× 80/100** = 441,600 fertilised eggs.

Incubation and embryo development

Incubation took place on spawning grounds made of wire in a pond with 30 cm of water depth. Temperature varied between 20 and 21°C and full hatching occurred after 3 to 4 days. Fertilisation is followed by a process of cellular cleavage that corresponds to a series of rapid mitotic divisions, allowing for the formation of numerous cells called blastomeres.

During incubation, the embryonic cycle continues within the egg shell, which the larvae break at the time of their hatching through the action of frontal enzymes.

As in all other fish, the embryonic phase of royal carp begins at the time of fertilisation of the egg. Egg development begins as soon as they have been fertilised and are in contact with water. It takes place in stages until the fish larvae hatch; this is the incubation period, and is characterised by the feeding (endogenous) of the embryo from the yolk.

In the egg swelling phase, the dry fertilised eggs absorb water for hydration and the peritelline space develops, the eggs grow in volume and become larger than the dry eggs. In the cleavage phase, embryonic development begins, as well as the stages morula, blastula and gastrula until organogenesis. The embryonic phase starts when organogenesis is

initiated and stops once hatching is completed. In the phase of cell division and germ development, the single-cell animal pole is subdivided into 2, 4, 8, 16, and 32 cells successively, all arranged in a single layer. Subsequent cell divisions produce a multi-layered blastoderm at the end of the morula phase. In the embryo development phase, the fish embryo forms around the yolk. The head and tail are formed, and the eyes can be distinguished. Fish eggs are very sensitive during the cell division phase and until the end of the morula stage.

Difficulties were encountered in monitoring embryogenesis, as not all stages of embryogenesis were determined. Several egg samples were used to determine the stages, and the majority of the eggs were in the morula stage, first movement stage and pre-hatch stage (Figure 12).

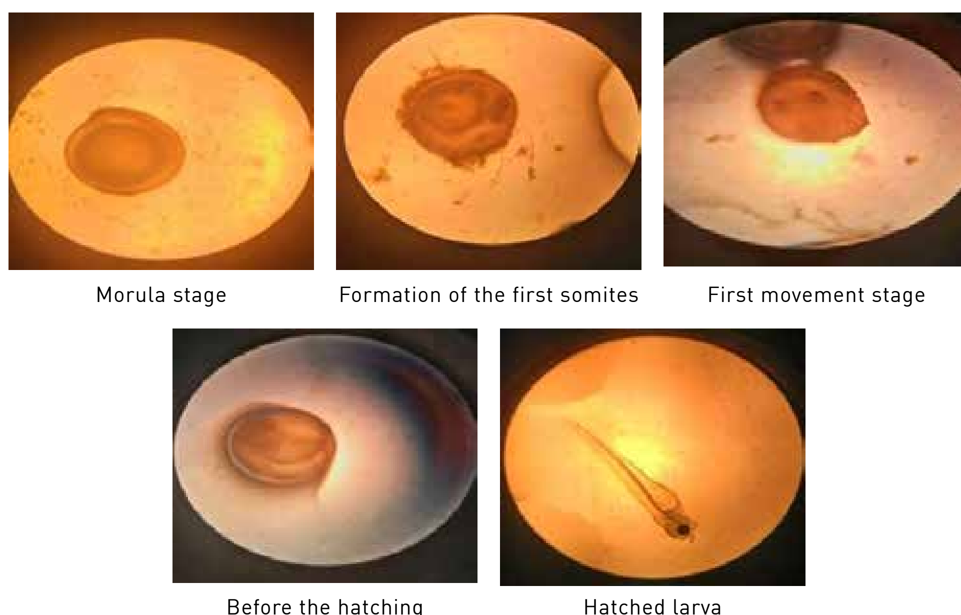


Figure 12. Embryo development

Figure 13. Heterogeneity of the sample of royal carp alvins at age 45 days in the same pond

Hatching

Hatching rate was always in the same sample of 100 eggs. Hatching rate = number of larvae × 100/number of eggs =80×100/100 =80%.

The total number of larvae = total number of eggs × hatching rate / 100 = 552,000 × 80 / 100 = 441,600.

Larval rearing

On day 3 post-hatching, larvae were fed egg yolk and *Saccharomyces cerevisiae* three times a day. From day 5 post-hatching, daphnia, phytoplankton and artificial food powder were given three times a day. After day 45 post-hatching, samples were taken at random from around the pond. We noticed that the fry were heterogeneous (in size and weight) despite originating from the same reproduction, fertilisation and hatching (Figure 13).

Discussion

The present findings indicate successful reproduction of *C. carpio*. *In vitro* fertilisation and incubation of eggs with the use of artificial reproduction techniques involving the use of natural or synthetic hormones promoting the final maturation enabled much data to be collected. According to several reports, the selection of the females to be induced is made on the basis of the homogeneity of fish size and of the ovules and their diameter, generally between 1.4 and 1.6 mm (Viveen et al., 1985; Graaf and Janssen, 1996; Gilles et al., 2001). In addition, the swelling of the fish's belly is a good indicator (Tamas et al, 1982).

In order to maintain high productivity when applying controlled and semi-controlled reproduction, it is necessary to control the reproductive cycle of biological species, especially its reproductive aspect, including the period of reproduction, size of sexual maturity, sex ratio and the environmental determinism (Hossain et al., 2017; Khatun et al., 2019). *C. carpio* grows rapidly, achieving sexual maturation in the second year of life, and is highly fertile (about 2 million eggs per female) (Hossain et al., 2016). The combination of these features allows this species to develop invasiveness potential (Troca and Vieira, 2012).

Our choice of the period of reproduction was dependent on the number of degree days which is generally 1200°C hour from January 1 until the date in March when the fish reaches this number of degree hour and correspond macroscopically (abdomen dilatation and oviduct or genital papilla hyperaemia). To confirm this probable ovulation, a biopsy is conducted to determine the stage of ovogenesis of the genitors and if it is not in dormant phase (meiosis II, metaphase II).

Cyprinus carpio is omnivorous fish that consumes food of animal (aquatic insects, macroinvertebrates, and zooplankton) and plant origin (phytoplankton, macrophytes) (Tessema et al., 2020). The broodstock reproduced in this study were given artificial feed rich in protein (33%) and in energy, with the supplementation of vitamins and trace elements, with the addition of foods that are naturally rich in chitin to accelerate the emptying of the intestinal tract and allow better assimilation and a very high coefficient of transformation from the head and pectoral fins. Live food makes up most of the fish larvae diet, and is particularly important in the rearing of larvae whose yolk sac is rapidly depleted while their digestive system is still rudimentary. Zooplankton have an effect on the physico-chemical and planktonic parameters of the water, increasing pH, conductivity and the nutrient load (ammonia, phosphorus, nitrite and nitrate), contributing to good growth of the plankton population (Tavares et al., 2009). Thus, the use of organic waste improves the phytoplanktonic and

zooplanktonic biomass of the fertilized water (Garg, 1996; Garg and Bhatnagar, 1999; Tavares et al., 2009). Rotifers are successfully used as live food for several fish species to improve larval growth and survival performance and to increase of production yield (Villegas et al., 1990; Reitan et al., 1993; Lim and Wong, 1997; Castell et al., 2003). It is important that there is a positive correlation between natural food rich in chitin and essential amino acids, vitamins and trace elements, and artificial food, since according to Horvath (2002), the effect can be epistatic (interaction between several genes) or pleiotropic (quality of a protein with the ability to govern several traits and determine physiological effects).

GnRH gave good results as an inducer in different at in the aquaculture farm of Ain Sultane. In particular in the royal carp, the dose of 3 mg (one ball) /1 kg of female weight was sufficient to induce ovulation; in this study, a female of 5.7 kg was injected with 5.7 mL solution of 6 balls of GnRH in 6 mL saline solution. The egg mass was about 20% of the fish mass (1104 g of eggs). This quantity indicates that the broodstock were obviously well fed with artificial food and live food (zooplankton). Males were injected with hCG because it gives satisfactory results, about 5 mL milt was received despite the fact that males had a very large quantity of milt, since more was not required for the fertilisation of our eggs. hCG is available and costs less than GNRH. According to Munsur Ali et al. (2016), hormone injection into the muscular basal region of the pectoral fin gives the best induction result. Although induction by hormone therapy appears to be the most effective method in artificial reproduction (Zidan et al., 2020), this widely used method must be performed with great caution, respecting the concentrations prescribed for each fish species. In the same context, the main constraint to this form of reproduction for small producers is the high cost of commercial hormones (Géoffroy et al., 2019).

The latency time extended to 24 hours at temperatures 22±1°C. Eggs require around 360°C h and the females begin to release pheromones into the environment to signal their maturity (ovulation). This phenomenon is a bio-indicator for us to know the best time for stripping (Gilles et al., 2001).

In the current study, fertilisation was performed artificially according to the dry method described by Janssen (1985). Egg fertilisation depends on sperm quality (Blecha et al., 2016). Other studies have revealed a new method to deliver sperm to eggs in common carp and revealed that sperm injection into the ovary can also result in successful fertilisation (Müller et al., 2018, 2020).

In stripping, good quality eggs are in liquid form and do not contain many white eggs. The best egg colour ranges from brown to green, and the problem that arises in dry fertilisation is the agglutination of eggs. We used whole milk that is rich in fat to remove all the adhesion of the eggs.

Egg incubation took place in artificial spawning grounds to facilitate the separation of eggs and keeps eggs from falling into the mud to avoid asphyxiation, thus ensuring better aeration of the embryo and inhibiting the development of saprolegnosis. The incubation time required 84 h to 96 h until hatching. With an average of 22°C or 1848 to 2112°C/h. This difference in the time of hatching expresses the differentiation of the speed of embryo development from one egg to another due probably to the exposure of the egg on the spawning ground (oxygenation, temperature). The unfertilised eggs take on a white colour and degrade in the water over time and can cause mortality of viable eggs by saprolegnosis. Temperature and dissolved oxygen content are factors that

limit growth performance. Temperature is a crucial ecological factor for the survival of species in running waters, and which has significant ecological repercussions ((Makhoukh et al., 2011). It acts on the nitrogen retention coefficient, and therefore, on the weight growth of fish. Water temperature is very important for initial egg development, larval growth and survival (Cho et al., 2015). It directly affects aquatic life and the chemical conditions in water, in particular the absorption capacity of gases (Munsur Ali et al., 2016).

The first few hours after hatching, the larvae feed on their yolk and swim vertically to fill the swim bladder and become able to swim horizontally. The larval phase is a delicate and crucial stage of fish rearing (Park et al., 2017). During this phase, feeding plays an important role on the development of the larvae, including the development of the skeletal system. Several recent studies have correlated the increase or decrease in the rate of malformations in larvae and later in juveniles with the quality of the feed consumed (Mazurais et al., 2009; Darias et al., 2011). After 45 days of feeding, the fry reach sizes of 2 to 10 cm and over 20 grams in weight.

The heterogeneity of the larvae is explained by the availability of natural and artificial food that the larvae find in their environment (Adámek et al., 2015), and by their genetic predisposition.

Conclusions

This study examines the artificial reproduction (controlled) of royal carp *Cyprinus carpio* using two hormonal inducers GnRH and hCG at the aquaculture station of Laribi Sadak in Ain Sultane. Carp induction was performed successively by GnRH in females and by hCG in males with conclusive results. All the broodstock responded well to injection. During this experimental study, we achieved positive results, with good feeding of the broodstock and a correlation between natural zooplankton-based food and artificial food rich in amino acids and essential trace elements, energy and especially vitamins, which boosted (flashing) gametogenesis and allowed an appreciable hatching rate. It should be noted that in order to avoid cannibalism, it is necessary to proceed with the calibration of the larvae to ensure batches of fish and thus accelerated growth of alvins, while avoiding cannibalism which increases the rate of mortality in intensive breeding.

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Umjetno osjemenjivanje šarana (*Cyprinus carpio*)

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Cilj je ovoga bio rada utvrditi eksperimentalni protokol u svezi umjetnog osjemenjivanja šarana (*Cyprinus carpio*) s hormonalnom indukcijom mriještenja. U tu smo svrhu proveli studiju na akvakulturnoj farmi u Khemis Meliana (Ain Defla, Alžir). U ovom smo radu koristili šest šarana iz matičnog jata: 4 ženke i 2 mužjaka. Hormonalne injekcije dane su u dorzalni mišić ispod peraja. Indukcija šarana u ženki obavljena je sukcesivno putem GnRH pri dozi od 3 mg/kg i mužjaka dozom hCG po stopi od 500 IU po kg mase. Oplodnja je obavljena umjetno, suhom metodom. Nakon inkubacije jajašaca za provjeru stanja jajašaca i razvoja embrija tijekom vremena rabljeno je binokularno povećalo. Dobiveni rezultati pokazuju da je umjetno osjemenjivanje ove vrste moguće, kao i preživljavanje i rast ličinki s uspješnom ovulacijom, oplodnjom i izlijeganjem ličinki. Prikupljanje zametnih stanica obavljeno je nakon 24 sata od hormonalne stimulacije na temperaturi od 21 °C. Deagregacija jajašaca učinkovito je obavljena pomoću punomasnog mlijeka. Ukupni broj jajašaca bio je 552,000 jajašaca s masom od 1104 g. Prosječno vrijeme latencije bilo je 22 sata. Postotak izlijeganja nakon 3 dana inkubacije na umjetnoj podlozi za mriještenje na temperaturi od 21 °C +/- 1 bio je 441,600 ličinki (80 %). Naknadna hranidba ličinki započela je trećeg dana od izlijeganja s diversifikacijom između prirodne hrane na bazi rotifera i umjetne hrane. Na kraju pokusa, možemo zaključiti da je moguće ispravnom uporabom tehnika hormonalne stimulacije i poboljšanjem hranidbe i abiotičkih čimbenika koji su dominantni u uzgoju ribe poboljšati reprodukciju.

Ključne riječi: *šaran, GnRH, hCG, umjetno osjemenjivanje, hrana*