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Topic:

Study of spawning induction in Black Bass Micropterus salmoides (lacepéde. 1802)

Presented by:

GAMOUZI Mallek

HENNA Nibras Taquoua

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Mr. REFFAS Wahid.	Professor - ENSSMAL	President
Mr. Dilmi Ammar	Senior Lecturer B - CNRDPA	Examiner
Mrs. CHABET DIS Chalabia.	Senior Researcher B - CNRDPA	Supervisor
Mrs. MESLEM Nabila.	Senior Lecturer B - ENSSMAL	Co-Supervisor

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Dedication

To my dear family and friends, with heartfelt gratitude, I dedicate this work to you.

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Thank you for believing in me, especially during the most challenging moments.

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Dedication

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In solidarity with Palestine, a land of resilience and hope. May peace and justice prevail.

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Absract

This study investigated the controlled spawning of the largemouth bass, *Micropterus salmoides*, in order to contribute to sustainable aquaculture of the species in Algeria. Broodstock fishing was carried out at El Maouane Dam, setif province, where wild broodstock were caught and acclimated before hormonal induction that was carried out at the CNRDPA in Bou Ismail, in the Tipasa province.

Fish were sexed by external observation or by catheterization after anesthesia in order to monitor sexual maturity. Fish were weighed, measured, and administered injections of hormones (HCG and pituitary glande). Artificial nests filled with disinfected gravel were added to the tanks to improve spawning. Fertilized eggs were incubated in aerated bottles to provide good water flow and oxygen.

The water quality was within the recommended ranges for the reproduction of Black Bass. only 10% of mature oocytes were observed with an average diameter of 0.97 ± 0.01 µm. 30% of the female broodstock injected with HCG showed signs of successful spawning. Spawning was observed on average 72 hours after the hormone injection. no spawning was observed in the females injected with pituitary gland and all males wich injected only with pituitary gland responded. A strong correlation was observed between the diameter of the fertilized egg and the culture time with coefficient r = 0.97. The eggs obtained were severely affected by saprolegniosis. with a developmental arrest and total egg mortality

Keywords: Broodstock; HCG; Oocytes; Pituitary glande; Saprolegniosis

Résumé

Cette étude a porté sur la ponte contrôlée de l'achigan à grande bouche (*Micropterus salmoides*) afin de contribuer à une aquaculture durable de l'espèce en Algérie. La pêche des géniteurs a été réalisée au barrage d'El Maouane, dans la wilaya de Sétif, où des géniteurs sauvages ont été pêchés et acclimatés avant l'induction hormonale qui a été réalisée au niveau du CNRDPA de Bou Ismail dans la wilaya de Tipasa.

Les poissons ont été sexés par observation externe ou cathétérisme après anesthésie afin de suivre la maturité sexuelle. Les poissons ont été pesés, mesurés et ont reçu des injections d'hormones (HCG et hypophyse). Des nids artificiels remplis de gravier désinfecté ont été ajoutés aux bassins pour améliorer la ponte. Les œufs fécondés ont été incubés dans des bouteilles aérées pour assurer une bonne circulation de l'eau et un apport d'oxygène suffisant.

La qualité de l'eau était conforme aux recommandations pour la reproduction de Black Bass. Seuls 10 % des ovocytes matures ont été observés, avec un diamètre moyen de 0,97 ± 0,01 µm. 30 % des femelles reçu une injection d'HCG ont montré des signes de ponte réussie. La ponte a été observée en moyenne 72 heures après l'injection d'hormone. Aucune ponte n'a été observée chez les femelles ayant reçu l'injection d'hypophyse, et tous les mâles ayant reçu l'injection d'hypophyse seule ont répondu. Une forte corrélation a été observée entre le diamètre de l'œuf fécondé et le temps de culture, avec un coefficient r = 0,97. Les œufs obtenus étaient gravement atteints de saprolégniose, avec un arrêt du développement et une mortalité totale des œufs.

Mots-clés : Géniteurs ; HCG ; Ovocytes ; Hypophyse ; Saprolégniose

ملخص

بحثت هذه الدراسة في التبويض المُتحكم فيه لسمك القاروص كبير الفم (Micropterus salmoides)، بهدف المساهمة في تربية مستدامة لهذا النوع في الجزائر. تم صيد الأسماك الأم في سد الموان بولاية سطيف، حيث تم الحصول على الأسماك الأم البرية وتأقلمها قبل تحفيزها هرمونيًا على مستوى المركز الوطني للبحث والتنمية في الصيد البحري وتربية المائيات ببوإسماعيل ولاية تيبازة.

تم تحديد جنس الأسماك عن طريق الملاحظة الخارجية أو القسطرة بعد التخدير لمراقبة النضج الجنسي. تم وزن الأسماك وقياسها وحقنها بهرمونات (HCG والغدة النخامية). أُضيفت أعشاش اصطناعية مليئة بالحصى المُعقم إلى الخزانات لتحسين التبويض. حُضنت البيضات المُلقحة في زجاجات مُهواة لتوفير تدفق جيد للمياه والأكسجين.

كانت جودة المياه ضمن النطاقات المُوصى بها لتكاثر سمك القاروص كبير الفم. لوحظ وجود 10% فقط من البويضات الناضجة بمتوسط قطر 0.97 ± 0.01 ميكرومتر. أظهرت 30% من إناث الأسماك الأم التي حُقنت بهرمون HCG علامات تبويض ناجحة. لوحظ التبويض بعد 72 ساعة من حقن الهرمون في المتوسط. لم يُلاحظ أي تبويض في الإناث المحقونة بالغدة النخامية، بينما استجابت جميع الذكور التي حُقنت بالغدة النخامية فقط. لوحظ ارتباط قوي بين قطر البويضة المخصبة ووقت التربية، حيث بلغ معامل r = 0.97. تأثرت البيضات الناتجة بشدة بداء السابروليجنيا، مع توقف نموها ونفوق إجمالي للبيض.

الكلمات المفتاحية: الفحول؛ هرمون موجهة الغدد التناسلية المشيمائية البشرية؛ بويضات؛ الغدة النخامية؛ داء السابروليجنيا

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List of abreviations

- %: percent

- °C: Celsius

- µm: micrometer

- PSU: Partical Salinity Unit

- ‰: permille

- cm: centimeter

- CNRDPA: National Center for Research and Development in Fisheries and Aquaculture

- CPE: Carp Pituitary Extract

- Gn: pituitary gonadotropic hormone

- Gn-rh: Gonadotropin-Releasing Hormone

- h: hour

- ha: hectare

- HCG: Human Chorionic Gonadotropin

- Kg: kilogram

- km: kilometer

- L: liter

- LH-RHa: Luteinizing Hormone Releasing Hormone Analogue

- TL: total length

- m: meter

- m2: square meter

- m3: cubic meter

- mg: milligram

- ml: milliliter
- T° : temperature
- pH : hydrogen potential
- r : correlation coefficient.
- Max:Maximum
- Min : Minimum
- SD: Standard Deviation

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General Introduction

Introduction

The mastery of artificial reproduction in fish species is a major challenge in aquaculture, fishery resource conservation, and the sustainable management of aquatic environments. Among the species of growing interest, the Black Bass *Micropterus salmoides* (Lacepède, 1802), also known as Largemouth Bass, has attracted particular attention due to its recreational value, sporting qualities, and its ability to adapt to various environments.

Originally from North America, this species has been introduced into several countries, including Algeria in 1956, to reinforce fish populations and diversify exploited species. Its ecological plasticity and appeal for sport fishing have contributed to its increasing presence in both scientific literature and management practices worldwide.

However, its natural reproduction in controlled environments remains unpredictable and depends on numerous environmental factors, limiting the possibilities for effective management and farming development. In this context, hormonal induction of reproduction represents a promising alternative to better control the reproductive cycle, synchronize spawning, and improve juvenile yield.

This study is part of that approach. Its main objective is to induce spawning in *Micropterus salmoides* under controlled conditions using hormonal treatments. Two types of hormones were used for this purpose: human chorionic gonadotropin (HCG), widely used in aquaculture for its effectiveness in triggering ovulation, and pituitary extract (hypophysis) from other fish species, known for its broad action on the gonadotropic axis.

CHAPTER I: Overview of the Black Bass

Overview of the Black Bass:

1. Species Presentation

Micropterus salmoides is a known as largemouth bass in English and achigan à grande bouche in French. commonly scientific name *M. salmoides.* The largemouth bass *(Micropterus salmoides)*, referred to in China as "California bass," (Shoubing Wang et al., 2024). Is a freshwater species originally native to east-central North America (Kim, et al., 2022). Classified within the class Actinopterygii, order Perciformes, and family Centrarchidae, this species has since been widely introduced across the globe (Brown, et al., 2009), specifically from the Mississippi River basin and the Great Lakes region (Bruslé, et al. 2001). This species ranks among the largest freshwater predators, comparable in size and diet to perch, pike, or zander. Moreover, largemouth bass has spread globally due to numerous introductions in the late 19th and early 20th centuries, primarily aimed at promoting recreational fishing (Bruslé and Quignard, 2001). It is a very important species in aquaculture and recreational fishing worldwide, representing 56% of the total production of Perciformes Centrarchidae, with the majority of production occurring in China (Cai et al., 2019).

2. Taxonomic Classification of *Micropterus salmoides* (Lacepède, 1802)

The systematics of Black Bass according to WoRMS is as follows:

Kingdom: Animalia

Phylum: Chordata

Class**:** Actinopterygii

Order : Perciformes

Family : Centrarchidae

Genus : *Micropterus*

Species : *Micropterus salmoides* (Lacepède, 1802)

3. Morphological description of the largemouth bass (*Micropterus salmoides*)

The largemouth bass (*Micropterus salmoides*) is a freshwater predatory fish belonging to the Centrarchidae family. It is distinguished by its compact, stocky, and robust silhouette, reminiscent of that of the common perch. Its body is oblong, moderately high and slightly compressed laterally, giving it a massive appearance (Flouhr and Mary, 2010).

Its head is broad and powerful, representing about one-third of the total body length. The mouth, located at the end of the head, is very developed, broad and oriented obliquely upward. The lower jaw is slightly prominent, while the upper jaw clearly extends beyond the rear of the eye, an essential characteristic for distinguishing this species from its close relative *Micropterus dolomieu* Both jaws have small, inconspicuous teeth (Bruslé and Quignard, 2001).

This fish has two distinct dorsal fins. The first is relatively low, armed with 10 spiny rays, and often has a dark spot at its posterior end. The second dorsal fin, higher and rounded, is composed of about 12 soft rays (Brown et al., 2009). The pelvic fins are short and rounded, with one spine and five soft rays. The pectoral fins are broad, with rounded tips and 13 to 15 rays. The caudal fin is slightly indented, adapted for rapid movemen (Bruslé and Quignard, 2001).

The dorsal coloration varies from dark green to bright olive green (Flouhr and Mary, 2010). The flanks are pale green to golden in color, enhanced by a broad, uniform black longitudinal stripe that sometimes extends to the snout, passing through the eye and operculum (Fey and Corolla, 2022). The sides of the head range from green to olive, while the ventral surface ranges from milky white to light yellow, the fins are generally greenish-gray, without bright pigmentation (Fey and Corolla, 2022).

Figure 1. *Micropterus salmoides* (Largemouth Bass) photographed by Rémi Masson, May 2010. Image source : DORIS (Données d'Observations pour la Reconnaissance et l'Identification de la faune et la flore Subaquatiques).

4. Size and lifespan

In North America, the largest specimens can reach 80 to 97 cm in length and weigh up to 10 kg. In Europe, and particularly in France, individuals rarely exceed 60 cm in length and 4 kg in weight (Fey and Corolla, 2022).

As part of the study conducted at the Keddara Dam (Boumerdès, Algeria), biometrie measurements revealed modest sizes, yet consistent with those typically observed in Mediterranean environments. In December 2019, the total length (TL) of the specimens ranged from 14.2 cm to 35.6 cm, with an average of 28.6 ± 0.38 cm, while weights varied between 326.16g and 392.05 g. (Chabt Dis et al., 2023).

The life expectancy of the species is generally 6 to 8 years, although it can reach up to 15 years in favorable environmental conditions (Bruslé and Quignard, 2001).

5.Geographic Distribution

Micropterus salmoides is native to eastern North America, particularly to Canada and the United States, its natural distribution includes the St. Lawrence–Great Lakes basin, the Hudson Bay drainage via the Red River, and the Mississippi River basin, extending from southern Quebec and Minnesota southward to Texas, the Gulf Coast, and southern Florida, it is also native to Atlantic coastal drainages from North Carolina to Florida and to Gulf drainages from southern Florida to northern Mexico (Page and Burr, 1991).

Outside its native range, the species has been widely introduced for recreational fishing and aquaculture purposes, it is now established throughout much of the United States and southern Canada beyond its original range, as well as in numerous regions around the world, including Europe, Asia, Africa, and South America (Froese and Pauly, 2023).

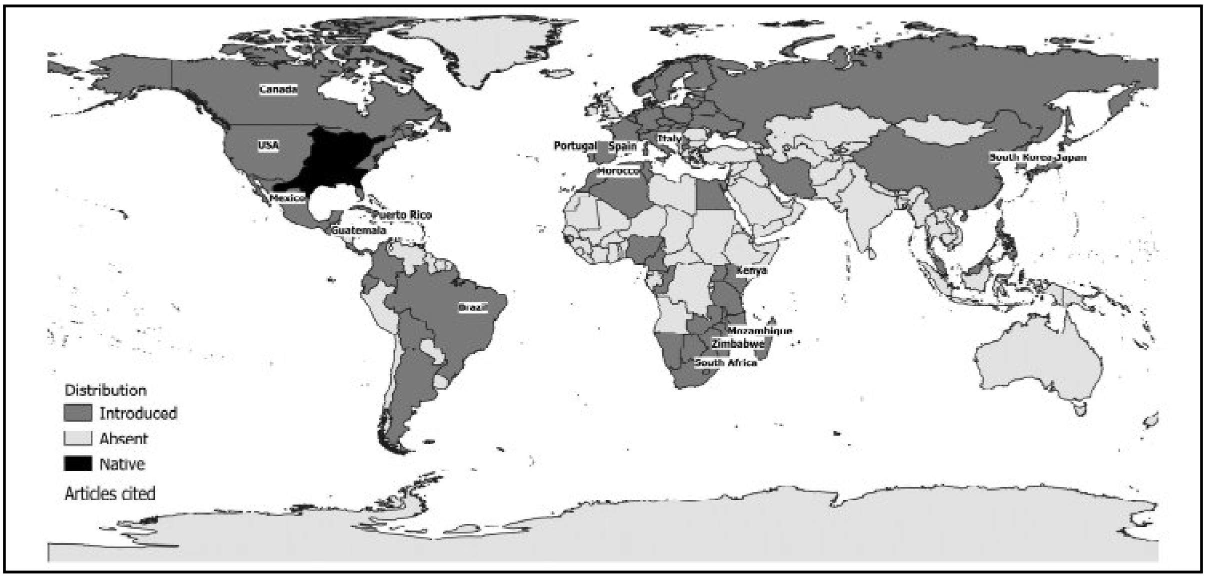


Figure 2. Distribution of *Micropterus salmoides* according to GISD 2021. Native distribution according to Brown et al. 2009. The labels indicate the names of the countries of origin of the literature describing M. salmoides as an invasive species cited in this review.

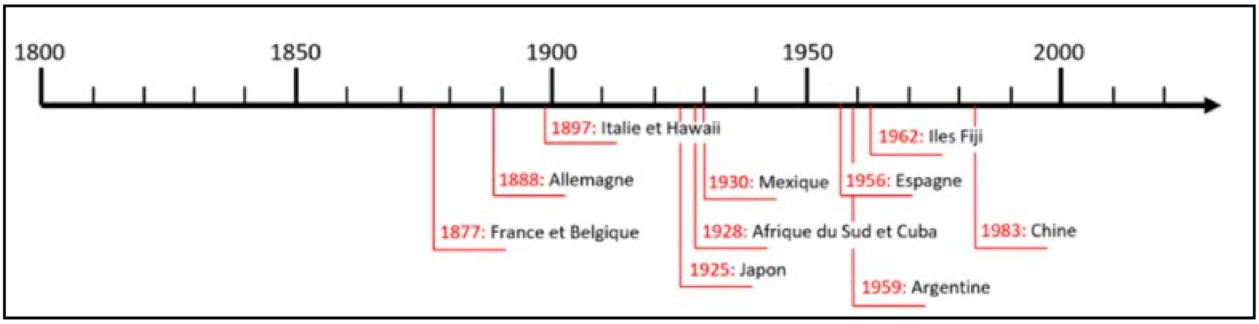


Figure 3. Timeline of the introduction of the largemouth bass (*Micropterus salmoides*) worldwide. Adapted from Gauvin, A. (2020).

French authorities in 1956 in Algeria, followed by a second wave in 1970, specifically for the development of recreational fishing in freshwater environments that were originally devoid of natural predators and characterized by relatively cool temperatures (Lévêque and Bruton, 1994). Since then, numerous stocking and restocking efforts have been implemented (see Table 01), using fry imported from abroad or produced through controlled breeding in local hatcheries.).

Table 1 . Presence and History of Largemouth bass stocking and restocking operations (Fry or Broodstock) in Algeria from 1982 to 2023 (according to Meddour and CNRDPA reports)

Region	Province	Water body	Year	Quantity
West	Tlemcen	Beni-Bhdel Dam	1985	2000 Fry
			2015	1000 Fry
		Meffrouche Dam	1985	500 Fry
			2015	1000 Fry
		Boughrara Dam	2015	500 Fry
	Sidi Bel Abbes	Lake Sidi M’hamed Ben Ali	1985	500 Fry
			2015	1000 Fry
			2016	17 Brooders
		Sarno Dam	1985	400 Fry
			2015	800 Fry
			2016	11 Brooders
	Mascara	Bou-Hanifia Dam	1985	400 Fry
	Ain-tmouchnet	Chaabat el leham	2016	1000 Fry
Centre	Ain defla	Sidi M’hamed Ben Tayba	2014	1000 Fry
	Boumerdes	Naceria Hill Dam Reservoir	1985	30 Brooders
	Tizi Ouzou	Boukhalfa Dam	1982	500 Fry
	Béjaia	Techyhaf	2014	2000 Fry
East	Sétif	Ben Alleg Hill Dam Reservoir	2014	1800 Fry
			2015	2000 Fry
South	Béchar	Joref el toba Dam	2018	7 Broodstock

Also, *Micropterus salmoides* shows remarkable adaptation in Keddara Dam in boumerdes province, with high potential for growth and reproduction due to favorable environmental conditions (Chabet dis et al., 2023).

6. Ecology

6.1. Habitat

Although the largemouth bass is primarily associated with lakes, it can also be found in rivers. This species requires large aquatic environments such as expansive lakes, wide rivers, or streams (Bruslé and Quignard, 2001). In natural settings, *Micropterus salmoides* can adapt to a wide range of habitats, both lotic (flowing waters) and lentic (still waters), although it clearly prefers calm, slow-flowing, and relatively deep waters (Flouhr and Mary, 2010). Generally, it favors mesotrophic waters found in the lower courses of rivers and streams, avoiding fast-flowing areas (Bruslé and Quignard, 2001). The largemouth bass displays wide environmental tolerances that allow it to survive in a variety of native and invaded ecosystems (Costantini et al., 2023), which explains its adaptability to such diverse habitats.

According to Lasenby and Kerr (2000), habitat preferences for this species in Ontario include ponds with a surface area greater than 0.1 hectares, featuring muddy or gravelly substrates. It typically inhabits depths of less than 6 meters, favoring clear, slow-moving, and shaded waters, which also offer refuge from light (Brown et al., 2009). Aquatic vegetation plays a crucial role in structuring the habitat of the largemouth bass: dense plant cover can significantly reduce predation on juveniles, thus improving their survival rates (Brown et al., 2009).

6.2. Abiotic factors

Temperature

The thermal requirements of the largemouth bass (*Micropterus salmoides*) vary depending on its developmental stage and physiological activity. For adult individuals, the optimal temperature range for growth is between 24 and 30 °C (Venables et al., 1978; Stuber et al., 1982). Optimal individual growth is generally achieved at temperatures of 24–30 °C and the optimal temperature for reproductive activity is 20–21 °C (Costantini et al., 2023). The minimum temperature allowing growth is 15 °C, while the maximum threshold is 36 °C (Stuber et al., 1982). Regarding egg incubation, the optimal temperature is estimated between 20 and 21 °C (Clugston, 1964), with a tolerance range of 13 to 26 °C (Kelley, 1968). Survival of eggs and embryos becomes unlikely above 30 °C or below 10 °C (Kelley, 1968; Kramer and Forgeron, 1960).

Salinity

Micropterus salmoides is a stenohaline species primarily associated with freshwater environments, although it can occasionally be found in brackish waters. *Micropterus salmoides* prefers salinities below 5 PSU, although it is sometimes found in brackish waters (Costantini et al., 2023). It is notably present in certain estuaries where salinity does not exceed 12 PSU (Heidinger, 1976; Marquet et al., 2003). The salt content of the environment has a significant impact on the species' biological characteristics (Heidinger, 1976). When salinity exceeds 10 PSU, the reproduction rate drops considerably, although egg hatching can still occur at salinities ranging from 20 to 30 PSU. Furthermore, largemouth bass stops feeding when salinity reaches 25PSU (Flouhr and Mary, 2010).

pH

Micropterus salmoides shows low tolerance to fluctuations in the pH of its environment (Flouhr and Mary, 2010). This species can only develop in waters where the pH ranges between 6.1 and 9.5, with optimal conditions being close to neutrality, between 7.0 and 7.5 (Heidinger, 1976), and pH values of 6.5 to 8.5 (Costantini et al., 2023). Although it can temporarily tolerate more acidic (5–6) or more alkaline (above 10) conditions, a significant decrease in reproductive activity has been observed under such circumstances (Heidinger, 1976). The species can withstand short-term exposure to extreme pH values ranging from 3.9 to 10.9 (Brown et al., 2009). However, it will not spawn at a pH below 5.0, and eggs do not survive at a pH above 9.6 (Stuber et al., 1982). A mortality rate of 50% has been recorded when pH levels fall below 4.2 or exceed 10.4 (Heidinger, 1976; Flouhr and Mary, 2010).

Oxygen

The largemouth bass has low tolerance to decreases in dissolved oxygen levels in its habitat. It can tolerate low dissolved O_2 concentrations, but it avoids values below 1.5 mg/L (Costantini et al., 2023). It tends to avoid waters where dissolved oxygen concentrations fall below 3 mg/l and cannot survive at 1.5 mg/l, even under optimal temperature conditions (Scott and Crossman, 1973). Concentrations below 1.0 mg O_2/l are considered lethal (Stuber et al., 1982; Brown et al., 2009). According to Heidinger (1976), the critical survival threshold for this species is around 1 mg/L. However, this threshold may vary depending on water temperature: it is estimated at 0.92 mg/L at 25 °C, 1.14 mg/L at 30 °C, and 1.19 mg/L at 35 °C.

Growth is reduced by 25 to 30% at an oxygen concentration of 4 mg/L compared to that observed at 8 mg/L (Heidinger, 1976). Similarly, reproduction and egg hatching rates are significantly reduced when dissolved oxygen levels are limited to 2.0 mg/L, 2.1 mg/L, and 2.8 mg/L at respective temperatures of 15, 20, and 25 °C (Flouhr and Mary, 2010).

Turbidity

The largemouth bass is primarily a visual predator whose hunting efficiency strongly depends on water clarity (Mark et al., 2010). Increased turbidity reduces its reaction distance, leading to a significant decline in overall prey consumption (Brown et al., 2009). This species is intolerant of high turbidity levels and prefers environments with suspended solids concentrations below 25 ppm. Nevertheless, growth can still occur in ponds with turbidity levels ranging from 25 to 100 ppm, although the optimal range is considered to be between 5 and 25 ppm (Brown et al., 2009). In general, the largemouth bass exhibits greater tolerance to turbid water than the smallmouth bass (Carter et al., 2010).

7. Reproduction

7.1. Reproduction in its natural habitat

Largemouth bass exhibit a polyandrous mating system, where a single female mate with multiple males within a single breeding season (Dewoody et al., 2000). Their reproductive cycle occurs annually, with spawning taking place between January and March in southern regions and from May to June in northern areas (Mearelli et al., 2002; Orlando et al., 1999). Males are responsible for nest construction, which typically occurs on the bottom substrate composed of hard-packed sand, pea gravel, clay, or marl, all covered by a thin layer of mud (Orlando et al., 1999). While males prepare the nests, females remain in deeper water until the nests are complete. (Mearelli et al., 2002).

Its reproductive cycle is largely dependent on seasonal variations, specifically temperature and photoperiod. The spawning period generally occurs in the spring when the water temperature reaches 16 to 18 °C (Bruslé and Quignard, 2001).

Once the nest is ready, the male actively seeks out a female and guides her to the nest to deposit her eggs (Orlando et al., 1999). To encourage her to stay near the nest, the male swims in circles around it and nudges her repeatedly to prompt egg release (Dewoody et al., 2000). After the female lays her eggs, the male fertilizes them externally (Mearelli et al., 2002). The spawning period for largemouth bass typically occurs in late winter in southern U.S. regions and late spring in northern areas when water temperatures reach around 30 degrees Celsius (Dewoody et al., 2000; Mearelli et al., 2002; Orlando et al., 1999).

Largemouth bass generally reach sexual maturity between 1 and 2 years of age, they continue reproducing until around 12 years old, and spawning occurs from March to June, peaking when water temperatures range from 20 °C to 24 °C (Wang et al., 2024). At slightly cooler temperatures (17.4 °C to 19.5 °C; mean 18.3 °C), reproductive behavior has also been observed, including nest-building and parental care by males, who guard the eggs and fry. A single female can produce between 3,000 and 45,000 eggs per spawning event, with an average around 4,000, and egg quantity is positively correlated with female size (Wang et al., 2024).

Fertilized eggs are laid in the nest constructed by the male and then externally fertilized (Orlando et al., 1999; Mearelli et al., 2002). Hatching occurs within 1 to 5 days, depending on water temperature, with warmer conditions accelerating the process (Hambright et al., 1986; Hambright, 1991). Fry become independent in approximately 7 to 10 days, although this period can extend up to 5 weeks (Mearelli et al., 2002). At birth, they typically measure between 4 and 6 mm in length (Page and Burr, 1991).

Parental Investment and Care

In *Micropterus salmoides*, parental care is exclusively performed by the male, who plays a central role in reproductive success (Brown et al., 2009). After preparing the nest and ensuring fertilization, the male remains to guard the eggs, cleaning them with gentle fin movements to prevent silt accumulation (Flouhr and Mary, 2010). Incubation lasts between 2 and 6 days depending on water temperature accelerated by warmth and delayed by cold with durations ranging from 2 days at 28°C to up to 13 days at 10°C (Heidinger, 1976; Bruslé and Quignard, 2001; Flouhr and Mary, 2010). Once hatched, the transparent fry measure about 3 mm and stay in the nest for 6 to 7 days, feeding on their yolk sac until they reach approximately 6 mm in length (Heidinger, 1976; Bruslé and Quignard, 2001; Marquet et al., 2003; Flouhr and Mary, 2010). During this period, the male continues to protect them, often displaying aggressive behavior to deter predators (Bruslé and Quignard, 2001). However, as the male becomes increasingly hungry 4 to 5 days after hatching, he may resort to filial cannibalism, consuming some of his offspring (Johnke, 1995). By 7 to 10 days post-hatching, the surviving fry are capable of independent swimming and may reach up to 30 mm before leaving the male's protection (Flouhr and Mary, 2010). The success of this parental investment is strongly influenced by environmental conditions, particularly water temperature and flow stability, with optimal scenarios resulting in up to 80% egg hatching success (Flouhr and Mary, 2010; Brown et al., 2009). Conversely, temperature drops can inhibit spawning and lead to nest abandonment, making temperature a critical factor in the reproductive biology and early survival of this species (Bruslé and Quignard, 2001; Brown et al., 2009).

7.2. Captive Breeding

According to Schlumberger (2002), triggering reproduction in captivity can be achieved by acting on three levels (Figure 4)

-Environmental manipulation**:** This involves adjusting water temperature and photoperiod. However, these factors alone are rarely completely effective. Breeding pairs must also be provided with suitable spawning substrates. Still, for certain species, these environmental cues are not sufficient to reliably induce spawning.

-Hormonal stimulation via the hypothalamic-pituitary axis: This is achieved through the injection of synthetic analogues of Gn-RH (gonadotropin-releasing hormone), which is naturally secreted by the hypothalamus. For instance, injecting LH-RHa stimulates the pituitary gland to produce gonadotropins.

-Direct increase of circulating gonadotropins: This involves injecting gonadotropic hormones directly into the bloodstream, either in the form of pituitary extracts (such as carp pituitary homogenates containing Gn) or human chorionic gonadotropin (HCG)

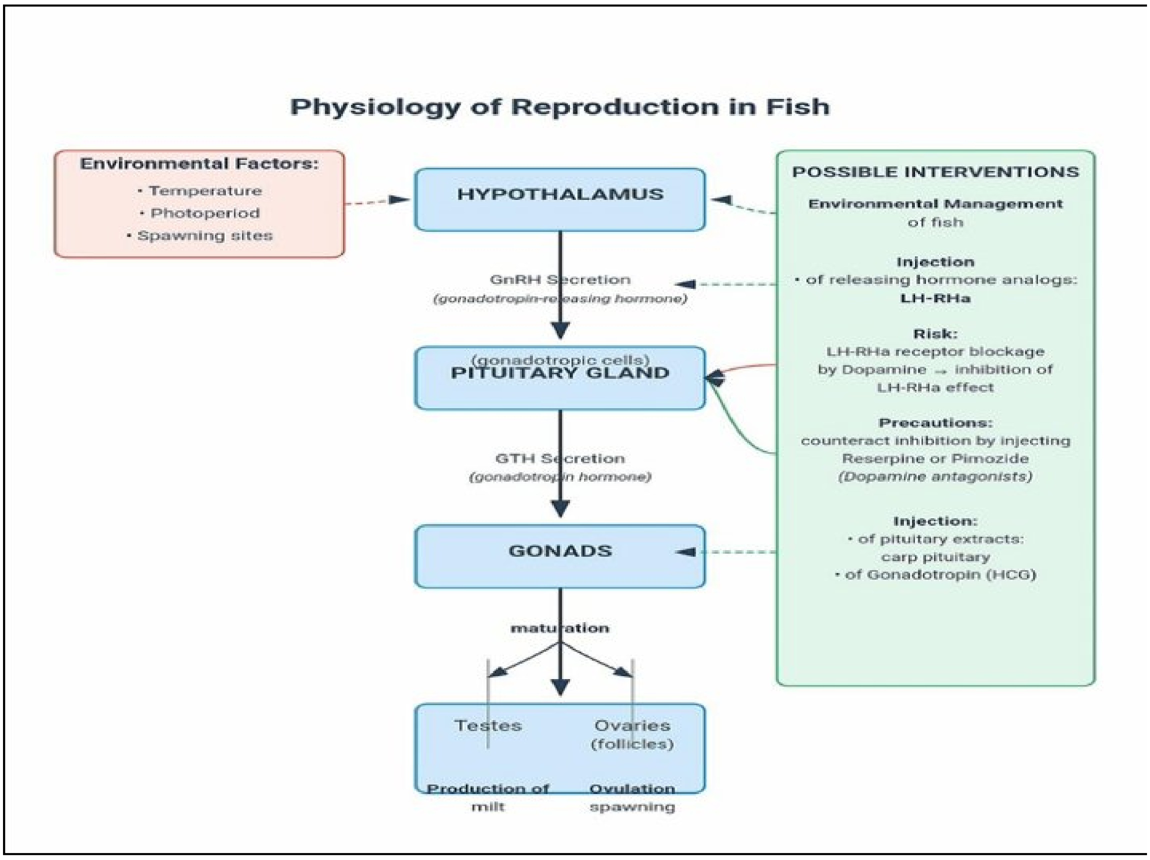


Figure 4. Reproductive Physiology in Fish: Organs and Hormones Involved in Reproduction (Schlumberger, 2002).

7.3. Pond Spawning

This is a traditional and simple method in which brooders stock are placed in a pond to reproduce freely, at a density ranging from 25 to 100 individuals per hectare (Glenewinkel et al., 2011). Artificial nests may be added to encourage spawning (Glenewinkel et al., 2011). The Broodstocks are left in the pond until the fry reach the desired size, typically after 30 to 45 days Glenewinkel et al., 2011).

However, due to cannibalism, this method is not very effective for producing fry larger than 25 mm in total length (TL) (Glenewinkel et al., 2011). The ponds are fertilized to stimulate primary production, which promotes the development of zooplankton and insect larvae to feed the Largemouth bass larvae (Glenewinkel et al., 2011).

7.4. Pond spawning with egg transfer

The ponds are prepared in advance, and brooders stock are introduced as described previously for pond spawning (Glenewinkel et al., 2011). Nests are monitored each morning to check for egg deposition (Glenewinkel et al., 2011). Once eggs are laid, the nests containing them are transferred to another pond that has been previously fertilized (Glenewinkel et al., 2011). There, the eggs are left to hatch, and the larvae are allowed to develop. However, a major drawback of this method is the inability to accurately determine the number of resulting larvae (Glenewinkel et al., 2011).

7.5. Pond spawning with larval transfer

The steps of this method are similar to those of the previous technique. After hatching, the larvae are transferred from the spawning pond to a separate pond designated for their growth. This transfer is carried out either by net collection or pond drainage (Glenewinkel et al., 2011). The larvae are then stocked at a density ranging from 125000 to 500000 larvae per hectare to produce fingerlings measuring 38 to 50 mm in total length (TL). Under optimal conditions, the growth rate ranges from 1.0 to 1.5 mm per day. Fingerlings can reach 38 mm TL within 30 to 45 days (Glenewinkel et al., 2011).

This method offers several advantages: stocking density can be adjusted according to the intended production plan, and fingerlings of uniform size can be raised together to reduce cannibalism, which can otherwise significantly lower the yield per hectare (Glenewinkel et al., 2011).

7.6. Intensive reproduction

Intensive reproduction, also known as controlled reproduction, was developed to address the limitations of traditional methods. The brooders stock density in tanks is similar to that used in other techniques, although the fish can also be placed in small tanks or raceways. Hormonal injections may be applied to synchronize spawning. In this method, eggs are collected and incubated in hatchery systems under controlled environmental conditions

(Glenewinkel et al., 2011). However, artificial reproduction through stripping has produced poor results due to the asynchronous maturation of oocytes in Black Bass (Jacquemond, 1996).

8.Production

The global production of Black Bass, specifically largemouth bass (Micropterus salmoides), has seen significant growth, with China being the dominant producer noted by FAO 2021. In 2022, China's largemouth bass aquaculture production reached approximately 802,486 tons, accounting for over 99% of the global production of this species according to Yu et al. (2024). Worldwide, largemouth bass production increased about 20-fold between 1999 and 2018, with most of this growth occurring in China (Lutz 2025).

In the United States, largemouth bass farming is smaller in scale, fewer than 200 of the nearly 3,000 fish farms in the U.S. produce largemouth bass with 193 farms reported in 2023 faces challenges such as high mortality rates during production, which limits supply both for food markets and for stocking recreational fishing lakes. Efforts are underway to improve hatchery practices to expand production domestically (Cletzer, 2015).

CHAPTER II: Materials and Methods

1.Description of the Study Site El Maouane Dam

The El Maouane Dam has an estimated total storage capacity of 148 million m³. Following recent rainfall and snowfall, the water level rose to 28 million m³, which is expected to sufficiently meet the domestic water needs of the local population during the upcoming summer season (Arslan, 2024).

Figure 5. El Maouane Dam, Sétif Province, Algeria.

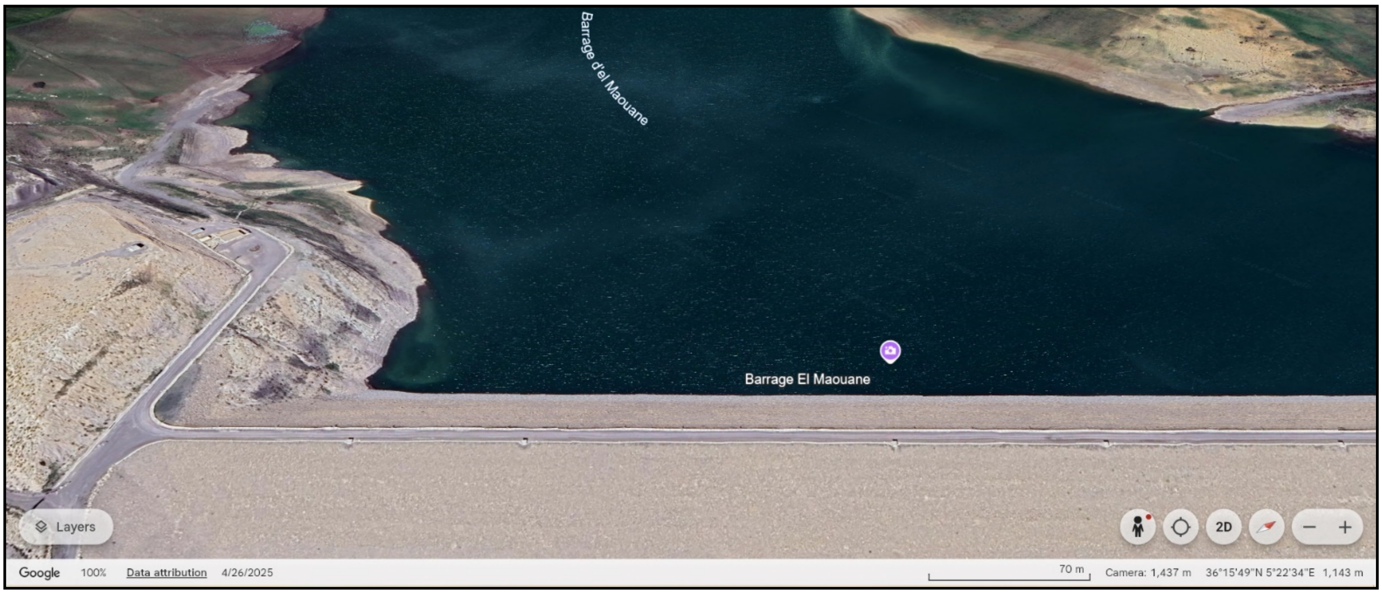


Figure 6. Aerial View of El Maouane Dam (Sétif, Algeria) Captured from Google Earth (26 April 2025) — Latitude: 36°15′49″N, Longitude: 5°22′34″E, Altitude: 1,143 m

2. Physicochemical quality of El Maouane dam

The physico-chemical parameters (Temperature, pH, salinity, conductivity, turbidity, and oxygen) of the water at El Maouane Dam were analyzed on May 27, 2025, at 10:15 a.m. using a Hanna-type multi-parameter (Figure 07), in order to better characterize the environmental conditions influencing the artificial reproduction of largemouth bass (*Micropterus salmoides*).

Figure 7.Multiparamétre

3. Obtaining broodstock

To obtain broodstock, fish were caught from El Maouane Dam with using nets of 45 mm empty mesh and transported alive to El Ourissia hatchery. There they are stocked either in broodstock tanks for 3 days then transported to CNRDPA in Bou Ismail by Tabouache fouad (Figure 09).

The broodstock were transported in a live well, a specially designed tank intended to keep captured fish alive until they reached their destination. The tank had a cubical shape with an approximate volume of 1 m³, and was filled to two-thirds with water from the dam to replicate the natural habitat of the fish.

It was equipped with a top access hatch for the safe placement of the fish, and an aeration system consisting of a motor and diffusers to provide continuous oxygenation throughout the journey. This system was essential for maintaining water quality and reducing stress during the transport to the CNRDPA (313-kilometre) (National Centre for Research and Development of Fisheries and Aquaculture) in Bou Ismaïl (Figure 09).

Figure 8. Nets used for fishing

Figure 9. Unload the broodstock from the tanks

4. Broodstock health treatment

Broodstock are typically housed in separate plastic basins based on sex. To prevent any disease upon arrival, fish underwent a 5-minute prophylactic bath in methylene blue solution at 1 ppm concentration (Figure 10). This preliminary treatment eliminates external pathogens and parasites, while concurrently minimizing physiological stress prior to placement in spawning tanks.

Figure 10. Broodstock treament using methylene blue

5. Broodstock acclimatization

Prior to any handling, both male and female broodstock were gradually acclimated for 2 hours to optimal reproductive temperatures. The ideal temperature range was maintained between 15°C and 18°C, with adjustments made progressively not exceeding ±3°C at a time—to minimize thermal stress and prevent physiological shock.

During the acclimation period, males and females were kept in separate plastic basins to avoid premature interaction and ensure proper conditioning before hormonal induction.

6. Anesthesia of broodstock

To minimise stress and prevent handling-related injuries that could jeopardise spawning success, fish were routinely anaesthetised before every manipulation (weighing, hormonal injection and maturity checks). Eugenol served as the anaesthetic at 0.3–0.4 ml/L. At 22 °C, fish lost equilibrium after 2 à 4 min of immersion (Figure11); they were promptly removed at that point and transferred to fresh, oxygenated water, where full recovery occurred within a few minutes (Glenewinkel et al., 2011). Tranquilisation is particularly critical when working with wild specimens, as even minor injuries can compromise the entire operation.

Figure 11. Eugenol using for anesthesia

7. Sex identification, weighing, of broodstock

The sex of each broodfish was determined through careful visual inspection of the abdominal region (Figure 17). As described by Woynarovich and Horváth (1981), the external indicators of sexual maturity are generally consistent across species.

- Males exhibit a flat, firm abdomen with no redness around the urogenital opening. Slight pressure on the abdomen results in the release of milt.
- Females are identified by a soft, distended belly and a noticeably reddened genital area. with slight abdominal pressure we will have oocytes (Figure 12).

Figure 12. Male vs. Female Comparison (female on the right)

Total length and total weight were recorded using an ichthyometer and an electronic scale, respectively (Figure 18 and 19).

Following the methodology described by Berkani et al. (2017), we measured the following morphometric parameters for each fish:

Total length (TL): the distance from the anterior-most point of the head to the tip of the longest ray of the caudal fin.

Standard length (SL): the distance from the anterior-most point of the head to the base of the caudal fin (at the caudal peduncle articulation).

Body height: the vertical distance between the dorsal and ventral fin insertions.

Figure 13. Fish weighting measurement

Figure 14. Fish

8. Oocyte Biometry and Maturity Stage:

to identify the stage of maturation, an obstetric catheter was used (Figure 15). The instrument, with a diameter of 1.2 to 1.8 mm, was gently inserted through the urogenital opening and carefully advanced into the body cavity. It was then withdrawn while applying slight suction to collect reproductive products (oocytes) from the gonads. All catheterizations were carried out under anesthesia to minimize stress and prevent internal tissue damage.

Figure 15. An obstetric catheter

An observation of oocyte development is carried out on a sample of females from each broodstock group (Figure 16). This monitoring aims to assess the maturity stage of the oocytes prior to initiating reproduction. The value of this approach lies in facilitating the tracking of ovarian maturation and in providing a relatively accurate estimation of the reproductive period.

Figure 16. Microscopic Observation of Largemouth bass's Oocytes.

Each collected sample was split into two parts. The first part, used for measuring oocyte diameter, was fixed in a 4% formalin solution (Figure 18) then mounted on a graduated slide and examined under a microscope at 4x magnification. The second part was placed in SERRA solution (Figure 17) as described by Schlumberger (2002).

SERRA solution: Prepared by mixing 6v of 95% ethanol, 3v of formalin, and 1v of acetic acid.

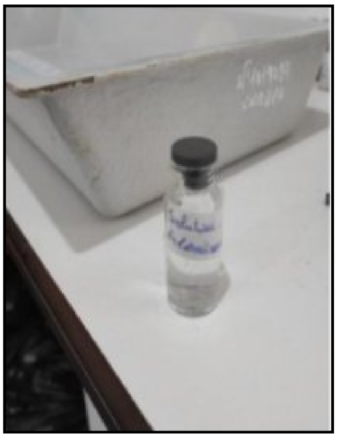


Figure 18. SERRA Solution

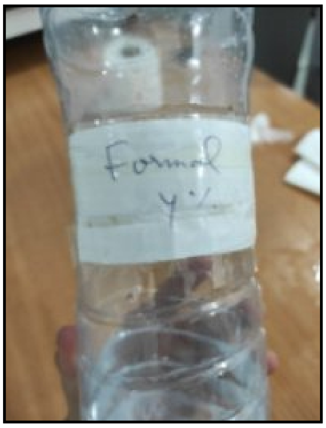


Figure 17. SERRA Solution

After 5 to 10 minutes, the oocytes become translucent, allowing the nucleus referred to as the germinal vesicle to be observed by transparency (at 4x or 10x magnification). As final maturation progresses, the germinal vesicle gradually migrates from the center toward the periphery of the cell

9. Induced Spawning

The equipment used during the various stages of the experiment included:

Sex hormones (HCG and pituitary extract) (Figure 24 and 25).

Syringes for hormonal injections.

Mortar and pestle for the preparation of pituitary extracts.

Landing nets for fish handling.

Plastic basins for temporary holding of broodstock.

Freshwater to maintain broodstock under appropriate conditions.

Precision balance for weighing pituitary glands (Figure26).

High-capacity scale for weighing fish.

Methylene blue, used as an antiseptic in rearing water.

Figure 19. HCG

Figure 20. Carp pituitary extract

Figure 20. Precision balance

Two types of hormonal treatments were prepared for the induction of reproduction in broodstock: Carp pituitary extract (CPE) and Human chorionic gonadotropin (HCG).

The hormones were donated by the CNRDPA. The required quantity of carp pituitary extract, was first weighed using a precision electronic balance. The granules were then finely ground using a dry porcelain mortar and pestle, ensuring a homogeneous powder. The resulting material was diluted in 1 ml of sterile physiological saline (0.9% NaCl) and thoroughly mixed to ensure complete dissolution. The suspension was then aspirated into a sterile syringe, taking care to avoid any residual tissue or sediment remaining in the mortar. All procedures were conducted under aseptic conditions to prevent contamination.

The Human Chorionic Gonadotropin (HCG) was provided in lyophilized 5000 IU vials. Each vial was reconstituted just prior to use by adding 1 ml of sterile physiological saline, and the solution was gently swirled to fully dissolve the hormone while avoiding denaturation. Once reconstituted, the hormone was immediately drawn into a sterile syringe (Figure 27).

All hormone solutions were kept in refrigerator and used within a few hours of preparation to maintain their biological integrity and efficacy. The dose administered was calculated based on the individual body weight of each broodfish, in accordance with standard aquaculture practice.

Figure 21. Preparation of the injections

We injected 100% of the males with pituitary extract (CPE) only, while the females received both CPE and HCG. For each male or female, CPE was administered at a dose of 5 mg/kg, and for the females, HCG was additionally injected at a dose of 4000 IU/kg.

The hormonal injection was administered intramuscularly following the method described by Huet (1971). The injection site was located beneath the first ray of the dorsal fin, toward the anterior part of the body and approximately halfway down the lateral line (Figure 23). Using a graduated plastic syringe, the needle was inserted at a 45° angle to a depth of 2 to 3 cm, with only the first third of the needle penetrating the muscle tissue between the scales. Alternatively, the injection can be performed under the pelvic fin, but this requires a fine needle and a small syringe, along with great caution from the handler to avoid injuring the fish’s internal organs**.**

Figure 22. Fish injection

10. Introduction of broodstock into Spawning Tanks

Before introducing the broodstock, the tanks were thoroughly cleaned, rinsed carefully, and then left exposed to sunlight for 24 hours to ensure proper disinfection and eliminate any potential pathogens (Figure 24).

Figure 23. cleaning the tanks before the arrival of the broodstock

The characteristics of tanks are shown in table 02.

Table 2. Tanks characteristics and conditions used for broodstock rearing

Pond	Surface Area (m^2)	Total Capacity (m^3)	Water Level (cm)	Light Source	Oxygenation
B1	3	1.8	60	Natural	Permanent
B2	3	1.8	60	Natural	Permanent
B3	3	1.8	60	Natural	Permanent
B4	3	3	100	Natural	Permanent

11. Installation of Artificial Nests

To facilitate egg collection from largemouth bass, artificial nests were installed in the breeding tanks. These nests consisted of plastic crates equipped with floatation devices and filled with gravel, simulating natural spawning sites. The gravel, serving as a spawning substrate, was disinfected using boiling water and then rinsed thoroughly with clean running water. The entire nest structure, including the crates, was also treated with boiling water to ensure proper hygiene (Figure 25).

Figure 24. Nests disinfection and preparation

The nests were arranged within the tanks at intervals of approximately 1.5 m to reduce territorial conflicts among males (Figure 31). The number of nests was adjusted according to the number of males introduced, following the recommendations of Jacquemond (1996).

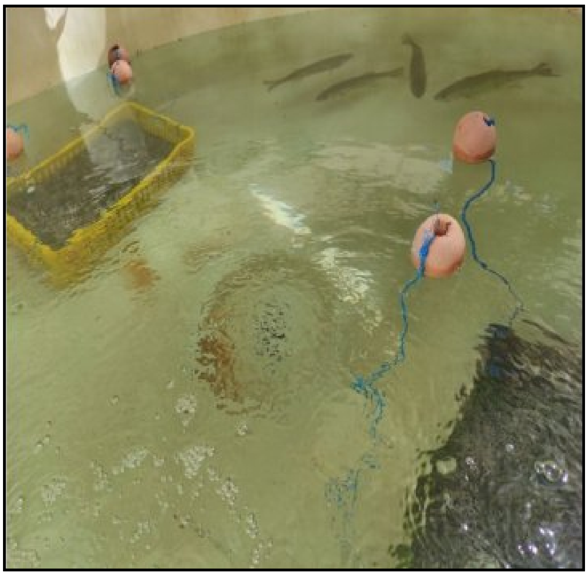


Figure 25. Nest

Placement

12. Hormonal Injection Timeline

The schedule of hormonal injections was established based on water temperature and gonadal development to ensure effective induction.

13. Egg Incubation

Fertilized eggs were incubated in artificial plastic bottles made by cutting plastic bottles and adapting them to function as vertical incubators (Figure 32). A continuous supply of oxygen was provided using an air tube inserted in the bottom of each bottle to ensure constant water movement and adequate aeration. This setup allowed for the suspension of eggs in the water column, reducing the risk of fungal contamination and improving oxygen exchange. The incubation system was kept under controlled conditions until hatching.

Figure 26. Egg

Incubation

CHAPTER III: Results and Discussion

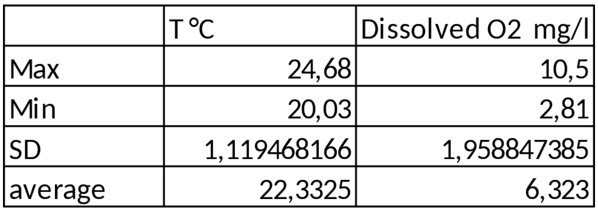
1. Environmental Conditions

The environmental conditions measured in the El Maouene Dam on the day of fishing and in the rearing tanks which were monitored daily throughout the experiment to ensure a stable and optimal environment for the broodstock are shown in Table 03 and Figure 28 and 29, respectively.

Table 3. Physico-chemical parameters in El Maouane Dam.

Parameter	Value
Temperature °C	20.07
pH	8.34
Salinity (PSU)	0.51

Table 4. Water quality parameters in reproductive tanks



	T °C	Dissolved O2 mg/l
Max	24,68	10,5
Min	20,03	2,81
SD	1,119468166	1,958847385
average	22,3325	6,323

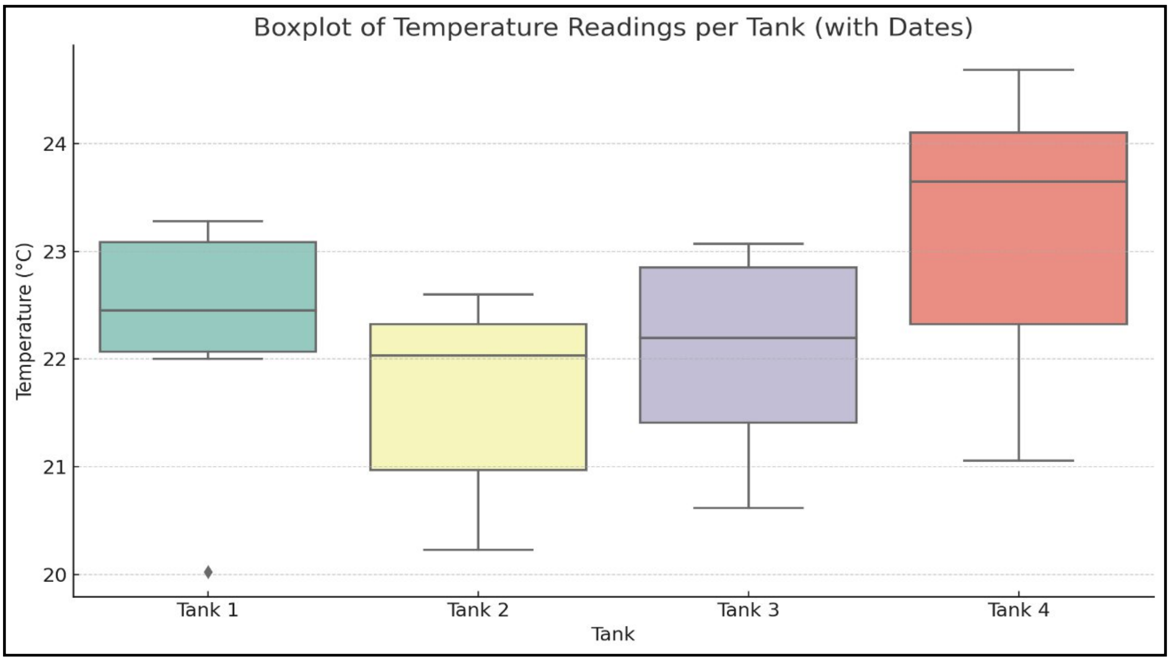


Figure 27. Variation in temperature T(°C) at the reproduction tanks.

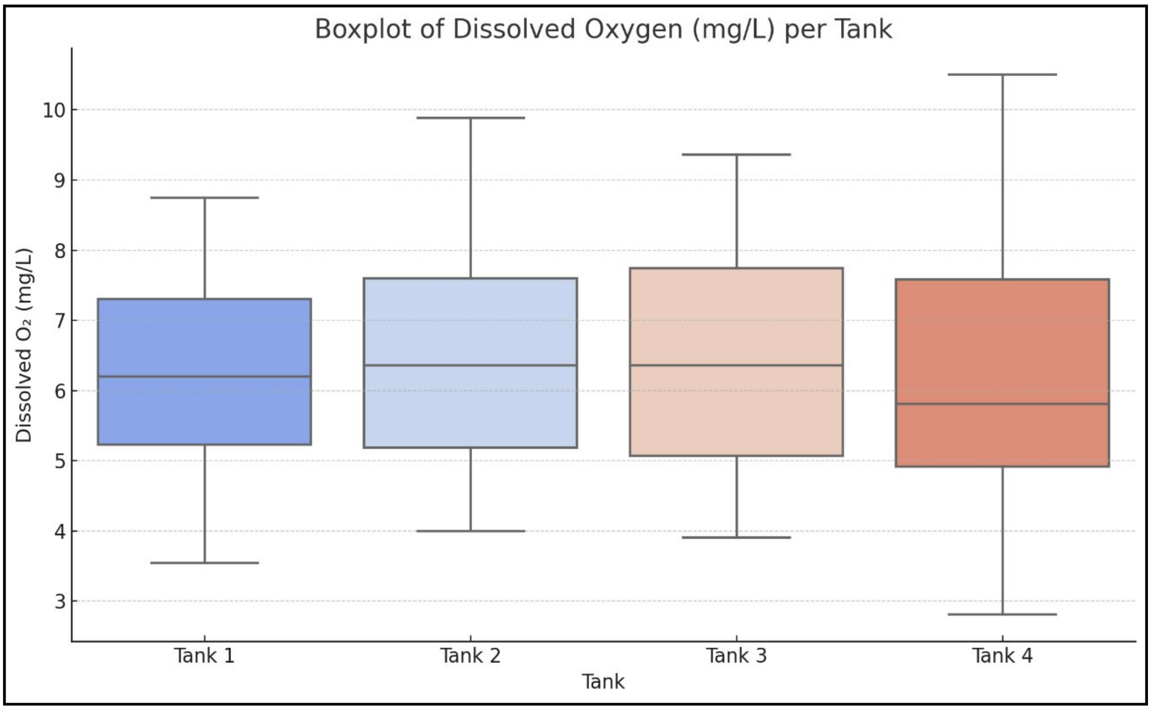


Figure 28. Variation in dissolved oxygen (mg/L) at the reproduction tanks.

The figure 27 shows the daily variation in water temperature measured in the four spawning tanks (Tank 1, Tank 2, Tank 3, Tank 4) from April 29 to May 8, 2025. During this period, the average temperature observed across all tanks was 22.33 °C, ranging from 20.03 °C to 24.68 °C. These values fall within the optimal temperature range generally recommended for the reproduction of largemouth bass (*Micropterus salmoides*). The uniformity of temperatures among the different tanks, as indicated by the near-overlapping bars for each day, reflects good thermal stability throughout the experiment.

Largemouth bass reproduction is dependent on water temperature, they typically spawn in the spring when water temperatures are between 15 and 24°C, optimal growth for adult largemouth bass occurs between 24 and 30°C (Wang et el.; 2024).

Largemouth bass reproduction is sensitive to pH levels, successful spawning requires specific pH conditions where the optimal pH for largemouth bass reproduction is between 6.5-8.5, with pH levels below 5.0 inhibiting spawning and levels above 9.6 harming egg survival (Stuber et la., 1982).

Micropterus salmoides are primarily freshwater fish, but can tolerate some salinity and preferred habitat being waters with salinity levels of 3PSU or less, also salinity can also negatively affect reproductive success where high salinity levels can hamper spawning and reduce the number of eggs produced (Reinert and Peterson, 2008).

Dissolved oxygen concentration is another fundamental hydrological parameter, essential for fish respiration and overall well-being, especially during the spawning period. The figure 28 illustrates the daily variations in dissolved oxygen levels (in mg/L) recorded in the four spawning tanks (Tank 1, Tank 2, Tank 3, Tank 4) from April 29 to May 8, 2025. During this period, the average dissolved oxygen concentration across all tanks was 6.32 mg/L, with values ranging from 2.81 mg/L to 10.5 mg/L. These levels are considered adequate and non-stressful for largemouth bass (*Micropterus salmoides*) broodstock, ensuring an environment conducive to their metabolic and reproductive activity. Although slight variability between tanks was observed on certain days, oxygen concentrations remained generally stable and above the critical thresholds necessary for fish survival and health.

Micropterus salmoides reproduction is sensitive to oxygen levels. Dissolved oxygen levels below 3 mg/L must be avoided, though they can survive at 1.5 mg/l under optimal temperatures but levels below 1.0 mg/Lare lethal (Trippel et al., 2017).

2. Broodstock fishing

The number of largemouth bass broodstock captured is shown in Table 04.

Table 5. number of largemouth bass broodstock captured.

Day	Number of broodstock captured
Sunday 27/04/2025	18
Monday 28/04/2025	19
Tuesday 29/04/2025	21
Wednesday 30/04/2025	18
Total	76

A total of 76 largemouth bass individuals were caught in the dam. The present study reports for the first time the presence of *Micropterus salmoides* in El Maouene Dam (Setif province).

Chabet dis et al. (2023) reported for the first time the presence of *Micropterus salmoides* in Keddara Dam (Boumerdes province) and they revealed that *Micropterus salmoides* adopts feeding and spawning strategy at the water body which is an adaptation of the largemouth bass contributes to ensuring the success of its reproduction and survival in this biotope.

3. Sex identification, weighing and measuring

The average weight of the broodstock showed good signs for reproduction is 0.47 ± 0.1 kg for male and 0.44 ± 0.04 kg for female, with a maximum of 0.58 kg for male and 0.5 Kg for female, and a minimum of 0.32 kg for male and 0.4 kg for female. For the total length, the average length is 34.6 ± 1.55 cm for male and 32.3 ± 0.9 cm for female with a maximum of 36.5 cm for male and 33.2 for female, and a minimum of 33 cm for male and 31 cm for female.

The total sex ratio of males to females was 1.25:1 (Figure 29).

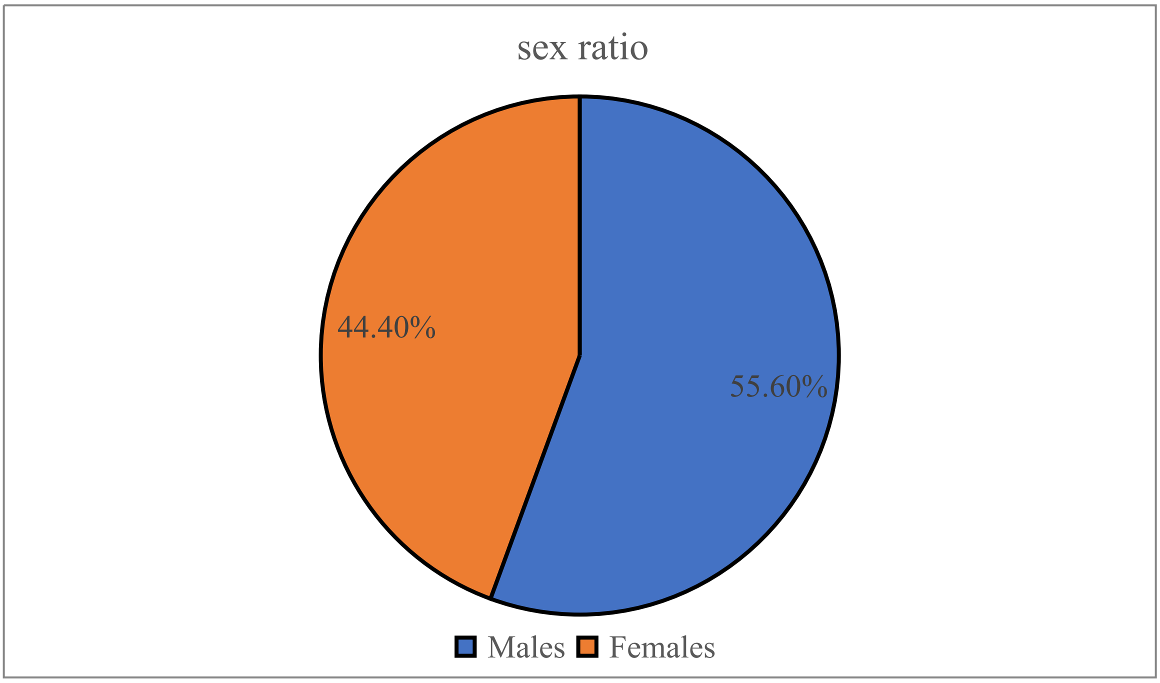


Figure 29. Percentage of Male and Female of largemouth bass.

The average weight for *Micropterus salmoides* reproduction varies depending on the age and sex of the broodstock, generally, males reach sexual maturity and are capable of reproduction around 2 years of age, while females mature around 3 years of age (Lorenzoni et al., 2002). For males, this often corresponds to a length of around 22 cm and a weight of 160g. For females, it's typically around 30 cm and 397g. However, larger, older fish tend to produce more eggs, and females generally need to be larger than males to reproduce successfully. In some studies, females around 452.36g have been used in experiments related to reproduction (Lorenzoni et al., 2002).

Chabet dis et al (2023) reported that during the pre-spawning period, the sex ratio is largely in favor of males with a value around 2 males for 1 female. On the other hand, Attou (2018) noted that during the breeding period, the sex ratio is in favor of females with 0.22 males for one female.

4. Oocyte biometry and maturity stage

Several stages of maturation of largemouth bass oocytes caught at the El Maouene Dam, which are defined by the growth of the oocyte and the formation of its yolk, were observed. only 10% of mature oocytes with a single lipid globule taking a peripheral position were observed in the sample (Figure 30) with an average diameter of 0.97 ± 0.01 µm.

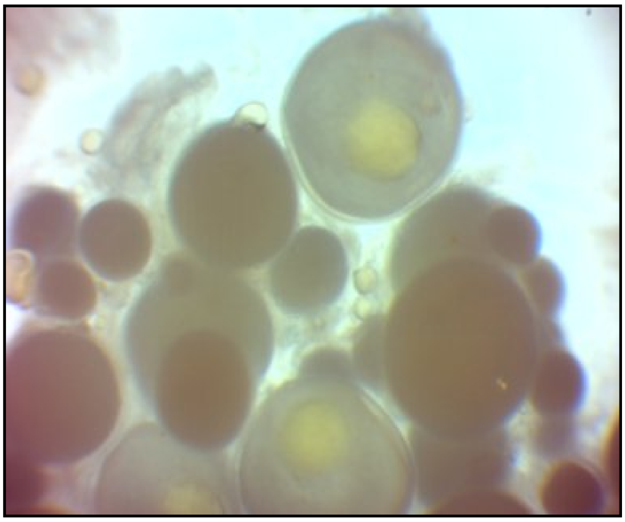


Figure 30. Largemouth bass oocytes (4× magnification)

Rejwan et al., (2007) noted that largemouth bass oocytes cannot all mature at the same time and their spawning is fractional, the male ensures fertilization when partial oocyte release is complete.

5. Hormonal Treatments

In the group treated with HCG (female), 30% of the female broodstock showed signs of successful spawning. Spawning was observed on average 72 hours after the hormone injection (Figure 30).

Figure 31. Response of a female to HCG injection

In contrast to the HCG group, no spawning was observed in the females injected with pituitary gland extract. However, all males responded (100% of males were injected with the pituitary gland) with milt after slight abdominal pressure (Figure 31) and they exhibited reproductive behavior, such as nest guarding and courtship interactions between individuals (Figure 33)

Figure 32. Milt after slight abdominal pressure.

Figure 33. Reproductive behavior (nest guarding, courtship interactions between individuals).

In 2018, Attou studied hormonal injection of largemouth bass using HCG and the pituitary gland, where he reported that HCG produced positive and better results than the pituitary gland.

Regarding time response after hormonal injection, Attou (2018) noted a variable time response ranging from 36 hours to 130 hours, and that this variability can be explained by the role of hormones in gonad maturation.

6. Egg development

The fertilized eggs of largemouth bass, with a diameter of 1.007 mm, adhere to the substrate, their coloration was between yellow and orange (Figure 34). The fertilized egg began its embryonic development and underwent a series of rapid cell divisions. The fertilized egg's development (diameter) of the largemouth bass is represented in Figure 35. A strong significant correlation was observed between the diameter of the fertilized egg and the culture time with a correlation coefficient r = 0.97.

Figure 34. The fertilized eggs in the artificial nests.

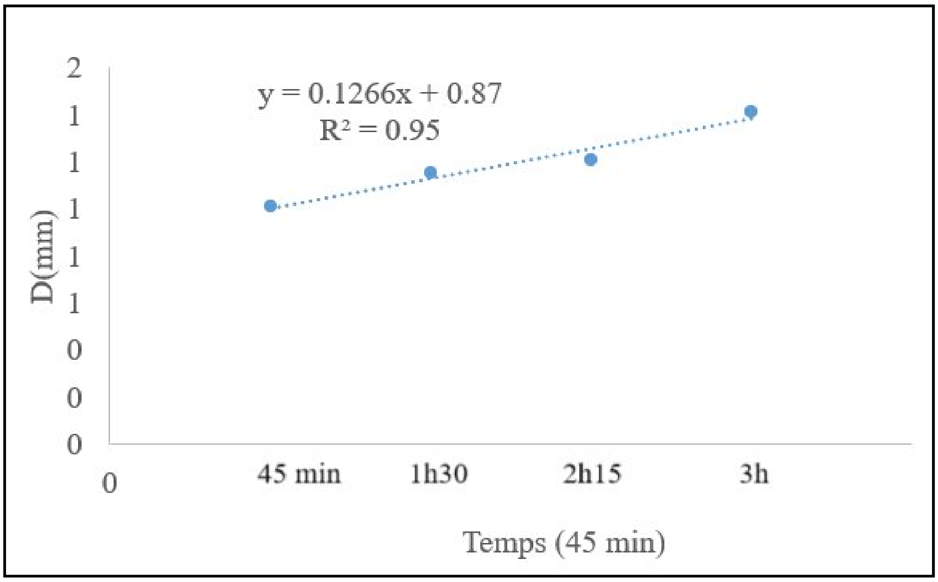


Figure 35. Development of the fertilized eggs.

7. Saproligniosis Issue

Despite the successful induction of spawning in the HCG group, the eggs obtained were severely affected by saprolegniosis, a fungal infection. This infection was characterized by the presence of white, cotton-like mycelium covering a significant proportion of the eggs, estimated at over 100%. The proliferation of these fungi led to near-total egg mortality. Dead eggs turned an opaque whitish shade and were uniform in size (Figure 35)

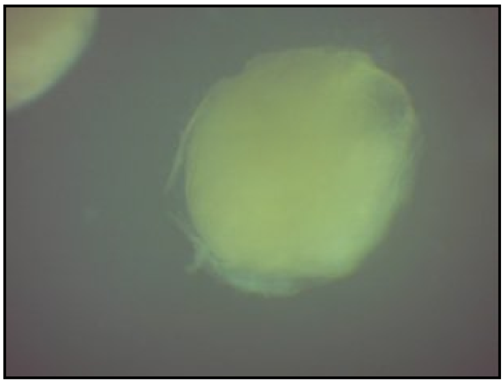


Figure 36. Fertilized egg infected with saprolegniosis.

The success of spawning induction by HCG was unfortunately overshadowed by the massive egg mortality caused by saprolegniosis. This fungal infection, primarily caused by oomycetes of the genus *Saprolegnia,* is a recurring and economically significant problem in aquaculture, especially following the ban on certain antifungal agents (Lindholm-Lehto and Pylkkö, 2024). The high incidence of saprolegniosis observed in this study can be directly linked to the capture and handling conditions of the broodstock. Fishing with fine-mesh nets likely caused physical injuries (abrasions, skin lesions) to the broodstock. These micro-injuries serve as ideal entry points for fungal spores present in the water and provide attachment sites for mycelial development (Doğan, 2024; van West et al., 2022).

Moreover, the acute stress caused by fishing operations and prolonged transport may have weakened the fishes' immune systems. Physiological stress renders organisms more vulnerable to opportunistic infections, including fungal ones (Wang et al., 2023). Eggs from stressed broodstock may also be of lower quality and thus more susceptible to rapid fungal colonization (Gallinat et al., 2022). Once the first eggs are infected, the fungi spread quickly, contaminating healthy eggs and resulting in mass mortality, as observed in this experiment (Hussein et al., 2001).

Conclusion

Conclusion

This study aimed to evaluate the induction of spawning in *Micropterus salmoides* under controlled conditions using broodstock collected from El Maouene Dam.

Females were injected with HCG and CPE, while males were injected with only the CPE. The breeding was carried out in circular tanks exposed to natural light and constant oxygenation. The fish and water quality were monitored.

The physicochemical parameters measured in situ in either wild or captivity shows that the measured values are within the recommended ranges for the reproduction of largemouth bass and this reflects that the fishing period was well chosen and the control of the environmental conditions for reproduction in captivity.

Largemouth bass oocytes cannot all mature at once, and spawning is split. Furthermore, this study confirms the effectiveness of HCG on females, as no female injected with CPE responded to the injection. All males responded to the CPE injection, ensures fertilization and nest protection.

In the present study, a strong correlation was observed between egg size and embryonic development time with coefficient r = 0.97. However, saprolegniosis resulted in a 100% mortality rate.

The hormonal injection of largemouth bass with HCG yielded positive results in females allowing us to obtain eggs while CPE produced favorable outcomes in males but not in females. However, a more detailed study on hormonal injection using other hormones such as Ovaprim is recommended to determine the most effective hormone for spawning induction of this species.

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المدرسة الوطنية العليا لعلوم البحر وتهيئة الساحل

École Nationale Supérieure des Sciences de la Mer et de l’Aménagement du Littoral

Étude technico-économique en vue de l'obtention

du diplôme CDE

Thème :

Unité pilote de production d’Artémia

Présenté par :

Gamouzi Mallek

Henna Nibras Taqouoa

Soutenue le ./../2025, devant le jury composé de :

Carte d'information

- **Equipe d'encadrement**

Equipe d'encadrement (à titre indicatif)

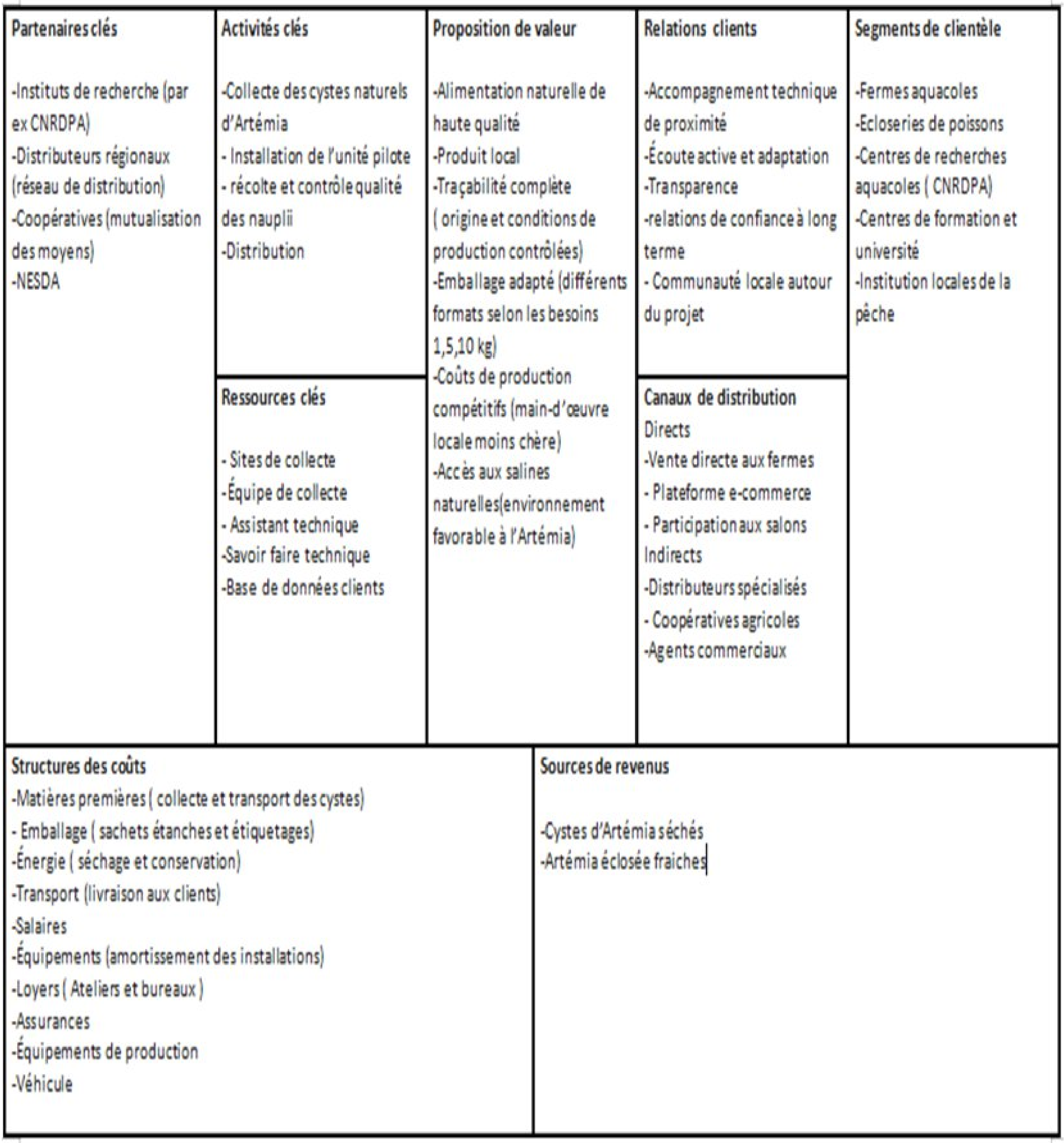
Encadrant principal : Mme Chabet Dis Chalabia	**Spécialité :** Aquaculture
Co-encadrant 01 : Mme Meslem Nabila	**Spécialité :** Aquaculture

- **Equipe de projet :**

Etudiante	**Faculté**	**Spécialité**
Gamouzi Mallek	ENSSMAL	Aquaculture
Henna Nibras Taquoua	ENSSMAL	Aquaculture

Code d'activité : 107608

BUSINESS MODEL CANVAS



Partenaires clés

-Instituts de recherche (par ex CNRDPA)
-Distributeurs régionaux (réseau de distribution)
-Coopératives (mutualisation des moyens)
-NESDA

Activités clés

-Collecte des cystes naturels d'Artémia
- Installation de l'unité pilote
- récolte et contrôle qualité des nauplii
-Distribution

Ressources clés

- Sites de collecte
-Équipe de collecte
- Assistant technique
-Savoir faire technique
-Base de données clients

Proposition de valeur

-Alimentation naturelle de haute qualité
-Produit local
-Traçabilité complète (origine et conditions de production contrôlées)
-Emballage adapté (différents formats selon les besoins 1,5,10 kg)
-Coûts de production compétitifs (main-d'œuvre locale moins chère)
-Accès aux salines naturelles(environnement favorable à l'Artémia)

Relations clients

-Accompagnement technique de proximité
-Écoute active et adaptation
-Transparence
-relations de confiance à long terme
- Communauté locale autour du projet

Canaux de distribution
Directs
-Vente directe aux fermes
- Plateforme e-commerce
- Participation aux salons
Indirects
-Distributeurs spécialisés
- Coopératives agricoles
-Agents commerciaux

Segments de clientèle

-Fermes aquacoles
-Ecloseries de poissons
-Centres de recherches aquacoles (CNRDPA)
-Centres de formation et université
-Institution locales de la pêche

Structures des coûts
-Matières premières (collecte et transport des cystes)
- Emballage (sachets étanches et étiquetages)
-Énergie (séchage et conservation)
-Transport (livraison aux clients)
-Salaires
-Équipements (amortissement des installations)
-Loyers (Ateliers et bureaux)
-Assurances
-Équipements de production
-Véhicule

Sources de revenus

-Cystes d'Artémia séchés
-Artémia éclosée fraiches

Introduction

L’artemia, également appelée crevette de saumure ou brine shrimp, est un petit crustacé aquatique appartenant au genre Artemia et à la famille des Artemiidae. Cette espèce est particulièrement remarquable pour sa capacité à vivre dans des environnements hypersalins, tels que les lacs salés et les bassins de saumure, où la salinité peut atteindre des concentrations extrêmes, jusqu’à 25% ou plus. Cette adaptation unique lui permet d’évoluer dans des milieux où la plupart des autres organismes ne peuvent survivre, ce qui fait de l’artemia un modèle d’extrêmophile en biologie.

L’artemia est largement utilisée en aquaculture, notamment sous forme de nauplii (larves fraîchement écloses), comme aliment vivant pour la nutrition des larves de poissons et de crustacés. Sa capacité à produire des œufs dormants appelés cystes, qui peuvent être stockés pendant de longues périodes et éclosent à la demande, facilite grandement son utilisation commerciale. Ces cystes sont récoltés en grande quantité, notamment dans des sites naturels comme le Grand Lac Salé de El Melah, et constituent une ressource essentielle pour l’alimentation vivante dans les élevages aquacoles à travers le monde.

Outre son intérêt économique, l’artemia présente des caractéristiques biologiques fascinantes, telles qu’un système d’osmorégulation très efficace et une capacité à adapter son mode de reproduction selon les conditions environnementales, alternant entre oviparité (production de cystes) et ovoviviparité (production de nauplii). Ces traits lui confèrent une grande résilience face aux conditions fluctuantes de son habitat naturel.

Ainsi, la production d’artemia constitue un élément clé dans le développement durable de l’aquaculture, offrant une source nutritive de haute qualité, facilement stockable et adaptable aux besoins des élevages larvaires. Cette introduction vise à présenter les principales caractéristiques biologiques et écologiques de l’artemia, ainsi que son importance dans le secteur aquacole.

1. L’idée du projet

Le projet consiste à mettre en place une unité pilote de production d’Artémia, un zooplancton d’une importance capitale dans l’élevage larvaire des poissons et des crustacés. Reconnue mondialement comme source de nourriture vivante de haute qualité, l’Artémia est indispensable aux premières phases du développement des espèces aquacoles.

En Algérie, l’absence totale de production locale constitue un frein au développement durable du secteur aquacole, fortement dépendant des importations. Pourtant, certaines zones naturelles comme les sebkhas d’El Melah et de Bahira (Sétif) abritent naturellement des populations d’Artémia, offrant ainsi une ressource biologique locale encore non exploitée. Face à la demande croissante des écloseries et fermes aquacoles – qu’elles soient marines ou continentales – ce projet vise à répondre à ce besoin en développant une production locale, accessible et maîtrisée. La démarche est pensée de manière progressive : commencer à petite échelle, avec des installations simples et un processus de production contrôlé, basé sur la collecte et la valorisation durable de souches locales d’Artémia. Cette phase expérimentale permettra d’acquérir une expertise technique, d’évaluer les rendements, de former une équipe qualifiée, et de poser les bases d’une future filière nationale de production d’aliments vivants pour l’aquaculture.

2. Les valeurs ajoutées

Nous avons identifié les principales valeurs ajoutées que notre unité de production d’Artémia apportera aux clients cibles (écloseries, fermes aquacoles, institutions de recherche, collectivités et services de l'État) :

- Implanter une unité de production d’Artémia opérationnelle à l’échelle locale, avec des infrastructures adaptées (bassins, systèmes de culture, incubation, récolte et conditionnement).

- Maîtriser l’ensemble du cycle de production (éclosion, élevage, récolte, conditionnement) dans des conditions contrôlées, garantissant un produit stable et conforme aux normes aquacoles.

- Atteindre un volume de production annuel cible (ex. : X tonnes/an de nauplii ou d’œufs d’Artémia), permettant de couvrir une part significative de la demande nationale.

- Développer un protocole technique reproductible, incluant les paramètres physico-chimiques optimaux, les régimes alimentaires, les taux de survie, etc.

- Mettre en place un système de contrôle qualité rigoureux, assurant la traçabilité, la pureté biologique, et la viabilité des nauplii livrés.

- Créer un réseau de distribution efficace, reliant l’unité de production aux principaux bassins aquacoles du pays, en garantissant rapidité et conservation du produit.

- Former une équipe locale qualifiée, composée de techniciens et biologistes spécialisés dans la culture et l’utilisation de l’Artémia.

- Évaluer l’impact économique et écologique du projet, à travers des indicateurs de rentabilité, de satisfaction client et de durabilité.

3. Les valeurs proposées

Notre projet de production d’Artémia repose sur un ensemble de valeurs fondamentales qui guident notre approche et renforcent son impact sur le secteur aquacole :

- **Valorisation d’une ressource naturelle locale :** Utilisation responsable et durable des sebkhas algériennes (El Melah, Bahira...) riches en Artémia, encore inexploitées à ce jour.

•**Pionnier en Algérie :** Première initiative structurée visant la production d’Artémia à l’échelle locale, en réponse à une dépendance totale à l’importation.

•**Accessibilité pour les petites structures aquacoles :** Offrir une solution économique et adaptée aux réalités du terrain pour les écloseries et fermes aquacoles algériennes.

•**Approche progressive et réaliste :** Démarrage en unité pilote pour tester, maîtriser et ajuster les techniques de production avant une éventuelle expansion.

•**Respect de l’environnement :** Production propre sans produits chimiques, en valorisant un écosystème existant sans le perturber.

•**Transfert de compétences locales :** Formation de jeunes techniciens et développement de savoir-faire nationaux en production d’aliments vivants.

4. Objectifs du projet

•Mettre en place une unité pilote de petite échelle pour la culture et la production d’Artémia en conditions contrôlées.

•Identifier, prélever et tester des souches locales d’Artémia issues de sebkhas naturelles (El Melah, Bahira...).

•Maîtriser les différentes phases de production : incubation des cystes, élevage des nauplii, récolte, conditionnement.

•Fournir les premiers volumes d’Artémia à des fermes ou écloseries locales pour tests zootechniques.

•Évaluer la faisabilité technique et économique du projet à petite échelle (rendements, coût, qualité du produit).

•Former une équipe locale à la gestion et au suivi de la production.

5. Activités clés

•Collecte de souches naturelles d’Artémia dans les sebkhas locales (El Melah, Bahira).

•Installation de l’unité pilote : bassins, systèmes d’incubation, matériel de récolte.

•Culture et suivi des paramètres (salinité, température, oxygène, pH).

•Récolte et contrôle qualité des nauplii (viabilité, taille, valeur nutritive).

•Tests de distribution auprès de fermes ou écloseries partenaires.

•Formation du personnel local aux techniques de production.

•Suivi des rendements et analyse technique des résultats.

•Préparation à l’extension : rapport d’évaluation et plan de développement.

6. Analyse stratégique du marché

1. Segments cibles

•Écloseries publiques et privées : Besoin direct d’Artémia pour l’alimentation larvaire (tilapia, bar, daurade, crevette...).

•Le CNRDPA (Centre National de Recherche et de Développement de la Pêche et de l’Aquaculture) :

→ Client institutionnel potentiel, notamment pour l’approvisionnement de ses stations expérimentales, unités pilotes d’écloserie, ou dans le cadre de projets de recherche appliquée en aquaculture.

→ Peut utiliser l'Artémia produit localement pour ses travaux scientifiques, essais d'alimentation, ou programmes de formation.

•Centres de formation et universités : Utilisation pour des TP, essais pratiques et stages de recherche.

•Petits aquaculteurs : Intéressés par une source fiable et locale d'Artémia à faible coût.

•Institutions locales de la pêche (DPA, chambres de pêche) : Pour accompagner les petits porteurs de projets aquacoles.

2. Canaux de communication

- Visites et démonstrations sur site

→ Accueillir des écloseries, aquaculteurs, chercheurs ou institutions pour observer les étapes de production en conditions réelles.

- Page Facebook professionnelle

→ Plateforme accessible pour publier des annonces, disponibilités, conseils d'utilisation, photos, vidéos, et recevoir des commandes ou messages directs.

- WhatsApp Business

→ Canal rapide et direct pour interagir avec les clients (devis, questions, livraison, assistance technique).

- Partenariats académiques et institutionnels

→ Collaboration avec le CNRDPA, universités et instituts pour appuyer la crédibilité technique du projet et diffuser les résultats.

- Journées techniques et salons aquacoles

→ Présence dans les événements régionaux (ex : journées de la pêche, foires agricoles) pour élargir le réseau professionnel et établir des contacts directs.

- Supports de communication imprimés

→ Fiches techniques simplifiées (en arabe et en français), dépliants de présentation du projet, guides d'éclosion d'Artémia.

- Réseau relationnel local

→ Utilisation du bouche-à-oreille, contacts des directions de la pêche (DPW), enseignants chercheurs, et anciens stagiaires du domaine.

3.Relations clients

Pour établir et maintenir une relation de confiance avec les premiers utilisateurs (écloseries, aquaculteurs, centres de recherche), le projet adoptera une approche de proximité et d'accompagnement personnalisé :

Accompagnement technique de proximité

→ Fourniture de conseils pratiques sur l'éclosion et l'utilisation de l'Artémia (oralement, par téléphone ou via WhatsApp).

→ Possibilité d'assistance directe sur site pour les premiers clients locaux.

Écoute active et adaptation

→ Récolte des retours des clients pour améliorer le produit et les méthodes de conditionnement.

→ Ajustement progressif de l’offre (quantité, forme : œufs secs ou nauplii vivants, emballage...).

Transparence

→ Communication claire sur l’origine locale des souches, la méthode de production et les conditions de stockage.

→ Affichage des prix, du mode d’élevage et des délais de livraison.

Relations de confiance à long terme

→ Construction d’un lien durable avec les écloseries et institutions partenaires (ex : CNRDPA, centres universitaires).

→ Priorité donnée aux clients réguliers ou aux collaborations expérimentales.

Communauté locale autour du projet

→ Création d’un petit réseau d’utilisateurs (groupes WhatsApp ou Facebook) pour échanger des pratiques, poser des questions, partager des retours d’expérience.

Plan de production et organisation :

1. Production

La production d’Artémia se fera selon un cycle simple et itératif : incubation des cystes, élevage des nauplii, récolte, et nettoyage des bassins. Chaque cycle durera environ une semaine. Des ajustements seront réalisés à chaque étape pour améliorer progressivement les rendements (densité, oxygène, température, qualité de l’eau).

2. Approvisionnement

Les ressources utilisées proviendront :

- De la collecte de cystes dans les sebkhas locales (ex : Bahira ou El Melah),

L’eau utilisée sera enrichie en sel si nécessaire pour atteindre les conditions favorables (30–35 PSU).

3. Besoins humains

Une petite équipe polyvalente assurera la gestion du projet :

- Responsables techniques (les deux ingénieures) :

Pilotage du projet, suivi des cultures, contrôle qualité, relation avec les partenaires, gestion des livraisons, communication technique.

- Un opérateur ou assistant technique (éventuel) :

Appui pour les travaux quotidiens (nettoyage des bassins, récolte, aération, alimentation, conditionnement).

- Appui administratif ponctuel :

Pour la gestion des documents, commandes, livraisons, relations avec les administrations ou structures de financement.

4. Technologies et matériel

Le projet nécessitera les équipements suivants :

- Bassins de culture en plastique ou en béton d’une capacité de 1 à 3 m³

- Systèmes d’aération composés de pompes à air et de diffuseurs

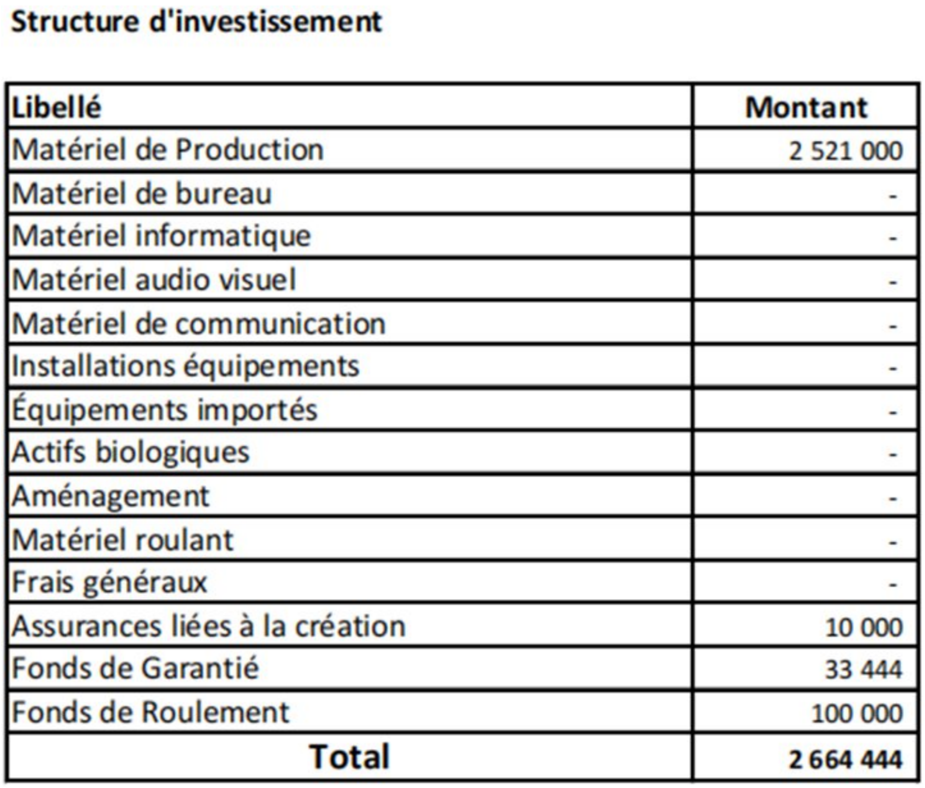
- Incubateurs coniques pour l’éclosion des cystes (volume de 10 à 15 litres)

- Matériel de récolte : filets à mailles fines, tamis, seaux

•Sondes de suivi (si disponibles) pour la température, le pH et la salinité

•Zone de conditionnement pour le tri et la préparation des nauplii

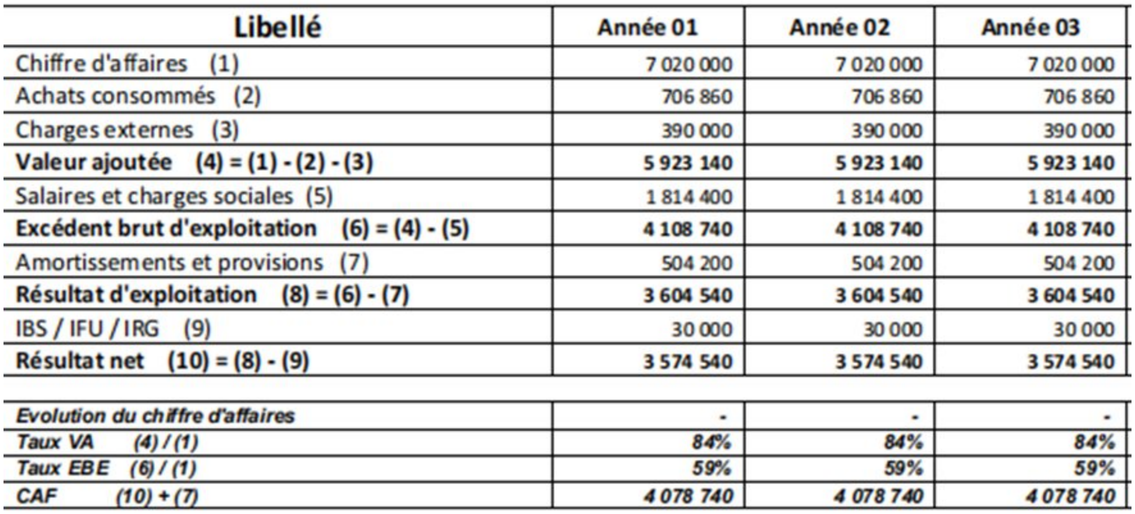
•Étuve pour le séchage ou le traitement des cystes (optionnelle mais utile pour le stockage à sec).



Structure d'investissement

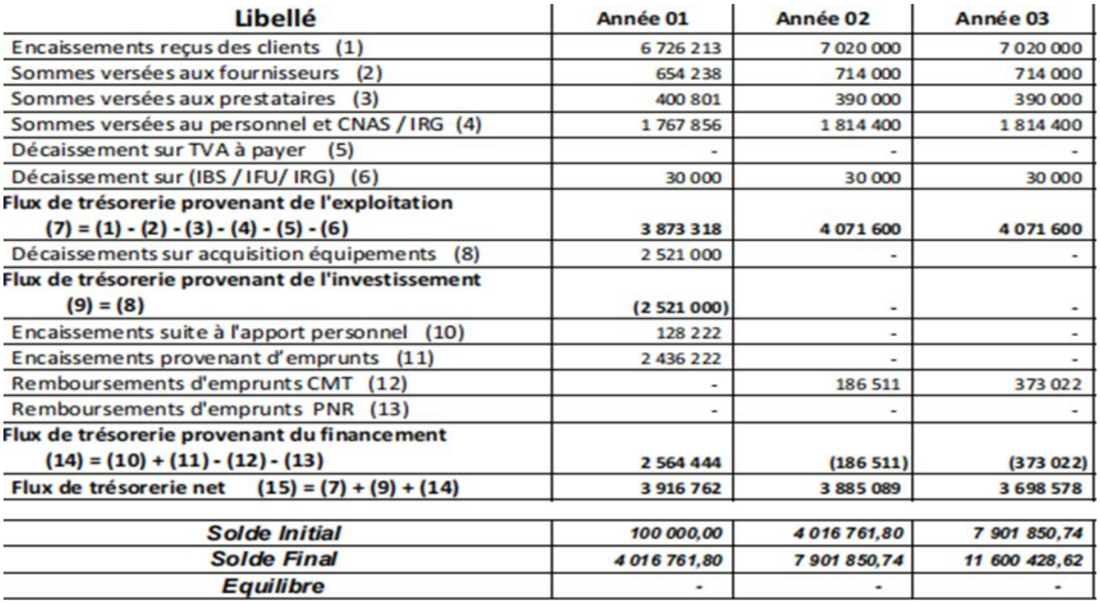
Libellé	Montant
Matériel de Production	2 521 000
Matériel de bureau	-
Matériel informatique	-
Matériel audio visuel	-
Matériel de communication	-
Installations équipements	-
Équipements importés	-
Actifs biologiques	-
Aménagement	-
Matériel roulant	-
Frais généraux	-
Assurances liées à la création	10 000
Fonds de Garantié	33 444
Fonds de Roulement	100 000
Total	**2 664 444**

COMPTE DE RESULTAT PREVISIONNEL



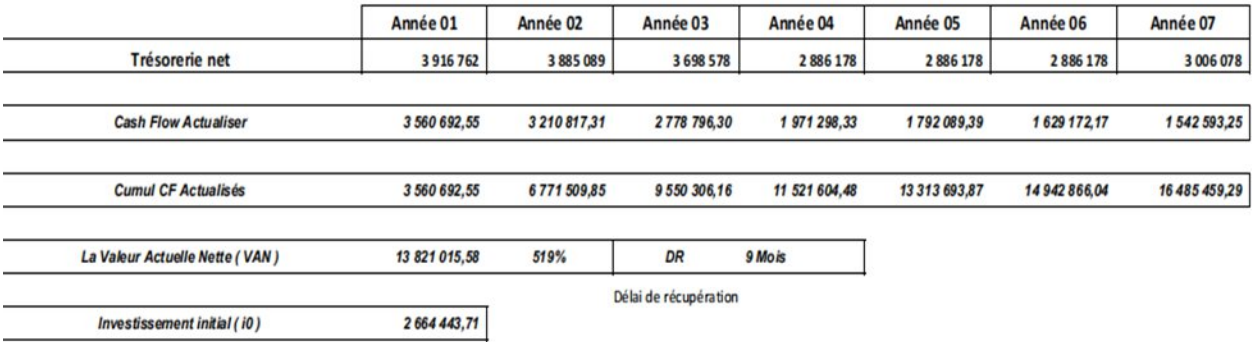
Libellé	Année 01	Année 02	Année 03
Chiffre d'affaires (1)	7 020 000	7 020 000	7 020 000
Achats consommés (2)	706 860	706 860	706 860
Charges externes (3)	390 000	390 000	390 000
Valeur ajoutée (4) = (1) - (2) - (3)	**5 923 140**	**5 923 140**	**5 923 140**
Salaires et charges sociales (5)	1 814 400	1 814 400	1 814 400
Excédent brut d'exploitation (6) = (4) - (5)	**4 108 740**	**4 108 740**	**4 108 740**
Amortissements et provisions (7)	504 200	504 200	504 200
Résultat d'exploitation (8) = (6) - (7)	**3 604 540**	**3 604 540**	**3 604 540**
IBS / IFU / IRG (9)	30 000	30 000	30 000
Résultat net (10) = (8) - (9)	**3 574 540**	**3 574 540**	**3 574 540**
Evolution du chiffre d'affaires	-	-	-
Taux VA (4) / (1)	***84%***	***84%***	***84%***
Taux EBE (6) / (1)	***59%***	***59%***	***59%***
CAF (10) + (7)	***4 078 740***	***4 078 740***	***4 078 740***

TABLEAU DE FLUX DE TRESORERIE (Méthode Directe)



Libellé	Année 01	Année 02	Année 03
Encaissements reçus des clients (1)	6 726 213	7 020 000	7 020 000
Sommes versées aux fournisseurs (2)	654 238	714 000	714 000
Sommes versées aux prestataires (3)	400 801	390 000	390 000
Sommes versées au personnel et CNAS / IRG (4)	1 767 856	1 814 400	1 814 400
Décaissement sur TVA à payer (5)	-	-	-
Décaissement sur (IBS / IFU/ IRG) (6)	30 000	30 000	30 000
Flux de trésorerie provenant de l'exploitation (7) = (1) - (2) - (3) - (4) - (5) - (6)	**3 873 318**	**4 071 600**	**4 071 600**
Décaissements sur acquisition équipements (8)	2 521 000	-	-
Flux de trésorerie provenant de l'investissement (9) = (8)	**(2 521 000)**	**-**	**-**
Encaissements suite à l'apport personnel (10)	128 222	-	-
Encaissements provenant d'emprunts (11)	2 436 222	-	-
Remboursements d'emprunts CMT (12)	-	186 511	373 022
Remboursements d'emprunts PNR (13)	-	-	-
Flux de trésorerie provenant du financement (14) = (10) + (11) - (12) - (13)	**2 564 444**	**(186 511)**	**(373 022)**
Flux de trésorerie net (15) = (7) + (9) + (14)	**3 916 762**	**3 885 089**	**3 698 578**
Solde Initial	***100 000,00***	***4 016 761,80***	***7 901 850,74***
Solde Final	***4 016 761,80***	***7 901 850,74***	***11 600 428,62***
Equilibre	-	-	-

ÉVALUATION PROJET, VAN, DR



	Année 01	Année 02	Année 03	Année 04	Année 05	Année 06	Année 07
Trésorerie net	3 916 762	3 885 089	3 698 578	2 886 178	2 886 178	2 886 178	3 006 078
Cash Flow Actualiser	3 560 692,55	3 210 817,31	2 778 796,30	1 971 298,33	1 792 089,39	1 629 172,17	1 542 593,25
Cumul CF Actualisés	3 560 692,55	6 771 509,85	9 550 306,16	11 521 604,48	13 313 693,87	14 942 866,04	16 485 459,29
La Valeur Actuelle Nette (VAN)	13 821 015,58	519%	DR	9 Mois			
			Délai de récupération				
Investissement initial (i0)	2 664 443,71						

Conclusion

L'unité pilote de production d'artémia en Algérie représente une opportunité stratégique majeure pour développer une filière locale à forte valeur ajoutée. Avec un avantage concurrentiel naturel grâce à l'accès aux salines nationales et un marché aquacole en pleine croissance, ce projet répond à un besoin réel de substitution aux importations coûteuses.

Le projet contribuera significativement au développement économique local tout en créant des emplois qualifiés dans les régions sahariennes. Cette initiative peut positionner l'Algérie comme acteur clé dans la production d'artémia en Méditerranée.