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REARING OF ROPȘA CARP, OBTAINED BY SELECTIVE BREEDING AND EPIGENETIC PROGRAMMING, IN AN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEM

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Abstract

Integrated Multi-Trophic Aquaculture (IMTA) systems, incorporating carp and mussels, represent a promising approach to aquaculture that enhances environmental sustainability by promoting nutrient recycling and reducing ecological impact. The Ropşa carp was used in the experiment for its adaptability to variable environmental conditions and precocity, reaching sexual maturity at 2-3 years. Selective breeding and epigenetic programming allow fish with improved growth performance and efficient feed conversion, contributing to increased productivity and reduced environmental impact. After selection of the specimens that showed good adaptability to high temperature conditions and a critical concentration of dissolved oxygen in the water, their growth was carried out in eight cages, in polyculture (Ropşa carp and Sinanodonta woodiana mussels), and in the rest of the water body in the experimental pond the population was mixed (three summers aged fish of various species and S. woodiana mussels). At the end of the experiment, a good percentage of growth and survival in cages was obtained for both studied populations, with monthly recording of production data and testing of water quality parameters.

Key words: *bivalves, cages, epigenetics, fish, IMTA, sustainability.*

INTRODUCTION

The aquaculture sector in Romania is primarily characterized by extensive cypriniculture units, which include both growers and combined units (broodstock nurseries and growers). These units account for over 70% of the total number of aquaculture facilities in the country. The main species cultivated are carp *Cyprinus carpio* (Linnaeus, 1758), silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), and bighead carp *Hypophthalmichthys nobilis* (Richardson, 1845) (Bucur et al., 2016).
In Romania, most fish production occurs in ponds, lakes, and reservoirs through extensive and semi-intensive cyprinid farming, often in polyculture with other species (Dobrotă et al., 2012). These systems are highly vulnerable and significantly impacted by climate change (Gancea et al., 2024). The effects of climate change manifest directly, through challenges related to water availability and maintaining necessary levels amid rising temperatures, prolonged droughts, evaporation, and extreme weather events. Additionally, there are indirect impacts, such as physiological stress on fish populations due to increased temperatures, oxygen depletion, reduced metabolic rates and feeding, and a higher incidence of diseases.
Freshwater ecosystems are highly threatened by climate change (Ahmed et al., 2019). Climate change is one of the primary drivers of disturbances in these ecosystems, leading to alterations in the structure, composition, and functioning of biodiversity. To develop effective strategies for breeding, production intensification, adaptation, and conservation using multitrophic technologies, it is important to understand how freshwater species can tolerate the environmental fluctuations induced by climate change (Azhar & Memiş, 2023).
Therefore, studying the adaptive potential of freshwater species and their cultivation in combination with related aquatic species is essential (Azevedo et al., 2011; Dobrotă et al., 2024). This research will help us comprehend

the effects of climate change on fish species and its broader implications for ecosystem structure and function (Behera et al., 2012). Epigenetics is defined as the branch of genetics that examines variations in traits and phenotypic characteristics of organisms resulting from changes in environmental conditions, which subsequently affect their behaviour (Eslam et al., 2018). It focuses on potentially heritable changes in gene expression that do not involve alterations to the DNA sequence (Granada et al., 2018). Epigenetic mechanisms work by modifying gene behaviour, either inhibiting or activating gene expression in response to environmental stimuli, while leaving the genome and DNA structure unchanged (Roy et al., 2022).

In recent years, the role of epigenetic mechanisms has gained prominence, particularly concerning gene-environment interactions (Moghadam et al., 2015). Important performance and production traits in aquaculture include growth rate, feed conversion efficiency, disease resistance, stress tolerance (such as low oxygen tolerance), reproductive success, harvesting capacity, and processing yields, among others (Makvandi-Nejad & Moghadam, 2023). Internationally, terrestrial agricultural production has limited resources, while aquaculture presents significant potential for food production, as 72% of the Earth's surface is covered by water (Alexopoulos et al., 2011; Borghese et al., 2025). Currently, inland freshwater bodies, which account for only 1% of the Earth's surface area, are the primary areas used for aquaculture (FAO, 2023). It is undeniable that aquaculture production is influenced by growing conditions and the uncertainties linked to climate change (Agostinho et al., 2008; Neculita & Moga, 2015) facing the entire planet. According to the Intergovernmental Panel on Climate Change (Bongaarts, 2019), sustainable development that balances environmental protection, economic prosperity, and social well-being is closely linked to the impacts and response strategies related to climate change (Boyd, 2017; Boyd & Tucker, 2014). Achieving sustainability in aquaculture production is impossible without directly addressing the effects of climate change.

Sustainability refers to the management of technological, financial, institutional, natural, and social resources to ensure a reliable supply of human needs, not only for the present but also for future generations (Petrea et al., 2019). For aquaculture to be sustainable, it must endure across generations. The sustainability of aquaculture systems (Ohia, 2025) can be assessed using three key indicators: environmental, economic, and social indicators. Environmental sustainability indicators include the efficient use of natural resources, pollution prevention, and biodiversity conservation (Ridler et al., 2007; Hodosan et al., 2023; Ruiz-Vanoye et al., 2025).

Economic sustainability (Costache et al., 2021) emphasizes the efficient use of existing financial resources, the economic feasibility of undertaken activities, resilience in absorbing unforeseen external expenses, and the generation of funds for reinvestment (Wang et al., 2012). In contrast, social sustainability focuses on the ability of aquaculture to provide community benefits such as food security, employment, income equality, equitable opportunity distribution, and inclusion of vulnerable populations (Valenti et al., 2018). While aquaculture sustainability has primarily concentrated on the sustainable use of environmental resources, the significance of the economic and social dimensions has received comparatively less attention (Whitmarsh et al., 2006; Ahmed et al., 2019; Engle, 2019). To mitigate the climate change footprint and enhance the efficiency of aquaculture production systems, the implementation of Integrated Multi-Trophic Aquaculture (IMTA) can serve as an effective and innovative strategy in our country. This approach maximizes production capacity by utilizing the same resources as current technologies (Chopin, 2013; Kibria and Haque, 2018; Arcade et al., 2023; Cranford et al., 2013).

MATERIALS AND METHODS

To conduct the study, we utilized a classical fish pond that features a continuous inflow and outflow system. The pond is constructed with concrete embankments, and its bottom consists of alternating layers of soil, sand, and other specific materials. The size of the pond is

35,000 m^2. Within the pond, there is a platform containing eight floating cages (Degefu et al., 2011), stocked with carp from various species (Ineu, Frăsinet, Ropșa). This includes six cages with two summer-aged carp and two cages with two summer-aged Ropșa carp, which were obtained through selective breeding and epigenetic programming for intensive rearing. The selectively bred specimens exhibit a higher resistance to low dissolved oxygen levels in the water and demonstrate a good growth capacity in a temperature range exceeding the average normal limit of 26°C. The stocking density of the pond follows polyculture rearing practices, combining different carp species (Frăsinet, Ropșa, Ineu) with Asian cyprinids (*H. molitrix, Ctenopharyngodon idella* (Valenciennes, 1844)*, H. nobilis*) as well as predatory fish (Costache et al., 2018), including *Sander lucioperca* (Linnaeus, 1758), *Silurus glanis* (Linnaeus, 1758), and *Esox Lucius* (Linnaeus, 1758). The total biomass of fish in the experimental pond was 3.5 tons per hectare. Additionally, 300 individuals of *Sinanodonta woodiana* (Lea, 1834) mussels, with an average weight of 265 g, were introduced into the experimental pond. These mussels were harvested from another pond that is part of the experimental base of S.C.D.P-Nucet (Figure 1).

Figure 1. Research base S.C.D.P - Nucet
(Source: original)

To obtain production data, it was conducted monthly assessments of individual growth rates, survival rates, total production per vivarium, weight multiplication rates, and specific feed intake (Mogodan et al., 2021).

Concurrently, it was carried out physico-chemical analysis of the water and fishing actions to evaluate the aquatic biomass.

For this action it was utilized a portable oxygen meter from HANNA HI9829-11042, manufactured in Romania, which is equipped with specialized probes suitable for these measurements. This device recorded parameters such as dissolved oxygen, water temperature, saturation, and pH. It was calibrated at two points using globally recognized standard values. Additionally, the atmospheric temperature was monitored with a digital thermometer mounted on the floating frame of the cages to ensure greater accuracy in the recordings.

Short characterization of the biological material assessed

The carp is the most widespread species of cyprinid cultivated, having great economic importance in ponds across Europe, as well as in Asia, North America, and South America (Nicolae et al., 2009; Nicolae et al., 2012). It belongs to the Cyprinidae Family, which is one of the largest families of freshwater fish, comprising over 2,000 species worldwide. The carp is the most economically valuable freshwater species in Europe and the temperate zones generally. Its meat is tasty and has high nutritional value (Nicolae et al., 2015a; Nicolae et al., 2015b).

The Ropșa carp breed used in the experiment, originates from the former USSR, having been brought to Nucet in 1981 for crossbreeding with local breeds. This breed is known for its adaptability to changing environmental conditions and early maturation. Ropșa carp reach sexual maturity at 2 to 3 years of age, in contrast to local breeds, which mature at 4 to 5 years (Nicolae et al., 2016; Radu et al., 2018). This shorter maturation period decreases the interval between generations, thereby enhancing breeding efforts.

In addition to the Ropșa carp, an important component of the trophic chain being studied, in the experimental integrated rearing basin, was the freshwater mussel, *S. woodiana*.

S. woodiana belongs to the Unionidae Family and are among the largest freshwater mussels in terms of size (U.S. Fish & Wildlife Service, 2015).

At the Nucet studied site, the maximum recorded size of these mussels reaches 20 cm in total length, with a maximum average weight of 286 g, 26 cm circumference. The mussels have been introduced in all floating cages.

Distribution of biological material in cages

The cages, constructed of eight modules each, consist of HDPE (High-density polyethylene) pipes that form the skeleton, while a wooden pontoon serves as an access path. The nets used to isolate the body of the vivarium from the rest of the tank are knotless and treated to withstand weather conditions.

The first six cages were stocked with 30 bivalves/cage, averaging 260 g each, with a minimum weight of 223 g and a maximum of 272 g. The last two cages contained a total of 40 bivalves per culture, averaging 250 g each, with a minimum weight of 230 g and a maximum of 266 g (Figure 2.)

Figure 2. Weighing action of mussels *S. woodiana* (Source: original)

The stocking standards for the first series of six carp cages were established based on experiences from previous years.

Figure 3. Recording biometric data of Ropșa carp, two summers age (Source: original)

From earlier rearing experiments, it was concluded that the most appropriate stocking formulas, particularly in terms of economic efficiency, are as follows:

- For the summer growth of common carp in the cages: 5-10 fish per cubic meter (ex./m^3), with an average weight of 150 g per fish. This has been the standard value applied for six years;
- For all eight cages, the selected stocking variant is as follows: 8 fish per cubic meter (ex./m^3), with an average weight of 145 g per fish. This variant was used in the experiment conducted in the first six cages (Figure 3);
- 10 fish per cubic meter (ex./m^3), with an average weight of 120 g/ex - the variant applied for the experiment carried out in the last two cages (Figure 4).

Each cage has a useful volume of 72 m^3.

Figure 4. Ropșa carp of selective breeding and epigenetic programming (Source: original)

To calculate the specific production indices within the monitored rearing range we used the following formulas:

- Percent survival – d (%) - determined with the formula:

$$d = N/ N_i \times 100 (\%) \quad (1)$$

where: N_f, N_i - the final and initial number of individuals;

- The actual growth increment - (Sr) - determined gravimetrically, consists of weighing two samples at the time of stocking, determining the initial mass, and two samples at the end of the rearing period, determining the final mass. It is calculated with the formula:

$$Sr = B_f - B_i \text{ (kg)} \quad (2)$$

where: B_f, B_i - final and initial biomass of the batch (kg);

- Individual growth rate (IGR) - determined with the formula:

$$IGR = (W_f - W_i)/ N \text{ (g/ex.)} \quad (3)$$

where: Wf, Wi - final and initial average mass of the batch (g); N - number of specimens (ex.).

- Daily growth rate - (DGR) - determined by applying the formula:

$$DGR = (W_f - W_i)/ T \text{ (g/day)} \quad (4)$$

where: Wf, Wi - final and initial average mass of the batch (g); T - duration of the growth cycle (days), 92 days (Popescu, 2009).

The water chemical analyses (Hansen & Koroleff, 1999; Boyd, 2017; Boyd & Tucker, 2014) were carried out in the hydro-chemistry laboratory of the Research and Development Station for Fish Farming Nucet (S.C.D.P-Nucet), according to the standard methodology for the determination of chemical parameters of fish water (Marica et al., 2023). Parameters followed in the chemical analyses were: ammonium ion (NH_4^+), reported in mg/L (optimum 0.5-1 mg/L/maximum 2 mg/L); $KMnO_4^+$ oxidisation, in mg/L (optimum 20 mg/L/maximum 60 mg/L), Ca^{2+}/Mg^{2+} ratio, in mg/L (optimum 5 mg/L/maximum 10 mg/L).

RESULTS AND DISCUSSIONS

Following the combined farming of Ropșa carp and Chinese pond mussels in a controlled system, the evaluation of the established parameters was improved due to the restriction of the area of the target area. By enclosing the growing habitat, it is easier to carry out control fishing and assess the existing biomass. At the same time, since a pontoon is available to connect the 8 individual modules, water sampling for the investigation of physicochemical parameters was facilitated.

The recorded mean air/water temperature variation from July 1 to July 31, 2024, in the Nucet-Dâmbovița experimental base was 28.66°C in water, with a minimum temperature of 26.55°C and a maximum of 31.5°C. For the same period in the Nucet experimental area, the mean air temperature was 29.01°C, with a minimum of 17°C and a maximum of July of 40°C.

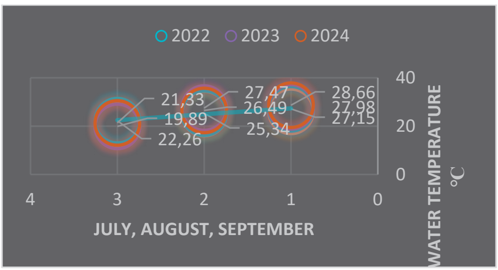

Figure 5. Water temperature (°C) in 2022, 2023, 2024 years

The month of August was characterized in terms of air temperature by a monthly average of 26.85°C, the lowest temperature recorded in August was 16°C and the maximum was 38°C. In case of water temperature, it averaged 26.49°C, with a minimum of 23°C and a maximum of 28.8°C. In September the average water temperature was 21.33°C, recording a maximum of 25°C and a minimum of 18°C. In terms of air temperature, the average air temperature for September was 20.81°C, the minimum was 12°C, and the maximum was 32.5°C. In correlation of these data collected, with the monthly average of 2022 and 2023, in the year 2024 all the temperatures are above the averages of those years with at least 1.5°C increase, in terms of water temperature (Figure 5).

In comparison to the years mentioned above, in 2024 the air temperature of July, August, and September were about 2°C higher, the only deviation from the rule being July 2019 when the difference between July 2024 and July 2019 temperature was about 3.2°C. On average, water and air temperatures have increased with 1.5 °C compared to previous years. According to data provided by Bongaarts (2019), these changes in temperature are based on a study examining climate change and its effects on aquatic ecosystems.

Under the conditions of the application of controlled rearing technology, the analysed biological material from the first six cages showed a surprising survival rate, given that the density of specimens per m^3 was quite high, thus resulting in a survival rate of 89%. In the case of the two stock cages with Ropșa carp obtained by epigenetic programming the survival percentage was higher, i.e. 92% compared to the first version. The higher rate of survival is due to the fact that the fish obtained following the application of epigenetic techniques, the characteristics of the selected specimens are clearly superior to standard fish material of the same species. High survival rates confirm the positive effects of genetic selection, such results are supported by studies on epigenetics in aquaculture (Granada et al., 2018; Makvandi-Nejad & Moghadam, 2023). Resistance to low oxygen content in the water and high-temperature tolerance above the optimal range of the species were the characters targeted during the study. Concerning the growth indicators after the completion of the retention-for-growing period July-August-September 2024, results obtained after the fish biomass assessment (biometric data recording - average weight g/ex. realized for samples of 200 fish, % survival, total production/cage) are presented in (Table 1).

The total production on each unit studied was successful yielding a real growth rate, an individual growth rate and a daily growth rate for each cage as shown in Table 2.

The data from the quantitative evaluations indicate that the rate of weight gain is higher in controlled-modulus rearing compared to classical earthen pond rearing. The survival rate of the studied variants, Ropșa from controlled breeding (cage 1-6), and Ropșa resulting from selective breeding (cage 7-8), was higher in the epigenetically programmed individuals with an average survival percentage of 92%; in the first 6 cages, the survival percentage was 89%.

Table 1. Tracked technological indicators (average weight, survival %, total production/cage)

Tracked indicator	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Cage 7	Cage 8
Average weight (g/ex)	2230	2110	2270	2244	2281	2310	2180	2240
Survival rate (%)	86	89	92	91	87	89	90	94
Total carp production (kg/cage)	1284	1215	1307	1292	1313	1330	1569	1612

Table 2. Tracked technological indicators (actual growth rate, individual growth rate, daily growth rate)

Tracked indicator	Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Cage 7	Cage 8
Growth rate (kg/cage)	1198	1129	1221	1206	1227	1244	1476	1519
Specific growth rate (g/ex)	2079	1960	2119	2093	2130	2159	2050	2109
Daily growth rate (kg/day)	13.05	12.30	13.30	13.14	13.36	13.55	16.04	16.51

On examination of the differences in survival rates in relation with total production for each cage, the average yield obtained in the last two modules was approximately 300.5 kg per module, higher than in earlier ones. This increased production in the last two modules can be attributed to the improved traits of the epigenetically programmed carp. These results are supported by a series of genetic research showing improvements in bio-economic and eco-economic traits whereby two-summer-old Ropsa carp outperform Frăsinet and Ineu carp (Nicolae et al. 2012). Despite the higher stocking density per cubic meter of water, the fish exhibited resilience to low dissolved oxygen concentrations below the critical limit (mg/L).

Additionally, temperature fluctuations in the water caused by atmospheric currents led to an average change of 2-6°C within 5-8 days during the summer. This fluctuation resulted in thermal discomfort and a constant state of stress for the aquatic biomass. Due to oxidative stress, the fish became apathetic and altered their behaviour. Linked with high water temperatures, the carp stopped feeding normally, became inhibited, and this could lead to mortalities.

The bivalves introduced in the monitored cages did not experience any mortalities and successfully fed under the environmental conditions present. The movements of the fish in the water column continuously stirred the suspensions, which favoured the feeding of the Chinese pond mussels. Their filtration capacity of bivalves it was tested in a recent study applied in a controlled environment, demonstrating their filtration performance and improved water quality parameters. In addition to stimulating the feeding process, these bivalves also utilized the submerged feeds, helping to maintain a biochemical equilibrium in the hypolimnion. After taking biometric measurements, the maximum weight of the mussels in the first six cages reached 282 g, which is an increase of 10 g compared to their peak weight at the time of stocking. The minimum weight at stocking rose to 234 g, reflecting an increase of 11 g. In the other two cages, the minimum weight increased by 9 g, while the maximum weight increased from 266 g to 276 g.

The highest concentration of ammonium ions (NH_4^+) was recorded during the second water sampling in July, with a level of 2.6 mg/L, which is 0.6 mg/L above the maximum limit, but within accepted limits for IMTA systems (Chopin, 2013). This concentration is considered normal within the context of intensive integrated multitrophic aquaculture systems (IMTA). These IMTA systems can respond to sudden changes in quality parameters, particularly during fluctuations in the food chain and increases in aquatic biomass density (m^3). Regarding organic matter oxidation, measured through $KMnO_4$ (TDS - mg/L), the maximum value was reported in the sample collected at the end of August, reaching 56.38 mg/L. The average ratio of calcium (Ca^{2+}) to magnesium (Mg^{2+}) across all collected water samples was approximately 4.0 to 4.2 mg/L. Ideally, this ratio should be maintained at 5:1 to ensure chemically optimal conditions for fish growth. According to the results of the water analysis, the obtained values in study are close to the reference value of 5 mg/L and the standard ratio of 5:1, ensuring optimal conditions for harmonious growth and development (Bucur et al. 2016).

CONCLUSIONS

Integrated multitrophic aquaculture (IMTA), enhanced by the variable of intensity, effectively addresses all the requirements of a successful aquaculture process. However, increasing the intensity of aquaculture often leads to irreversible environmental degradation. In this context, IMTA serves as a feasible alternative that helps prevent undesirable consequences associated with the depletion of aquatic resources.

One effective solution is the integration of carp farming in floating cages with freshwater mussels in a land-based polyculture pond. This approach allows a more efficient production. It enables fish to be harvested for various purposes, whether commercially or for periodic growth assessments, at any desired time with minimal effort. The ease of handling greatly reduces stress on the fish, as it lessens the frequency of fishing and harvesting activities. Moreover, bivalves are natural indicators of aquatic environmental quality due to their sensitivity to various physico-chemical imbalances in the water used for aquaculture.

The food chains involved in this integrated system cooperate to survive, which minimizes competition for feed, another compelling reason to implement integrated aquaculture practices.

In the rearing environment, bivalves help maintain biochemical balance by filtering water that is approximately eleven times their total body weight each day.

The notable growth and survival rates of Ropșa carp, achieved through genetic programming, demonstrate the effectiveness of selective breeding in this species. This breeding has resulted in fish that show improved resistance to low dissolved oxygen (DO) levels and better adaptability to fluctuations in water temperature. Consequently, this leads to higher yields per unit area with minimal financial effort involved.

To conserve existing aquatic resources and ensure the economic efficiency of aquaculture processes, implementing modern farming technologies, such as those proposed by Integrated Multi-Trophic Aquaculture (IMTA), is essential to address current challenges.

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