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# The influence of water temperature and the pH on controlled spawning and the hatchability of *Cyprinus carpio* (Linnaeus, 1758), in the conditions of the Republic of Iraq

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**Abstract.** Water temperature and pH are considered the most important criteria for aquatic hosts of aquatic organisms, which have a significant impact on the maturation of the gonads, reproduction, growth and vital activity in general, in addition to some other environmental factors. Therefore, this study aims to discover the effect of high water temperature in addition to changing the pH on fertilized eggs, embryo growth, hatching rate and larval survival rate after hatching for 85 days, and to research the ideal water temperature and pH for egg hatching and growth of Cyprinus carp larvae. It was found that the higher the temperature that is above 25°C and the pH that is higher than 7.5 or lower than 7.3, the lower the hatching rate and the higher the larval mortality rate. The experiment showed that the fertilization rate reached 97.81 at an average water temperature of 25°C, a pH level of 7.5 and an average incubation period of 75 hours, while the specific growth rate of larvae on the first day was 8.25% and the survival rate was 96%. Therefore, it is recommended to maintain the water temperature at 22-25°C and pH level at 7.3-7.5 for incubating eggs and raising Cyprinus carp fry.

## 1 Introduction

In recent years, there has been a significant decrease in the quantity and quality of local fish catches due to the degradation of the water quality in the Tigris and Euphrates rivers. This has been noted by various sources, including [1].

To address this issue, the Iraqi government, through the Fisheries Research Center in Zafarani, Baghdad, and its farms in the provinces of Latifiya/Babel and Hawija/Kirkuk, encouraged Iraqi farmers to breed widely cultivated carp in earthen ponds. They provided healthy and suitable fingerlings (3-5 grams) at subsidized prices for the initial stages of development and fattening.

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Currently, ecology has a well-established concept of the ecological optimum. This is generally understood as the value of a particular factor that meets the needs of an organism and provides the most favourable conditions for its survival [2]. Fish have been the subject of research on how environmental factors affect their behavior. However, in previous studies [3].most attention has been focused on juvenile and adult fish. However, many fish species encounter difficulties during the process of fertilization and the development of eggs and embryos. Hence in recent decades, our knowledge of this process has greatly increased. It is crucial to apply this knowledge promptly to address issues related to the fertility and reproduction of these species, as noted by [4].

Water temperature and hydrogen index (pH) are among the most important parameters of the aquatic habitat of aquatic organisms, which have a significant impact on their vital activity, this also applies to fish, which belong to poikilothermic animals [5]. For each of the species, there is a certain range of ecological optimum for the level of exposure to factors, in particular temperature and pH, preferred for gonad maturation, spawning and vital activity in general. There are many studies of embryonic and larval development at different water temperatures [6]. Also, many studies which have shown that extreme pH values negatively affect the growth and reproduction of fish and lead to the mass death of young roo [7-8].

The common carp *Cyprinus carpio* (L. 1758) is a thermophilic, fast-growing, unpretentious, omnivorous fish, which is a domesticated form of wild carp (sazan). Carp has a wide range of habitats; fish can live at low oxygen levels (0.3 – 0.5 mg/l) and even more oxygensaturation [9].However, ideal concentrations of dissolved oxygen in water always range from 5-7 mg/L to ensure good development and growth, especially in Hatcheries and fry ponds. When growing carp in Iraq, mainly two indicators are controlled: water temperature and pH. In the hot summer months, farmers try not to exceed the water temperature above 29 ° C, although during these months it often tends to rise to the upper limits, especially at night, reaching 31- 32 ° C [10].The best development of carp larvae occurs at a water temperature of 22-24 °C. However, they can also adapt to higher temperatures, for example, 25-27 °C [11].The optimal pH level for carp larvae ranges from 6.5 to 9 [12].Temperature influences the formation of specific morphological features, embryonic development, hatching rate, and larval behavior [5]. It is also known that temperature affects the process of yolk resorption. As is known, the growth rate of fry increases with increasing water temperature, but when the temperature gets too high, it negatively affects the development of fry [13]. In salmon *(Salmo salar),* being cold-blooded, high temperatures result in high mortality rates [14]. Optimal growth occurs between 10°C and 14°C, while temperatures above 20°C can be lethal due to increased metabolic stress, decreased dissolved oxygen levels. Similarly, zebrafish (Danio rerio): when exposed to varying temperatures, they have a high growth capacity at temperatures of 24–28°C, but outside this range, growth may stop completely [15]. Unfortunately, despite the long existence and activity of the central state fish hatchery (Essaouira) for about 40 years, Iraq lacks sufficient knowledge about optimal reproduction and cultivation conditions, especially about the optimal temperature and pH requirements necessary for incubation of eggs and larvae. The fault of this can be considered by the lack of its own functional protocols for the reproduction and cultivation of fry and of common carp and local fish. So the purpose of this study was to find out how combinations of temperature and acidity (pH) affect the development of eggs, hatching of larvae and their survival in the common carp *Cyprinus carpio* in Iraq.

## 2 Materials and methods

Experiments were conducted at the central state fish hatchery in the Essaouira district of Kut Province, in the Republic of Iraq.

The experiment was conducted on common carp (*C. carpio*) females and males, collected from maternal ponds in spring, in May 2023. For the experiment, we selected six mature females with an average body weight of 3.2 ± 0.05 kg and six males with an average weight of 2.5 0.05± kg. The selection of fish was based on their external secondary sexual characteristics, good health and condition factors. Both the males and females were 2-3 years old.

The absolute and actual fecundity of the females, ranged from 720 to 880 thousand eggs and 560 to 640 thousand eggs, respectively. To synchronize spawning, we applied an ovulation-stimulating hormone to both the female and male producers. This hormone is an extract of pituitary gland – gonadotropin.

Females received two doses of an ovulation-stimulating hormone. The first dose was 10% of hormone, and after 12 hours, a second dose of 90% of hormone was administered. The males received one dose at the same time as the second dose for the females. After 10 hours, the stripping method was employed to extract both eggs and milt from the ovulated females and males, respectively. Fertilization of the eggs was done using the dry method. For fertilization we used 10 ml of sperm per 1 kg of eggs.

To calculate the total number of eggs produced by a female fish, we use a straightforward method. First, we take a small sample of eggs weighing approximately one gram. By counting the number of eggs in this sample, we can determine the average number of eggs per gram.

Next, we weigh all the eggs released by the female fish to find out their total weight. Then, we multiply the average number of eggs per gram (which we found in the sample) by the total weight of all the eggs. This gives us an approximate estimate of the total number of eggs produced by the female fish; we call it striped fecundity or working fecundity.

After washing the fertilized eggs with saline solution to remove any sticky substances, and after 1 hour 20 minutes after the start of fertilization (before the blastula stage (late gastrula) [16], 200 eggs were taken from the fertilization container. These eggs were examined under a microscope to determine the number of unfertilized eggs. This was done to calculate the fertilization ratio using the following equation:

Fertilization ratio % = {Number of fertilized eggs / total number of eggs} X 100

Incubation was carried out by placing 500 grams of fertilized eggs in 10-liter Weiss Units (cylindrical-conical type incubators). This process was triplicate under the conditions of the planned experiment until the end of hatching.

Four different pH values were obtained: 5.5, 6.5, 7.5, and 8.5. The pH levels of 5.5 and 6.5 were achieved using sulfuric acid, while the alkaline levels of 7.5 and 8.5 were achieved by adding sodium hydroxide.

All four pH levels of fertilized eggs were exposed to water at three different temperatures: 25, 27, and 29 degrees Celsius. These temperatures were regulated using electric heaters. Constant ventilation was provided in all feeding bottles until the end of the experiment. The pH level was monitored using a pH meter (model Hana 175 E/C).

Hatching took place from 48 to 75 hours during the incubation period, with four different pH levels and three different temperatures.

The number of eggs hatched for each operation was calculated using the following equation:

Hatching ratio (hatchability) (%) = {total number of hatched eggs / total number of fertilized eggs} X 100

Survival Rate = (live larvae – dead larvae) / live larvae x 100

Live hatched larvae were developed after their estimated number - 185 units per 1 gram of larvae, for 42 days in glass aquariums in three repetitions. It was fed with artificial food containing 35% protein to find out the percentage of growth and survival rate.

Food preparation and nutrition.

Table (1) shows the amount of experimental diet components. The food ingredients were mixed well with the addition of water.

**Table 1.** Ingredient and proximate composition of experimental diet.

| Component | % | | Composition | % |
|---|---|---|---|---|
| Fishmeal | 61.03 | | Crude protein | 35.6 |
| Groundnut oil cake | 22.7 | | Lipid | 9.81 |
| Rice bran | 12.82 | | Moisture | 10.1 |
| Vitamins (premix) | 1.5 | | Ash | 13.89 |
| Cod liver oil | 0.45 | | Carbohydrates | 30.6 |
| Carboxymethil cellulose | 1.5 | | | |

Calculations and statistical analysis.

## 3 Results and Discussion

The mean working fecundity in ovulated fish was 137470 ± 4315 eggs. The main results of targeted concrete objectives of environmental main conditions for common carp were widely varying (tab. 2).

In general, the percentage of fertilized eggs was relatively high across all temperature and pH treatments, and did not fall below 87%.

The hatching rate varied across all treatments depending on temperature and pH, with the best hatching rates at pH 7.5 and 6.5 at all temperature ranges, but the best hatching rate was at 25°C and pH 7.5 (97.81±1.15) and was statistically significant ($P < 0.05$) (Tables 2).

The hatching period was prolonged at pH 7.5. The hatching rate ranged from 58.49±1.23 to 72.10± 1.02 at pH 5.5; 75.13±1.21 to 93.07±1.13 at pH 6.5; 82.91±1.09 to 97.81±1.15 at pH 7.5; 66.66±1.22 to 91.11±1.11 at pH 8.5 under three water temperatures.

**Table 2.** Effect of water temperature with different pH on the hatchability of *Cyprinus carpio* eggs.

| **temperature 25 °C** | | | | |
|---|---|---|---|---|
| **pH** | **5.5** | **6.5** | **7.5** | **8.5** |
| Fertilization% | 88±5.0 | 89±6.0 | 92±4.0 | 90±7.0 |
| Hatchability% | 72.10± 1.02 | 93.07±1.13 | 97.81±1.15 | 91.11±1.11 |
| Incubation period (h) | 70±1.11 | 73±1.23 | 75±1.30 | 72±1.21 |
| Av. fertilization | | 90.5 | | |
| Av.hatchability | | 95.94 | | |
| temperature 27°C | | | | |
| pH | 5.5 | 5.5 | 5.5 | 5.5 |
| Fertilization% | 88±5.0 | 88±5.0 | 88±5.0 | 88±5.0 |
| Hatchability% | 64.81±1.8 | 64.81±1.8 | 64.81±1.8 | 64.81±1.8 |
| Incubation period (h) | 51±1.08 | 51±1.08 | 51±1.08 | 51±1.08 |
| Av. fertilization | | 89.5 | | |
| Av.hatchability | | 90.02 | | |
| temperature29 °C | | | | |
| pH | 5.5 | 5.5 | 5.5 | 5.5 |

| | | | | |
|---|---|---|---|---|
| Fertilization% | 89±3.0 | 89±3.0 | 89±3.0 | 89±3.0 |
| Hatchability% | 58.49±1.23 | 58.49±1.23 | 58.49±1.23 | 58.49±1.23 |
| Incubation period (h) | 48±1.32 | 48±1.32 | 48±1.32 | 48±1.32 |
| Av. fertilization | | 89.5 | | |
| Av.hatchability | | 79.02 | | |

Among water temperatures of 25, 27 and 29°C, weight gain, specific growth rate and FCE were maximum at water temperature of 25°C and were observed to be statistically significant ($P < 0.05$), initial average weight 0.003 g (Tables 3-5).

**Table 3.** Effect of water temperature (25°C) and changes in water pH on growth and survival of fry *C. carpio*.

| **Parameters** | **pH** | | | |
|---|---|---|---|---|
| | **5.5** | **6.5** | **7.5** | **8.5** |
| Final average weight (g) | 1.210 ± 0.21 | 3.022 ± 0.46 | 3.345 ± 0.89 | 2.201 ± 0.37 |
| Gain in weight (g) | 1.207 ± 0.20 | 3.019 ± 0.42 | 3.342 ± 0.84 | 2.198 ± 0.35 |
| Specific growth rate (SGR%/day) | 7.06 ± 16 | 8.14 ± 0.22 | 8.25 ± 0.31 | 7.76 ± 0.20 |
| Initial average length (mm) | 5.21 ± 0.10 | 5.23 ± 0.15 | 5.23 ± 0.19 | 5.22 ± 0.11 |
| Final average length (mm) | 45.71 ± 1.56 | 62.44 ± 1.75 | 67.89 ± 1.96 | 55.32 ± 1.43 |
| Gain in length (mm) | 40.50 ± 1.47 | 57.21 ± 1.66 | 62.66 ± 1.81 | 50.10 ± 1.53 |
| Survival (%) | 62 ± 2.56 | 91 ± 3.15 | 96 ± 3.37 | 82 ± 2.90 |

**Table 4.** Effect of water temperature (27 °C) and changes in water pH on the growth and survival of fry *C. carpio*.

| **Parameters** | **pH** | | | |
|---|---|---|---|---|
| | **5.5** | **6.5** | **7.5** | **8.5** |
| Final average weight (g) | 1.126 ± 0.15 | 2.430 ± 0.22 | 2.802 ± 0.21 | 1.960 ± 0.30 |
| Gain in weight (g) | 1.123 ± 0.11 | 2.427 ± 0.32 | 2.799 ± 0.39 | 1.957 ± 0.21 |
| Specific growth rate (SGR%/day) | 6.97 ± 0.13 | 7.88 ± 0.18 | 8.05 ± 0.26 | 7.63 ± 0.17 |
| Initial average length (mm) | 5.35 ± 0.11 | 5.34 ± 0.24 | 5.34 ± 0.27 | 5.35 ± 0.19 |
| Final average length (mm) | 42.98 ± 1.33 | 54.56 ± 1.77 | 59.87 ± 1.54 | 50.88 ± 1.24 |
| Gain in length (mm) | 37.63 ± 1.54 | 49.22 ± 1.61 | 54.53 ± 1.69 | 45.53 ± 1.57 |
| Survival (%) | 55 ± 2.33 | 85 ± 1.77 | 93 ± 1.52 | 76 ± 1.25 |

**Table 5.** The effect of water temperature (29 °C) and water pH changes on the growth and survival of fry *C. carpio*.

| **Parameters** | **pH** | | | |
|---|---|---|---|---|
| | **5.5** | **6.5** | **7.5** | **8.5** |
| Final average weight (g) | 1.120 ± 0.17 | 2.130 ±0.24 | 2.542 ± 0.45 | 1.880 ± 0.20 |
| Gain in weight (g) | 1.117 ± 0.14 | 2.127 ± 0.22 | 2.539 ± 0.40 | 1.877 ±0.17 |
| Specific growthrate (SGR%/day) | 6.97 ± 0.15 | 7.72 ± 0.20 | 7.93 ±0.25 | 7.58 ± 0.18 |
| Initial average length (mm) | 5.11 ± 0.12 | 5.11 ± 0.08 | 5.11 ± 0.11 | 5.11 ± 0.10 |
| Final average length (mm) | 39.09 ± 1.29 | 50.19± 1.47 | 56.27 ± 1.71 | 43.75± 1.41 |
| Gain in length (mm) | 33.98 ± 1.21 | 45.08 ± 1.30 | 51.16 ± 1.45 | 38.65 ± 1.27 |
| Survival (%) | 52± 2.13 | 80 ± 2.10 | 89 ± 2.19 | 71 ± 1.95 |

## 4 Conclusion

Our results showed optimal temperatures and pH providing good indicators of fertilization, embryonic development and survival of carp larvae. However, such results are not exclusive; there are many similar results in the literature for different fish species [18-20]. [21], found the highest hatching rate of carp eggs at pH 7.5 and water temperature 25 °C. The pH that leads to carp mortality may be either below 5 or above 9. At these extremes, the water becomes either highly alkaline or highly basic, leading to acidic stress, which may cause decreased metabolic rates, impaired respiratory function and disturbance of ionic balance, which may cause fish mortality [22]. The ability of juvenile fish to survive in different pH environments depends on the type of incubation medium used. As demonstrated in a study by [23], the pH level of the incubation medium directly affects the development of fertilized eggs. If the pH level is too high or too low, it can slow down or stop the hatching of eggs. It can also lead to the birth of more deformities in offspring. For instance, in our experiment, we found that fish larvae hatched from eggs in pH groups 6 were more deformed compared to those in other groups.

According to another study by [24], a high pH value can cause stagnation and death in fish. On the other hand, a too low pH level can impair the transport of oxygen in the blood of fish, as shown in other studies by [24-25].

Increasing the temperature during the egg incubation period accelerates the growth rate and shortens the development period, while decreasing the temperature slows the growth and lengthens the development period [26]. The connection between the time it takes for an egg to develop and the temperature at which it incubates appears to be a common one. This is confirmed by numerous laboratory and field studies of various freshwater species from different thermal groups and families of fish. For example, common carp and white Amur [18].walleye [27], and many other species.

Water temperature is a key factor that determines the early development of fish However, fish are able to adapt to certain temperature ranges. An increase within the acceptable range can accelerate the development of fish embryos [28], shorten incubation time, and help newly hatched larvae feed for the first time. However, if the temperature exceeds this range, it can lead to malformations, developmental arrest, or even embryo death [29]. While our research and the aforementioned studies confirm the importance of the optimal indicator concept, this research is trying to find a broader understanding of it. The goal is to improve or expand the indicators for fish reproduction and early development, such as incubation and hatching conditions, and to increase the range of environmental options for enhancing production. In other words, the understanding of the optimum has evolved based on a wealth of experimental data, and is supported by modern research. The concept of ecological optimum is one of the central and fundamental ideas in ecology. Therefore, in many studies, the goal was initially to determine the optimal value of a factor for the survival of a particular type of organism. The results obtained from these studies often serve as a basis for selecting the best values of that factor for growing different groups of organisms [30]. There is a reasonable opinion that the ecological optimum is not a fixed set of conditions, but a range of intensity changes that a species can tolerate. These changes occur at different speeds, frequencies, and amplitudes, and are within the species' adaptive abilities [31].

In many methodological guides and experimental studies, it is often advised to maintain certain environmental parameters at specific, optimal levels. However, the significance of dynamic changes in these parameters for the early development of fish is often overlooked. Therefore, an experimental investigation into the impact of fluctuations in the most crucial abiotic environmental factors on the embryonic and larval development of fish is crucial. Such an investigation can provide insights into the role of astaticity at this stage of

development and help evaluate the potential for using it to produce more viable juveniles in both laboratory and industrial settings [30].

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