



Effect of Post-Fertilization Timing of a 40°C Heat Shock on the Hatching Rate of Common Carp (*Cyprinus carpio* Linnaeus, 1758) Eggs

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Abstract: This study aimed to evaluate the effect of the timing of a 40°C heat shock applied at 5, 7, 9, and 11 min post-fertilization on the hatching rate of common carp (*Cyprinus carpio*) eggs. The experiment was conducted at the Fish Seed Center of Binjai City from April to June 2026 using a completely randomized design with four treatments and three replicates. The experimental unit was the incubation container, with three containers per treatment, 20 eggs per container, and 240 eggs in total, all obtained from a single broodstock pair. Data were analyzed using analysis of variance, polynomial contrasts, and the Least Significant Difference (LSD) test, and the findings were further verified using binomial logistic regression. Heat-shock timing had a highly significant effect on hatching rate ($F(3,8) = 35.41$; $p < 0.001$; $\omega^2 = 0.90$). Mean hatching rates increased monotonically with later heat-shock application, reaching $35.00 \pm 5.00\%$, $50.00 \pm 10.00\%$, $76.67 \pm 5.77\%$, and $86.67 \pm 5.77\%$ at 5, 7, 9, and 11 min post-fertilization, respectively. The linear component accounted for 97.3% of the treatment variation ($p < 0.001$). However, the hatching rate at 11 min did not differ significantly from that at 9 min (difference = 10.00%; $LSD_{0.05} = 13.03$). Therefore, the 11-min treatment produced the highest mean hatching rate among the treatments tested, but it cannot be considered the optimal timing. Because the experiment did not include an unshocked control group, the observed differences reflect the effect of heat-shock timing rather than the effect of heat shock relative to untreated eggs. These findings suggest that earlier heat shock may be more detrimental to embryonic development and should be further evaluated using an untreated control and a broader range of post-fertilization times.

Keywords: *Cyprinus carpio*; fish hatchery; hatching rate; heat shock; post-fertilization timing

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INTRODUCTION

Common carp (*Cyprinus carpio* Linnaeus, 1758) is one of the oldest freshwater aquaculture species cultivated in Indonesia and remains economically important, while national aquaculture production continues to increase (Kementerian Kelautan dan Perikanan [KKP], 2024). In hatchery production, egg hatching rate is a critical determinant of larval availability for subsequent nursery stages, and poor egg quality can reduce hatching success and increase early larval mortality (Irianto, 2003). Small-scale hatcheries, including government-operated Fish Seed Centers (Unit Pelaksana Teknis Balai Benih Ikan; UPT BBI), still frequently encounter low and inconsistent hatching performance among production cycles. Among the environmental factors affecting embryonic development, temperature is particularly important because it regulates developmental rate, metabolic activity, enzymatic processes, and embryo survival during incubation (Djarajah, 2001; Massey & Hutchings, 2021). Thermal conditions during egg incubation can also influence developmental performance and later phenotypic outcomes in ectothermic vertebrates, including fishes (Massey & Hutchings, 2021).

The influence of incubation temperature on fish embryogenesis and hatching success has been widely documented. In common carp, El-Gamal (2009) reported that hatching occurred within a relatively narrow thermal range, with the highest hatching rate at 27°C and marked declines at higher temperatures. Similarly, Siregar et al. (2024) recorded the highest hatching rate at 29°C and a lower rate at 32°C. Comparable intermediate-temperature responses have also been reported in other cultured fish species, including Bastar strain giant gourami (*Osphronemus gouramy*) (Pratama et al., 2018) and Asian seabass (*Lates calcarifer*) (Hasibuan et al., 2018). At the cellular and molecular levels, thermal stress can alter developmental regulation and induce stress-response pathways in common carp embryos. Recent transcriptomic evidence indicates that thermal stress affects gene expression and alternative splicing in a stage-dependent manner during common carp embryogenesis, suggesting that thermal sensitivity changes as development progresses (Hu et al., 2024). At the cellular level, heat shock proteins also contribute to adaptive responses to thermal stress by protecting cellular proteins and maintaining protein homeostasis (Kayhan & Duman, 2010).

However, responses to incubation temperature are not always consistent among studies. Muslim et al. (2021), for example, reported no significant effect of temperatures ranging from 22 to 32°C on the hatching rate of common carp eggs. Such variation suggests that thermal effects may depend on the temperature range tested, embryonic developmental stage, parental condition, and experimental protocol. More broadly, responses of ectothermic eggs to thermal conditions are known to vary according to both the pattern of thermal exposure and the developmental context in which it occurs (Massey & Hutchings, 2021).

In contrast to continuous incubation temperature, the effect of the timing of post-fertilization heat-shock application remains less well characterized. Heat shock is a short-duration thermal treatment in which fertilized eggs are exposed to water substantially warmer than the normal incubation medium at a specific time after fertilization. Because early embryogenesis involves rapidly changing meiotic and mitotic events, the biological effect of heat shock can depend strongly on the developmental stage at which the treatment is applied. Previous work in ornamental common carp has demonstrated that embryo survival and chromosome-set manipulation outcomes vary markedly with the timing of heat shock during the second meiotic division and first cleavage (Cherfas et al., 1993; Cherfas et al., 1994). These findings indicate that heat-shock timing is biologically important and cannot be treated independently of embryonic developmental stage.

Despite this developmental sensitivity, studies specifically addressing heat-shock timing under common carp hatchery conditions remain limited. Djalilu et al. (2018) applied a 40°C heat shock for 2 min at 2.5, 5.0, and 7.5 min post-fertilization and reported hatching rates of 81.67%, 69.33%, and 61.67%, respectively, all lower than the 91% recorded in the untreated control group. These findings indicate that, within the tested interval, heat shock did not improve hatching success relative to the control and that later application within the early post-fertilization window was associated with progressively lower hatching rates. However, because the experiment ended at 7.5 min post-fertilization, it remains unclear whether this declining pattern persists, stabilizes, or reverses when heat shock is applied at later developmental times.

The novelty of the present study lies in extending the post-fertilization heat-shock window beyond that examined by Djalilu et al. (2018) and evaluating the response during a later developmental interval. The treatment range was extended to 5–11 min post-fertilization, while the 5-min treatment was retained as an overlapping time point

to facilitate direct comparison with the earlier study. This design enables assessment of whether the previously reported decline in hatching rate continues at later application times or whether the response changes as embryonic development progresses. Such information is important for clarifying the temporal sensitivity of common carp eggs to acute thermal exposure and for evaluating the biological relevance of heat-shock timing in hatchery applications.

Accordingly, this study aimed to evaluate the effect of the timing of a 40°C heat shock applied after fertilization on the hatching rate of common carp eggs and to identify which of the tested application times produced the highest mean hatching rate.

METHOD

Study Site and Experimental Design

This experimental study was conducted from April to June 2026 at the Fish Seed Center (Unit Pelaksana Teknis Balai Benih Ikan; UPT BBI), Food Security Service of Binjai City, North Sumatra, Indonesia. A one-factor completely randomized design was used to evaluate the effect of post-fertilization timing of a 40°C heat-shock treatment. Four treatment levels were tested: P1 (5 min post-fertilization), P2 (7 min), P3 (9 min), and P4 (11 min). Each treatment was replicated three times, resulting in 12 experimental units.

Each incubation container was treated as an independent experimental unit, whereas the 20 eggs within each container were treated as subsamples. Accordingly, the sample size for inferential analysis was $n = 3$ containers per treatment, and the response variable for each experimental unit was the hatching rate calculated from the 20 eggs within that container. This approach was used to avoid pseudoreplication by preventing individual eggs from being treated as statistically independent experimental units. The experiment did not include an untreated control group without heat shock; the implications of this design limitation are addressed in the limitations section.

Broodstock, Egg Collection, and Experimental Units

Fertilized eggs were obtained through artificial spawning from one sexually mature pair of common carp maintained at the UPT BBI of Binjai City. The broodstock consisted of one female and one male, each weighing approximately 1.5 kg. Gametes were collected by stripping and fertilized artificially. Immediately after fertilization, the eggs were gently mixed in a single holding container to homogenize the egg pool before allocation to the experimental units.

A single broodstock pair was used to minimize variation in genetic background and initial egg quality among treatments. However, the use of one broodstock pair restricts the generalizability of the findings beyond the parental combination used in this experiment. From the homogenized egg pool, 20 eggs were randomly selected for each experimental unit, giving a total of 240 eggs. Before sampling, the eggs were gently mixed to increase the likelihood that each egg had an equal chance of selection. Each subsample was then randomly assigned to a treatment–replicate combination.

Twenty eggs were stocked in each container because common carp eggs are adhesive, and a relatively low density facilitated individual observation and counting by minimizing overlap among eggs. The experiment used 12 cylindrical containers, each 20 cm in diameter with a capacity of 1,500 mL. Each container was filled with 0.5 L of water and supplied with aeration. Container positions on the experimental rack were randomized to reduce potential environmental variation unrelated to the treatments.

Heat-Shock Treatment

Before use, all containers were washed with detergent, thoroughly rinsed with freshwater, dried, filled with 0.5 L of water, and aerated. The time of fertilization was designated as $t = 0$ min. According to treatment allocation, egg subsamples were removed from the holding container at 5, 7, 9, or 11 min post-fertilization and immersed in water at 40°C. The water temperature was checked with a thermometer immediately before each heat-shock treatment. Following heat-shock exposure, the eggs were immediately transferred to their designated incubation containers.

The complete experimental procedure is illustrated in Figure 1. The duration of exposure to 40°C was not quantitatively recorded during the experiment. Therefore, the experimental factor evaluated in this study was the timing of heat-shock application after fertilization, not the duration of thermal exposure. Because exposure duration was not recorded, the exact thermal dose cannot be reconstructed, which limits precise replication of the procedure. This issue is further addressed in the limitations section.

During incubation, water temperature and pH were measured daily using a thermometer and a pH meter, respectively. Both instruments were calibrated according to the manufacturers' instructions before use.

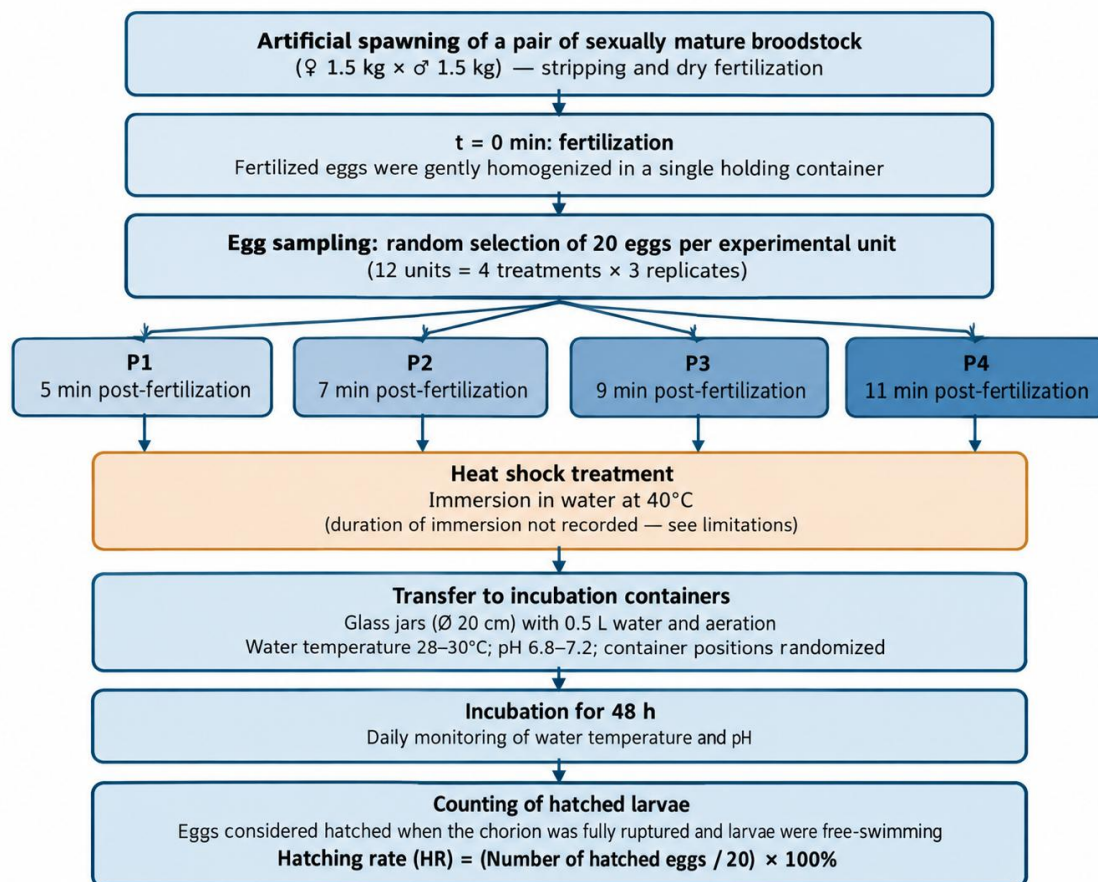


Figure 1. Flow diagram of the experimental procedure, from artificial spawning to hatching-rate assessment

Response Variable and Observation Criteria

The primary response variable in this study was the hatching rate (HR), defined as the percentage of eggs that successfully hatched in each experimental unit. Hatching rate was calculated by dividing the number of successfully hatched eggs by

the total number of eggs stocked in each container (20 eggs) and multiplying the result by 100, as follows:

$$HR = \left(\frac{\text{number of hatched eggs}}{\text{number of eggs stocked}} \right) \times 100$$

An egg was classified as successfully hatched when the chorion had ruptured completely, the larva had emerged fully, and the larva was capable of free movement. Eggs with a ruptured or cracked chorion from which the larva had not fully emerged were not classified as hatched. Larvae were counted 48 h after stocking using a fine-mesh scoop net and a light-colored plastic basin to facilitate observation, and the counts were documented photographically.

Fertilization rate was not assessed separately before the experimental treatments were applied. Consequently, the denominator used to calculate hatching rate may have included unfertilized eggs. The reported hatching rate therefore represents the combined outcome of fertilization success and subsequent hatching success rather than the proportion of fertilized eggs that successfully hatched.

Water temperature and pH were treated as supporting environmental variables. They were reported as observed ranges and were not subjected to inferential statistical analysis because their purpose was to characterize the incubation environment rather than to test treatment effects.

Statistical Analysis

Hatching-rate data were analyzed using one-way ANOVA after checking residual normality with the Shapiro–Wilk test and homogeneity of variances with Bartlett's and Levene's tests (Steel & Torrie, 2003). Treatment effects were summarized using eta squared (η^2) and omega squared (ω^2). To confirm the robustness of the results, ANOVA was repeated on arcsine square-root-transformed proportions, and binomial logistic regression was performed using the number of hatched eggs out of 20 eggs per container. Overdispersion was assessed from the residual deviance-to-degrees-of-freedom ratio. Orthogonal polynomial contrasts were used to evaluate linear, quadratic, and cubic trends across the equally spaced treatment times. Pairwise differences were assessed using the Least Significant Difference (LSD) test at the 0.05 and 0.01 significance levels (Hanafiah, 1991), with Tukey's test and Fisher's exact test used as supplementary checks.

RESULTS AND DISCUSSION

Hatching Rate across Heat-Shock Application Times

The hatching rate of common carp eggs ranged from 30% to 90% at the container level. Mean hatching rates were $35.00 \pm 5.00\%$ for P1 (5 min post-fertilization), $50.00 \pm 10.00\%$ for P2 (7 min), $76.67 \pm 5.77\%$ for P3 (9 min), and $86.67 \pm 5.77\%$ for P4 (11 min) (Table 1 and Figure 2). A clear monotonic increase was observed, with higher hatching rates associated with later heat-shock application within the 5–11 min post-fertilization window. However, the magnitude of the increase was not uniform. The largest increase occurred between 7 and 9 min, amounting to 26.67 percentage points, whereas the increase between 9 and 11 min was only 10.00 percentage points, suggesting that the response may begin to level off toward the upper end of the tested interval.

Orthogonal polynomial contrasts supported this pattern. The linear component accounted for 97.3% of the treatment variation and was highly significant ($F(1,8) = 103.31$; $p < 0.001$), whereas the quadratic ($F(1,8) = 0.39$; $p = 0.549$) and cubic

components ($F(1,8) = 2.51$; $p = 0.152$) were not significant (Table 2). Thus, within the tested interval, the relationship between heat-shock timing and hatching rate was best described by an increasing linear trend, with no statistical evidence of a peak within the observed range. The absence of a significant quadratic component further indicates that the 11-min treatment should not be interpreted as an optimum. If a true maximum exists, it may lie beyond the range examined in this study.

Variation among replicates was relatively moderate, with a coefficient of variation of 11.15%. The greatest standard deviation was observed in P2 (10.00%), whereas the remaining treatments showed standard deviations between 5.00% and 5.77%. Because each container held only 20 eggs, the hatching-rate measurement had a resolution of 5 percentage points; therefore, the hatching or failure of a single egg changed the estimated hatching rate by 5%. This limited resolution should be considered when interpreting relatively small differences among treatments.

Table 1. Hatching rate of common carp eggs at different post-fertilization heat-shock application times (%)

Replicate	P1 (5 min)	P2 (7 min)	P3 (9 min)	P4 (11 min)
1	35	40	80	80
2	30	60	70	90
3	40	50	80	90
Total	105	150	230	260
Mean $\pm$ SD	35.00 $\pm$ 5.00 ^a	50.00 $\pm$ 10.00 ^b	76.67 $\pm$ 5.77 ^c	86.67 $\pm$ 5.77 ^c

Note: Mean values followed by different superscript letters differ significantly according to the LSD test at the 0.05 significance level. SD = standard deviation; $n = 3$ containers per treatment.

Table 2. Summary of analysis of variance and orthogonal polynomial contrasts for common carp egg hatching rate

Source of variation	df	SS	MS	F	p-value	Significance
Treatment (timing)	3	5,089.58	1,696.53	35.41	< 0.001	**
– Linear	1	4,950.42	4,950.42	103.31	< 0.001	**
– Quadratic	1	18.75	18.75	0.39	0.549	ns
– Cubic	1	120.42	120.42	2.51	0.152	ns
Error	8	383.33	47.92	–	–	–
Total	11	5,472.92	–	–	–	–

Note: ** = highly significant ($p < 0.01$); ns = not significant. $F_{critical} 0.05(3,8) = 4.07$ and $F_{critical} 0.01(3,8) = 7.59$. Coefficient of variation = 11.15%; $\eta^2 = 0.930$; $\omega^2 = 0.896$.

Statistical Significance and Verification of Post Hoc Results

The assumptions of ANOVA were satisfied. Residuals were normally distributed (Shapiro–Wilk $W = 0.933$; $p = 0.408$), and error variances were homogeneous among treatments (Bartlett $\chi^2 = 1.061$; $p = 0.787$; Levene $W = 0.478$; $p = 0.706$). ANOVA showed that heat-shock timing had a highly significant effect on the hatching rate of common carp eggs ($F(3,8) = 35.41$; $p < 0.001$), with a very large treatment effect ($\eta^2 = 0.930$; $\omega^2 = 0.896$). These values indicate that approximately 90% of the observed variation in hatching rate within this experiment was associated with differences in heat-shock timing.

The same conclusion was supported by the supplementary analyses. ANOVA of the arcsine square-root-transformed data produced a comparable result ($F(3,8) =$

33.57; $p < 0.001$) and an identical pattern of pairwise differences. Binomial logistic regression of the egg-count data yielded a slope coefficient of 0.436 ± 0.072 per minute ($z = 6.09$; $p < 0.001$), corresponding to an odds ratio of 1.55 (95% CI: 1.34–1.78) for each 1-min delay in heat-shock application. The deviance-to-degrees-of-freedom ratio was 0.47, indicating no evidence of overdispersion. Overall, these analyses consistently showed that later heat-shock application within the tested interval was associated with a higher probability of hatching.

The complete LSD results are presented in Table 3. The LSD values were 13.03 at $\alpha = 0.05$ and 18.96 at $\alpha = 0.01$. The mean difference between P4 (11 min) and P3 (9 min) was only 10.00 percentage points, which was smaller than the LSD at the 0.05 level; therefore, the two treatments did not differ significantly ($t = 1.77$; $p = 0.115$). This conclusion was supported by Tukey's test ($p = 0.353$) and Fisher's exact test applied to pooled count data ($p = 0.238$). The 15.00-percentage-point difference between P1 and P2 was significant at the 0.05 level ($p = 0.029$) but not at the 0.01 level, whereas the differences between P1 or P2 and P3 or P4 were all highly significant ($p \leq 0.002$). Accordingly, the four treatments formed three statistical groups: P1 (a), P2 (b), and P3–P4 (c).

These findings require a cautious interpretation of the second study objective. Although the 11-min treatment produced the highest mean hatching rate among the tested treatments (86.67%), its apparent advantage over the 9-min treatment could not be distinguished from random variation at the available sample size. Therefore, the 11-min treatment should not be described as the best or optimal timing. The supported conclusion is that heat-shock application at 9 and 11 min post-fertilization produced statistically comparable hatching rates, both of which were higher than those obtained at 5 and 7 min.

Table 3. Least Significant Difference (LSD) test for pairwise comparisons among heat-shock application times

Treatment comparison	Mean difference (%)	t-value	p-value	Significance
P1 (5 min) vs P2 (7 min)	15.00	2.65	0.029	*
P1 (5 min) vs P3 (9 min)	41.67	7.37	< 0.001	**
P1 (5 min) vs P4 (11 min)	51.67	9.14	< 0.001	**
P2 (7 min) vs P3 (9 min)	26.67	4.72	0.002	**
P2 (7 min) vs P4 (11 min)	36.67	6.49	< 0.001	**
P3 (9 min) vs P4 (11 min)	10.00	1.77	0.115	ns

Note: ** = highly significant ($p < 0.01$); * = significant ($p < 0.05$); ns = not significant. LSD0.05 = 13.03 and LSD0.01 = 18.96; error degrees of freedom = 8.

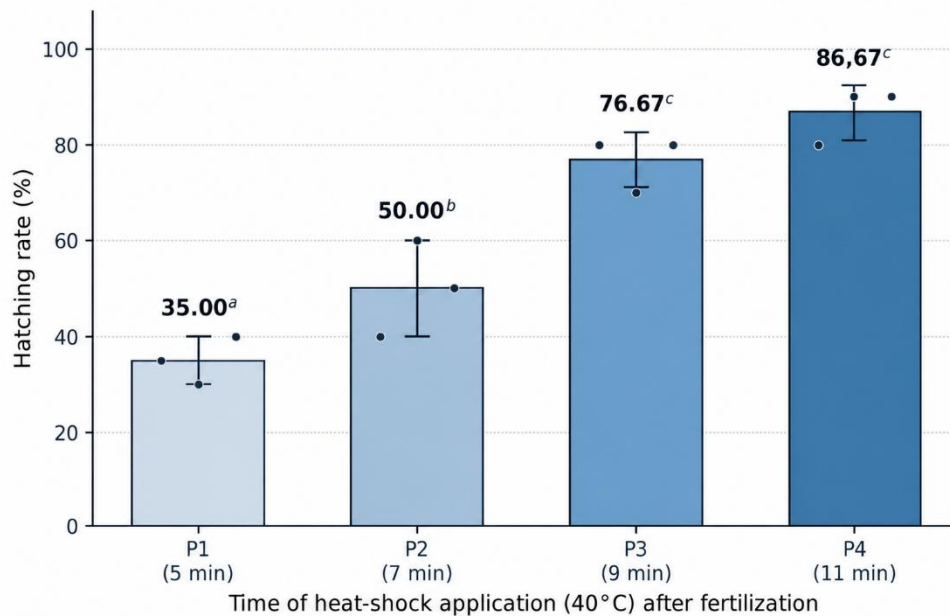


Figure 2. Mean hatching rates of common carp eggs following 40°C heat shock at different post-fertilization times. Error bars indicate SD (n = 3), and different superscript letters indicate significant differences (LSD, $p < 0.05$)

Comparison with Previous Studies

The observed increase in hatching rate with later heat-shock application contrasts with the pattern reported by Djalilu et al. (2018), the only previous study that directly varied the timing of a 40°C heat shock in common carp eggs. Djalilu et al. (2018) reported hatching rates of 81.67%, 69.33%, and 61.67% when heat shock was applied at 2.5, 5.0, and 7.5 min post-fertilization, respectively, indicating a decline with increasing application time. In contrast, the present study recorded 35.00% at 5 min and 86.67% at 11 min, indicating an increasing trend. This difference in response direction is a central finding and cannot be explained merely by differences in study location.

The overlapping 5-min treatment further highlights this discrepancy. At the same nominal post-fertilization time, Djalilu et al. (2018) reported a hatching rate of 69.33%, whereas the present study recorded only 35.00%. Such a large difference under nominally similar timing indicates that heat-shock timing was not the only factor that differed between the two experiments. At least three potential sources of variation can be identified. First, and most importantly, exposure duration differed between studies. Djalilu et al. (2018) applied a standardized 2-min immersion, whereas the immersion duration in the present study was neither controlled nor recorded. Because thermal dose depends on both temperature and exposure duration, differences in exposure time could shift the entire response curve. Second, egg quality and parental source differed because both experiments used different single broodstock pairs, and the initial fertilization rate was not determined in the present study. Third, stocking density differed substantially, with 100 eggs per container in Djalilu et al. (2018) compared with 20 eggs per container in the present study. Consequently, the precision of hatching-rate estimation per container was lower in the present experiment. A summary of these methodological and outcome differences is provided in Table 4.

Table 4. Comparison of experimental protocols and outcomes between the present study and previous studies on common carp eggs

Study	Factor Varied	40°C Exposure Duration	Unshocked Control	Eggs per Container	Highest HR (%)	Response Pattern
Present study	Application timing: 5, 7, 9, 11 min	Not recorded	No	20	86.67 (11 min)	Increasing
Djalilu et al. (2018)	Application timing: 2.5, 5.0, 7.5 min	2 min	Yes (91%)	100	81.67 (2.5 min)	Decreasing
Siregar et al. (2024)	Incubation temperature: 23, 26, 29, 32°C	nr	No heat shock	nr	87.67 (29°C)	Optimum at 29°C
Muslim et al. (2021)	Incubation temperature: 22–32°C	nr	No heat shock	nr	99 (32°C)	Not significant
El-Gamal (2009)	Incubation temperature: 20–38°C	nr	No heat shock	nr	74.33 (27°C)	Optimum at 27°C

Note: nr = not reported or not applicable; HR = hatching rate.

An additional point from Djalilu et al. (2018) requires particular caution when interpreting the present results. Their untreated control group achieved a hatching rate of 91%, which was higher than all heat-shocked treatments in that study. Based on that comparison, heat shock did not improve hatching rate in common carp but instead reduced it, although the magnitude of the reduction varied with treatment timing. The highest hatching rate obtained in the present study, 86.67%, was also lower than the 91% control reported by Djalilu et al. (2018) and was comparable to the 87.67% hatching rate reported for common carp eggs incubated at 29°C without heat-shock treatment by Siregar et al. (2024). Cross-study comparisons cannot substitute for an internal control because broodstock, egg quality, and environmental conditions differed among experiments. Nevertheless, these comparisons reinforce the conclusion that the present data do not demonstrate that heat shock improves hatching success. Rather, they show only that, among the tested heat-shock times, later application was associated with higher hatching rates.

The mechanisms underlying this pattern can only be proposed as literature-based explanations rather than conclusions directly demonstrated by the present experiment, because embryonic developmental stages were not examined microscopically. One possible explanation concerns the vulnerability of early developmental stages. During the first minutes after gamete activation in common carp, second polar body extrusion and preparation for the first cleavage occur, and this developmental window is commonly targeted by temperature shocks in ploidy manipulation to disrupt these processes (Linhart et al., 1991). Thus, heat shock applied at 5 min post-fertilization may have been more likely to interfere with these early cellular events and reduce embryonic survival than heat shock applied at 11 min, when the earliest developmental events may already have progressed further.

A second possible explanation involves cellular stress responses. Thermal exposure can induce heat shock proteins that protect and stabilize cellular proteins during thermal stress (Kayhan & Duman, 2010). It is therefore plausible that the capacity to tolerate acute thermal exposure changes as embryonic development progresses. By contrast, an explanation based on accelerated chorionase activity at higher temperatures (Djarajah, 2001), which is often used to explain earlier hatching under elevated incubation temperatures, appears less applicable here because heat

shock was applied only during the first few minutes after fertilization, well before the formation of hatching glands and secretion of chorionase. These proposed mechanisms remain speculative and require direct testing through observations of cleavage stages, ploidy assessment, and measurement of heat shock protein expression.

During incubation, water temperature ranged from 28 to 30°C and pH from 6.8 to 7.2. This temperature range falls within conditions reported to support common carp embryonic development, with favorable hatching generally occurring at approximately 27–29°C (El-Gamal, 2009; Siregar et al., 2024). The observed pH values were within the near-neutral range commonly reported as suitable for freshwater fish egg incubation (Yunani et al., 2024). Because temperature and pH remained within similar ranges across all experimental containers and were not manipulated as treatments, they are reported only as indicators of appropriate incubation conditions and should not be interpreted as explanations for differences among heat-shock treatments. Dissolved oxygen was not measured, although all containers received uniform aeration; therefore, adequate oxygen availability can only be assumed and cannot be confirmed from direct measurements.

Study Limitations and Implications

Several limitations constrain the strength and generalizability of the conclusions. First, the absence of an untreated control group prevents assessment of the effect of heat shock itself; therefore, the study supports only comparisons among heat-shock application times. Second, the duration of immersion in 40°C water was not recorded, preventing quantification of the thermal dose and limiting precise replication by other researchers. Third, all eggs were obtained from a single broodstock pair, meaning that genetic and parental variation in egg quality was not represented and the findings cannot be generalized confidently to a broader broodstock population.

Fourth, the small number of replicates, with only three containers per treatment and eight error degrees of freedom, limited statistical power. The non-significant difference between the 9- and 11-min treatments may therefore partly reflect limited power; however, whether the observed 10-percentage-point difference would become significant with greater replication cannot be determined from the present data. Fifth, the small number of eggs per container restricted measurement resolution to 5-percentage-point increments. Sixth, fertilization rate was not assessed independently, meaning that the reported hatching rate also incorporates potential fertilization failure. Seventh, the study ended at hatching and did not assess subsequent larval survival, morphological abnormalities, or ploidy status. Therefore, there is no basis for concluding that a heat-shock timing associated with a higher hatching rate also produces higher-quality offspring. The relationship between hatching success and subsequent seed quality requires additional data that were not collected in this study.

Given these limitations, the practical implications should be interpreted conservatively. The present findings should not be regarded as evidence supporting the use of heat shock to improve hatching success in small-scale common carp hatcheries because the benefit of heat shock relative to no treatment was not tested, and the available evidence from Djalilu et al. (2018) suggests the opposite. A more defensible practical implication is precautionary: when heat shock is required as part of another manipulation procedure, application during very early post-fertilization stages may be associated with substantially lower hatching rates than application at later times.

From a scientific perspective, the study provides additional data for the previously underexamined 5–11 min post-fertilization window. When considered alongside the 0–

7.5 min observations reported by Djalilu et al. (2018), these findings suggest that the relationship between heat-shock timing and common carp hatching rate may not follow a single monotonic pattern across the entire post-fertilization period. This possibility should be evaluated using a single experimental design that includes an untreated control and covers the full range of relevant post-fertilization times under standardized heat-shock conditions.

CONCLUSION

The timing of a 40°C heat-shock treatment applied between 5 and 11 min post-fertilization had a highly significant effect on the hatching rate of common carp eggs ($F(3,8) = 35.41$; $p < 0.001$; $\omega^2 = 0.90$). Hatching rate increased linearly across the tested interval, with no evidence of a peak within the observed time range. The 11-min treatment produced the highest mean hatching rate ($86.67 \pm 5.77\%$), but this value did not differ significantly from that obtained at 9 min ($76.67 \pm 5.77\%$; $LSD_{0.05} = 13.03$; $p = 0.115$). Therefore, the 9- and 11-min treatments were statistically comparable, and the 11-min treatment should not be regarded as the optimal application time.

These conclusions are limited to the experimental conditions evaluated in this study, namely eggs from a single broodstock pair, a heat-shock temperature of 40°C with an unquantified exposure duration, treatment application between 5 and 11 min post-fertilization, and incubation conditions of 28–30°C and pH 6.8–7.2. Because the study did not include an untreated control group, the findings should not be interpreted as evidence that heat shock improves the hatching rate of common carp eggs.

RECOMMENDATION

Future studies should include an untreated control, evaluate a broader post-fertilization window (e.g., 0–15 min), and standardize heat-shock duration to distinguish the effects of timing from exposure length. Increasing replication, egg number per container, and the number of broodstock pairs would improve statistical power and generalizability. Further assessments should also include fertilization rate, larval survival, morphological abnormalities, and, where possible, ploidy status. Until such controlled evidence is available, heat shock should not be recommended as a routine method for improving hatching success in small-scale common carp hatcheries.

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The authors used AI-assisted technologies solely to improve the readability and language quality of the manuscript. The authors reviewed and edited the final content and take full responsibility for the manuscript.

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