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Effects of Water Temperature on Embryonic Development, Hatching Success and Survival of Larvae of Siamese Mud Carp *Henicorhynchus siamensis* (Sauvage 1881)

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Abstract

Temperature is an important environmental factor for aquatic animals especially fishes, in particular during the early life stages. The objectives of this study are to investigate (a) the effect of different water temperature (26, 28, 30, 32 and 34 °C) on embryo development and newly hatched larvae of Siamese mud carp *Henicorhynchus siamensis* (Sauvage 1881) and (b) the effect of acute temperature change to the newly hatched *H. siamensis* larvae. The development was divided into two phases *viz.*, firstly, from zygote to gastrula periods and, secondly, segmentation to hatching periods. The *H. siamensis* larvae did not successfully hatch at the incubation temperatures of 26 and 34 °C. The development times of the three remaining temperatures were relatively closed at the first phase, in contrast to the second phase, which were quite varied. The hatching times at 28, 30 and 32 °C were about 652, 485 and 457 min, respectively. The percentages of hatching success of the three respective temperatures were 73.76±2.37%, 73.90±1.44% and 61.42±11.19%, respectively. For the effect of acute temperature changes, numbers of dead larvae were not significantly different between 30 and 28 °C (P-value = 0.30), but there was a significant difference between 30 and 32 °C (P-value < 0.01).

Introduction

Although it is too complicated to predict the impact of climate change to fishes, the primary effect of climate change will be through changes in water temperature (Brian et al. 2011). The physiological mechanisms of fishes are directly or indirectly temperature dependent, thus climate change will affect fish by altering physiological functions, such as growth, metabolism, food consumption, reproduction success, and their ability to maintain homeostasis in the face of a variable external environment (Roessig et al. 2004).

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Moreover, water temperature is the most important environmental factor that affects and governs the development of eggs, incubation time of embryos, hatching success, survival and growth of fish larvae (Hakim and Gamal 2009; Le et al. 2011; Ahn et al. 2012).

The countries in the Lower Mekong Basin (LMB) are expected to be impacted by climate change with significant changes in rainfall and temperature (MRC 2009). In Thailand, for example, it is estimated that the rainfall will be intense in a shorter period, and temperature will increase about 2 – 3 ^{o}C and be highly varied from season to season (Greenpeace 2006). Air temperatures have risen by 0.5 to 1.5 ºC in the past 50 years and continue to rise across the LMB and are likely to shift outside the present comfort zone, which is suitable for the LMB fishes for living (ICEM 2013). Continual temperature increase will inevitably impact the fish diversity and fisheries in the LMB, in which about 2 million tonnes of fishes and other aquatic animals are harvested each year, and which will eventually contribute to the food security in the LMB (ICEM 2010). There is another report by Lauri et al. (2012), which showed the difference between maximum- and minimum- daily average water temperature in the Mekong River could be more than 1 ^{o}C in 2032 onward. This is the cumulative impact of climate change and reservoir operation, i.e. the released water from dam.

The Siamese mud carp *Henicorhynchus siamensis* (Sauvage 1881) is a riverine species in mainland Southeast Asia. The species is a small-size cyprinid, i.e. about 20-25 cm TL (total length). It is the most abundant and most economically important fish in the LMB. It is the dominant species in the commercial set-net fisheries in Tonle Sap Lake, Cambodia, and the Khone Falls area in southern Laos (Rainboth 1996; Hai Yen et al. 2009; Fukushima et al. 2014). Not only does this fish provide protein, but also vitamins and minerals to the people in the LMB (Roos et al. 2007). *Henicorhynchus siamensis* also adapts to lentic environmental conditions, such as lakes and reservoirs, and contributes a significant portion in fish catches (Suvarnaraksha et al. 2010). Therefore, it is one of the most important candidates for a fish stock enhancement programme to increase fish production in inland waters (Jutagate 2009).

Henicorhynchus siamensis is a synchronous, i.e. single spawned species that clearly shows a single peak of gonadosomatic index in June to September and the highest in August (Suvarnaraksha et al. 2010). During the wet season, this species migrates into floodplains for spawning (Fukushima et al. 2014). Eggs and larvae grow in the floodplains and the larvae migrate back to rivers when the floodwaters begin to recede at the starting of dry season (Rainboth 1996; Fukushima et al. 2014). Therefore, suitable environmental conditions, during the wet season, are very important to guarantee the survival and recruitment to the fisheries of this species.

Little is known on fish distribution and their life history traits in Asia as well as possible impacts of climate change on fish, in comparison to fish in temperate zones (Ficke et al. 2007). Besides, it is widely accepted that the impact of climate change is unavoidable and is a serious concern to the developing world, including many Asian countries, where fish is among the major sources for protein food security (FAO 2007).

Henicorhynchus siamensis is a highly valuable fisheries resource throughout the LMB, and this study aims to explain the relationship between water temperature and embryonic development as well as the effect of acute changes in water temperature to mortality of the newly hatched *H. siamensis*. The findings will lead to a better understanding of the impact of temperature to this species which will help to improve climate risk management.

Materials and Methods

Broodstock

Broodstock of *H. siamensis* were from Ubon Ratchathani Inland Fisheries Research and Development Center in Ubon Ratchathani Province, Thailand. The parental fish were reared in a fibre-glass tank (60 L) for a week before breeding. The average water temperature was kept at 30±1 °C. The broodstock was divided into three lots (1 female: 2 males per lot) and each lot was kept in separate tanks. The average water temperature in each tank was 30±1 °C. The Suprefact was mixed with 10 mg of Motilium and then injected into the females (80- 150 g) and males (50- 100 g) at doses of 10 and 5 $\mu g \cdot kg^{-1}$, respectively. The parental fish spawned 8 h after injection.

Experiment and data analysis

Five water temperature treatments, i.e. at 26, 28, 30, 32 and 34 °C, were prepared in aquaria (24 x 40 x 28 cm) with a water volume of 25 L. One thousand eggs were rapidly transferred from the broodstock tanks into each experimental aquarium. The incubated eggs of *H. siamensis* were examined at 5 temperature levels from 26 to 34 °C at intervals of 2 °C in order to observe the effect of water temperature on embryonic development, hatching time and hatching success. The study was conducted in an air conditioning room, where the room- and air- temperatures were at about 20 and 24 °C, respectively. There were three replications for each treatment and the water temperatures were kept constant, at the set temperatures, by using the aquarium thermostat heater (Brand: EHEIM JAGER; model: TSRH 300 W). This experiment was conducted for 12 h, in which all the aquaria were aerated and the pH was maintained at 7.

The developmental periods of embryo from fertilisation to hatching were divided into seven periods *viz.*, zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching (Kimmel et al. 1995). The developmental periods were observed and recorded every 5 min in early periods (zygote, cleavage, blastula and gastrula) and then every 30 min until hatching. The study on the duration for embryonic development was conducted by sub-sampling about 0.1 L of water from each aquarium and then 20 eggs were taken to determine the periods of development which was considered complete when more than 60% of the sub-samples reached that period at each time interval. Hatching success (%), i.e. number of hatch in total number of eggs, was then calculated.

The analysis of variance (ANOVA) was applied to examine the effects of water temperature on hatching success. The Tukey's HSD test at 95% confidence interval was applied, when ANOVA revealed significance.

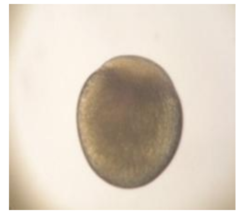
The effect of the acute change in temperature was examined by the experimental design "Before-After Control-Impact (BACI)". The setting of the experiment was similar to the first trial, i.e. effect of temperature on the embryonic development periods. The temperatures were set at 3 levels *viz.*, 30 ^{o}C as a control and the impacts were set at 28 ^{o}C and 32 ^{o}C, which respectively represented temperature at -2 and +2 ^{o}C of the control, since the estimated daily fluctuation of the water temperature in the Mekong could be beyond 1 ^{o}C in 2032 onward (Lauri et al.2012). Each temperature was replicated three times, in which 100 newly hatched *H. siamensis* were used in each replicate, in the aquaria (24 x 40 x 28 cm). The analysis was done by Randomized Intervention Analysis (RIA), on the difference in cumulative number of dead larvae between the control and each impact. The "before" was the period that the larvae were in control temperature for 20 min and then they were transferred, i.e. starting of the intervention of 100 larvae to the designated aquaria. Records were kept at 5, 15, 30, and 60 min then afterward every 60 minutes. The test was run by 999 iterations to random permutations of the impact-control data to generate the P-value. The statistical analyses were carried out with R software (R Development Core Team 2013).

Results

The durations for development at each period and hatching varied, depending on water temperature at their incubation (Table 1). Development time from zygote to gastrula period was relatively similar at all water temperatures. However, the development of blastula and gastrula periods at 26 °C took longer than the other treatments. The development period from segmentation to hatching, showed clear fluctuation. It is also worthy to note that the lower the temperature, the longer the time from segmentation period to hatching (Fig. 1). Development was stopped at the pharyngula period at 26 °C. Eggs did not hatch at 26 and 34 °C. For the remaining 3 levels, the time from the zygote stage until hatching, were obviously longer at 28 °C than 30 and 32 °C (Table 1).

The hatching success was highest at 30 °C (73.90±1.44%) but not significantly different at 28 °C (73.76±2.37%; Fig.2). Meanwhile, at the high temperature of 32 °C, hatching success decreased (61.42±11.19%) and was significantly different from the remaining two temperature levels. Dead larvae were observed after the intervention both in the control- and impact-manipulations. The average number of dead larvae at the control and 28 ^{o}C were relatively low, i.e. less than 10 individuals. However, the acute change in water temperature from 30 to 32 ^{o}C yielded high numbers of dead larvae, which was up to 30 larvae after 9 h. The RIA results indicated that there was no significant difference in average number of dead larvae between the control and 28 ^{o}C (P-value = 0.30; Fig. 3) but the difference between the control and 32 ^{o}C were significantly different (P-value < 0.01; Fig. 4).

Table 1. Average time (minutes) of embryonic development at five water temperature treatments.

Period	Temperature (°C)	Average time*(minutes)	Reference photographs	Description
Zygote	26	0.0		The chorion swells and cytoplasm streams toward animal pole to form the blastodisc
	28	0.0		
	30	0.0		
	32	0.0		
	34	0.0		
Cleavage	26	19.8		Blastodisc is vertically cleavaged from 1 to 64 blastomeres.
	28	21.0		
	30	19.8		
	32	19.8		
	34	19.8		
Blastula	26	150.0		Blastoderm covers about 30 % of egg and blastocoel is presented.
	28	96.6		
	30	90.0		
	32	96.6		
	34	91.8		
Gastrula	26	189.6		Germ ring is visible and covers 90 % of egg.
	28	144.6		
	30	144.6		
	32	139.8		
	34	139.6		
Segmentation	26	360.6		Fisrt somite expands to 26 somites and eyes vesicle, optic veasicle and tail bud are visible.
	28	309.6		
	30	274.8		
	32	259.8		
	34	256.8		
Pharyngula	26	600.0		Body is straight and well developed of somitesto the end of tail. Binary symmetry and pigment are visible.
	28	519.6		
	30	450.0		
	32	408.6		
	34	No hatching		
Hatching	26	No hatching		Basical organs are well developed, especially pectoral fin, jaws and gills and then hatching.
	28	652.2		
	30	484.8		
	32	457.2		
	34	No hatching		

* The average time (minutes) for each development period is considered to be completed when more than 60% of eggs had reached the period.

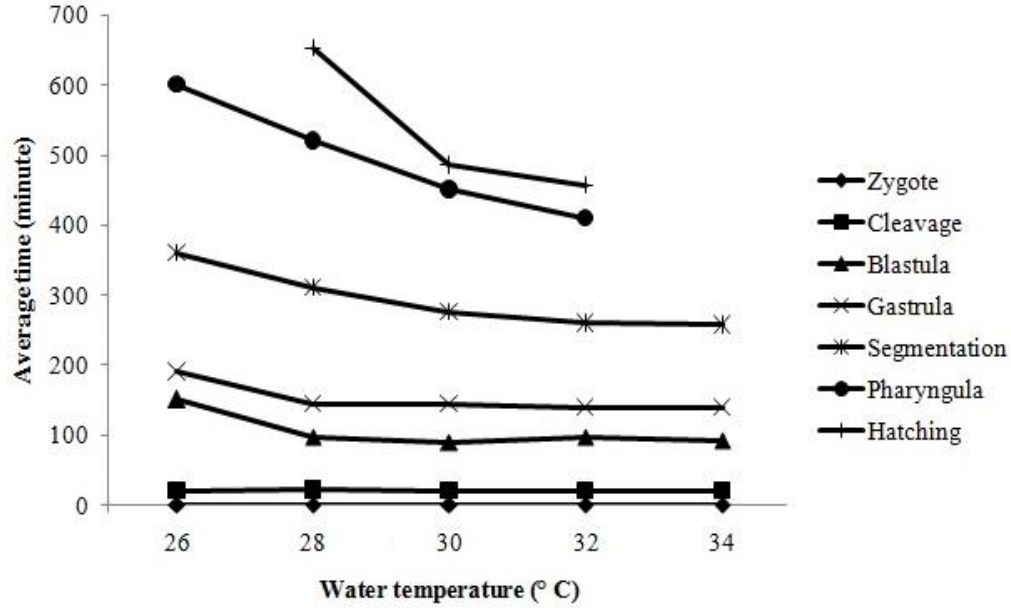


Fig. 1. The durations of *Henicorhynchus siamensis* embryonic development at each studied temperature.

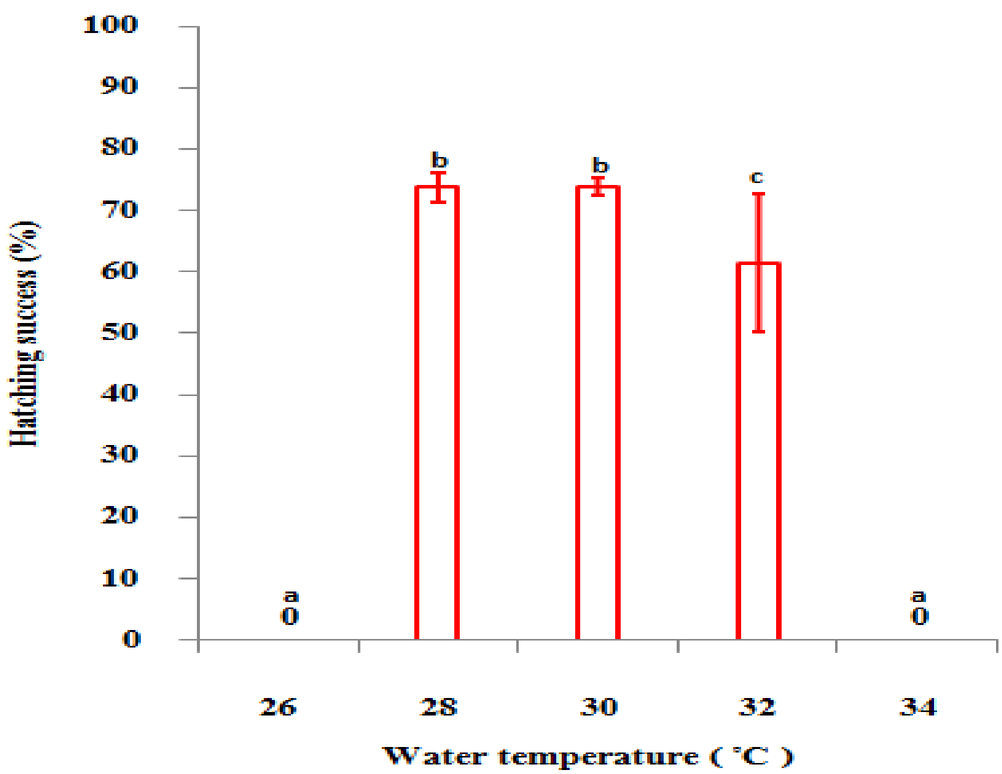


Fig. 2. Hatching success of *Henicorhynchus siamensis* at each studied temperature (The different letters, above each bar, indicate statistical difference at α = 0.05.)

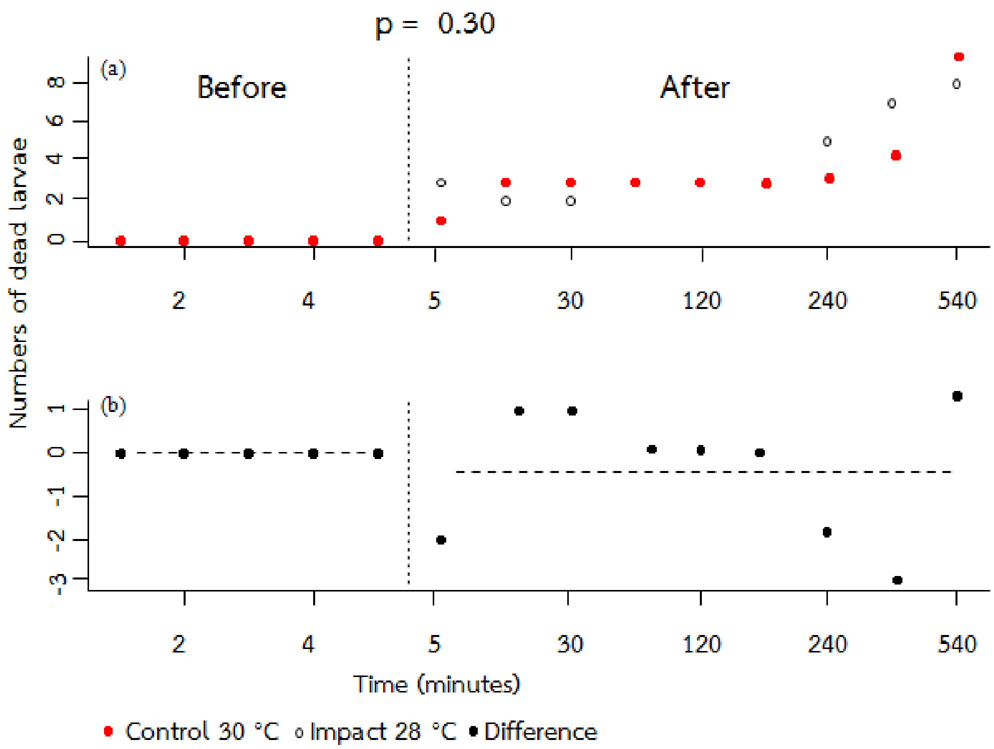


Fig. 3. Number of dead *Henicorhynchus siamensis* larvae of the control (30 °C) and impact (28 °C) treatments. Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

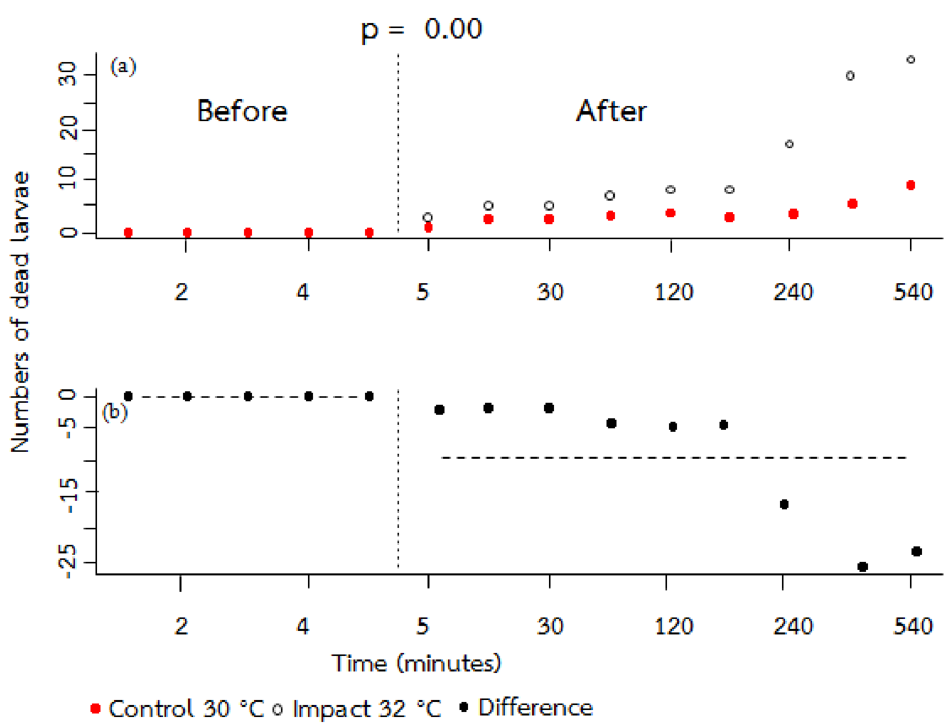


Fig. 4. Numbers of death *H. siamensis*larvae of the control (30 °C) and impact (32 °C). Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

Discussion

Changes in water temperature produced a strong effect on embryo development and hatching of *H. siamensis*. Temperatures, which are beyond the tolerance range, results in unsuccessful development and hatching failure (Kucharczyk et al. 1997; Kupren et al. 2011; Ahn et al. 2012). In this study, water temperatures at 24 and 34 °C are considered as the respective "minimum limiting"- and "maximum limiting"- temperatures (Cruz et al. 2002) for the egg development of *H. siamensis*. In general, the lower temperature causes unsuccessful egg development and the high temperature causes mortality of the embryo (Kupren et al. 2011; Ahn et al. 2012). The maximum limiting temperature is the main concern because water temperatures in Thai inland waterways can reach 34 °C during the rainy season (Choo-In et al. 2013), i.e. the spawning season for *H. siamensis*. Also increasing temperatures are expected on both daily maximum temperature and daily minimum temperature in the LMB, where the daily maximum temperature could be beyond 35 °C (TKK & SEA START RC 2009). The minimum limiting temperature may also be of concern, when cold water from dams is released (Lugg and Copeland 2014), and if this phenomenon overlaps into the spawning season.

Although there were non-significant differences in embryonic development time at each stage, there was a trend towards a decrease of development time when the incubation temperature increased for *H. siamensis*. This implies that a lower temperature retards the rate of embryonic development, and a higher water temperature accelerates the larval development, which conforms to the negative second order polynomial trend-line for the temperate- and most of marine fishes (Kawahara et al. 1996; Chambers and Tripple 1997; Yang and Chen 2005; Kupren et al. 2011). The reason for faster hatching of embryo at high water temperature is because of the increase of mobility in warmer water and early excretion of the hatching enzyme (Kupren et al. 2011).

The optimum temperature, which is suitable for fish embryo development and success in hatching as well as their survival, is usually correlated with spawning temperature (Kucharczyk et al. 1997; Ahn et al. 2012). The temperature experienced by the parents may influence the optimal temperature of egg incubation (Bermudes and Ritar 1999). In this experiment, the treatment at 30 °C, i.e. similar to the temperature in the spawning aquaria, yielded the highest hatching success. Meanwhile, the water temperatures in the treatments that prominently deviated from the average temperature in the spawning aquaria at ± 4 °C, i.e. 26 and 34 °C, damaged *H. siamensis* eggs and resulting in hatching failure. Notwithstanding this fact for *H. siamensis*, Landsman et al. (2011) reported that deviated temperature of ± 2 °C during the egg stage for Atlantic cod can generate huge variations in recruitment, i.e. can be up to 1000-folds. This probably results from elevated metabolic rate, causing accelerated consumption of energy store or from thermal degradation of proteins that impart a direct effect on cell function such as substrate binding activity and stress protein synthesis (Landsman et al. 2011).

Acute increase of water temperature causes significant decrease in survival of newly hatched larvae rather than acute decrease of water temperature (Landsman et al. 2011; Lahnsteiner et al. 2012). In general, deviations in metabolic rate could be observed in fishes during acute changes in temperature. The cold-acclimated individuals typically show a large increase in metabolic rate when exposed to warm temperatures, while warm-acclimated fish often show a considerable decrease when acutely exposed to colder temperatures (Killen et al. 2007). Hence, the daily development and mortality rates trend to increase with acute increasing temperature (Pepin 1991).

Conclusion

Although it is believed that tropical fishes have evolved to survive in very warm water and may seem less likely to suffer from increases in global temperature (Ficke et al. 2007), our results showed that the prominent deviation in water temperature affect embryonic developments and hatching of *H. siamensis*. The results also provide useful information for habitat management for protection of the local aquatic ecology as well as to aquaculture system. Further studies are needed to understand such impacts on the abnormality of the larvae and their growth performances.

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