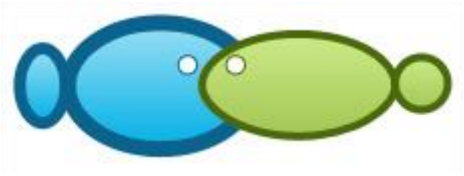

Effects of carbon dioxide (CO_2) at different temperatures on the embryonic development of striped catfish (*Pangasianodon hypophthalmus*)

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Abstract. The striped catfish, *Pangasianodon hypophthalmus* is a facultative air-breathing fish. This species is widely cultured in the freshwater areas of the Mekong Delta, Vietnam. This study reports the effects of elevated temperatures, carbon dioxide (CO_2) concentrations, and the combination of CO_2 with different temperatures on embryonic development, fertilization, and hatching. The first experiment included four temperatures (27, 30, 33, and 36ºC); the second experiment consisted of four concentrations of CO_2 (0.7±0.02, 7.05±0.22, 14.2±0.6 and 21.4±0.3 mmHg CO_2), and the third one was the combination of CO_2 and temperatures with five treatments including the temperature and CO_2 ≤ 0.7 mmHg) (control), 7 mmHg CO_2 at two temperatures (27 and 33ºC) and 14 mmHg CO_2 at two temperatures (27ºC and 33ºC). The fertilized eggs from artificial breeding were directly exposed to the tested treatments. The results show that the hatching rate of eggs (62.3%) was highest at 30ºC, and the time spent to complete embryonic development (20.2 hours) was shortest at 33ºC. The increase in CO_2 concentrations in the water did not affect the fertilization rate ($p > 0.05$), but the hatching rate decreased with the increased CO_2 concentrations. The embryonic development was significantly affected by high CO_2 concentrations at elevated temperatures. Particularly, the lowest hatching rate (40.8%) and the longest time for embryonic development (approximately 25.5 hours) were found in the treatment of 14 mmHg CO_2 at 33ºC. It is summarized that the increase in temperature combined with a high concentration of CO_2 in water affects the embryonic development of striped catfish.
Key Words: embryo, hatching, fertilization, hypercapnia.

Introduction. The Mekong Delta has been identified as one of the three hardest-hit deltas by climate change worldwide (IPCC 2007). Climate change manifests through the increasing frequency of droughts, hot weather, and heavy rain, as well as highly variable rainfall patterns and increases in CO_2. The climate change in this delta was forecasted that the rainfall would decrease by 10-20% in the early rainy season and increase at the end of the rainy season; the temperature would increase by 2ºC (up to 35-37°C) as highest between 2030 and 2040 (Tuan & Chinvanno 2009); the sea level rise was projected to be 75 cm (52-106 cm) at the end of this century, causing salinity intrusion into inland areas (MONRE 2016). These factors highly affect fish farming practices and have been seen in the past few years. High CO_2 concentrations in the water affect aquatic animals. Grottum & Sigholt (1996) found that 50% of *Dicentrarchus labrax* died within 96 hours in pCO_2 50 mmHg. Studies on the acid-base regulation under elevated CO_2 and temperature have been reported in three facultative air-breathing fish, *Pangasianodon hypophthalmus* (Damsgaard et al 2015), *Monopterus albus* (Thinh et al 2018) and *Chitala ornata* (Gam et al 2020) with typical responses. *P. hypophthalmus* completed compensation pHe (plasma pH) after 72 hours of exposure to 34 mmHg CO_2 by the associated increase of plasma bicarbonate (Damsgaard et al 2015).

P. hypophthalmus is a non-obligated air-breathing species identified as having high tolerance in extreme environmental conditions. The optimal fish growth was at 33ºC in the water environment (Phuong et al 2017). Phuc et al (2007) reported that *P. hypophthalmus* was not stressed in high temperatures combined with salinities and even

the growth performance improved in the water environment of 35ºC and 6 ppt. Studies about the effects of different temperatures or CO_2 concentrations and the combined impact of these two factors on the haematological functions of the *P. hypophthalmus*, have also been examined by Huong et al (2021).

There have been limited studies on the effects of temperature, CO_2 exposure, and CO_2 at elevated temperatures on fertilization, the time spent to complete embryonic development, and hatching in bimodal breathers, as seen in aquatic organisms and air-breathers. Therefore, this study aimed to investigate the effects of different temperatures, CO_2 concentrations, and combinations of CO_2 with various temperatures on the embryonic development of *P. hypophthalmus* to determine the adaptation of this species under high CO_2 levels and temperatures in water.

Material and Method

Time, eggs, and environmental parameters of the experiments. The study was conducted at the College of Aquaculture and Fisheries, Can Tho University from June to October 2022. Healthy matured broodstocks were used to produce eggs by artificial breeding. Eggs were fertilized by the drying method and transferred to test jars. During the experimentation, the temperatures and CO_2 concentrations in water of experimental jars were kept corresponding to test levels; temperatures were 26.9±0.03ºC, 30.0±0.04ºC, 33.2±0.07ºC, 36±0.08ºC, 39±0.06ºC and CO_2 concentrations were 0.7±0.02, 7.05±0.22, 14.2±0.6 and 21.4±0.3 mmHg CO_2. The jars were aerated well and also maintained other parameters within the suitable range, such as dissolved oxygen (7.05±0.15 mg L^{-1}), pH (7.75±0.06), total ammonia nitrogen (0.74±0.04 mg L^{-1}), and NO_2^- (0.04±0.01 mg L^{-1}). All the experiments followed national guidelines on the protection and experimental animal welfare in Vietnam (Law of Animal Health 2015).

Effects of different temperatures. The experiment was conducted with five different temperatures, including 27ºC (control condition), 30ºC, 33ºC, 36ºC, and 39ºC with three replicates. An amount of 0.8 g of eggs after fertilization (approx. 960 eggs) was randomly distributed to each 10 L glass jar (containing 8 L of water, approx. 120 eggs L^{-1} representing each replicate). In addition, 120 eggs were also distributed to 2 L glass bottles (containing 1 L of water) representing the same five temperature levels with three replicates each for observing fertilization and hatching rate. All experimental jars and bottles for each treatment were arranged in a big plastic tank of 300 L of water with a tested temperature to keep the desired water temperatures in the jars and bottles. The water temperatures in the big tanks were increased and controlled using Heaters (EHEIM professional 4+ 350T – Germany).

Ten eggs from each jar were sampled every 15 minutes from fertilization to the stage of multiple cells and 30 minutes for the later stages until hatching to observe embryonic development. The time spent to complete embryonic development was calculated from fertilization to hatching. The characteristics of the developmental phases of the embryos were recorded under a microscope (Olympus CX 21). The fertilization and hatching rates of eggs were observed at 2 hours and 30 hours after hydration from the bottles, respectively. The number of fertilized eggs that maintained translucence was determined at 2 hours after insemination, while the unfertilized eggs that turned whitish were removed from the bottles. The fertilization rate was determined by the number of fertilized eggs divided by the number of observed eggs. While the hatching rate was calculated by the number of hatched eggs divided by the number of fertilized eggs.

Effects of different CO_2 concentrations. The experiment was designed in 10 L glass jars (containing 8 L water) with four treatments, including control (below 0.7 mmHg CO_2), 7 mmHg CO_2, 14 mmHg CO_2, and 21 mmHg CO_2. As above, an amount of 0.8 g of eggs after fertilization (approx. 960 eggs) was randomly distributed to each 10 L glass jar (containing 8 L of water, approx. 120 eggs L^{-1} representing each replicate). Additionally, 120 eggs were distributed to 2 L glass bottles (containing 1 L of water) representing the same five CO_2 levels with three replicates each for observing fertilization

and hatching rate. CO_2 was added to experimental jars corresponding to the desired concentrations from oxygen tanks and CO_2 cylinders which were automatically controlled by the Oxyguard Pacific system coupled with a G10 ps CO_2 probe and a K01svpld pH probe (Oxyguard International A/S, Farum, Denmark). The time spent to complete embryonic development, fertilization rate, and hatching rate were observed as described above (Item: Effects of different temperatures).

Effects of CO_2 at different temperatures. The study was designed in 10 L glass jars (containing 8 L water) with five treatments, including the control (normal temperature and CO_2 < 0.7 mmHg), 7 mmHg CO_2 at two temperatures (27 and 33°C) and 14 mmHg CO_2 at two temperatures (27°C and 33°C). An amount of 0.8 g of eggs after fertilization (approx. 960 eggs) was randomly distributed to each 10 L glass jar (containing 8 L of water, approx. 120 eggs L^{-1} representing each replicate). Besides, 120 eggs were also distributed to 2 L glass bottle (containing 1 L of water) representing the same five CO_2 levels with three replicates each for observing fertilization and hatching rate. All experimental jars and bottles for each treatment were placed in a big plastic tank of 300 L of water with a tested temperature to keep the desired water temperatures in the jars and bottles. The desired temperatures of treatments were controlled by heaters (EHEIM professional 4+ 350T – Germany) and the set-CO_2 concentrations were controlled by an Oxyguard Pacific system coupled with a G10 ps CO_2 probe and a K01svpld pH probe (Oxyguard International A/S, Farum, Denmark). The time spent to complete embryonic development, fertilization rate, and hatching rate were observed as described above (Item: Effects of different temperatures).

Statistical analysis. All data were analyzed using PASW statistics (SPSS 18.0). A one-way ANOVA was used for embryonic development indicators (time of embryonic development, fertilization rate, and hatching rate) between treatments with a p-value of less than 5% ($p < 0.05$). All data were presented as means±standard error of the mean (SEM).

Results

Embryonic development of the striped catfish exposed to different temperatures. Hatching rates were significantly different between treatments 30°C (62.3%) and control (53.7%) and 33°C (49.9%) ($p < 0.05$) (Table 1). However, the fertilization rates among these temperatures were not significantly different, ranging from 83.0 to 85.7%. The time spent to complete embryonic development at the treatments of 30°C and 33°C (21.0 hours and 20.2 hours) were significantly shorter than those at 27°C (22.5 hours) (Table 1).

Table 1

Time of embryonic development, fertilization rate, hatching rate of *P. hypophthalmus* at different temperatures

Treatments	*Quota*		
	Time of embryo development (hours)	*Fertilization rate (%)*	*Hatching rate (%)*
27°C (Control)	22.5±0.06^a	85.7±0.87^a	53.7±1.07^a
30°C	21.0±0.06^b	83.0±1.56^a	62.3±2.37^b
33°C	20.2±0.23^c	83.0±2.08^a	49.9±2.35^a

All data are displayed as mean±standard error; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

It is noticeable that the embryos in the 36 and 39°C treatments were just able to reach the multiple-cell and 16-cell stages, respectively. Normal development of the embryos is shown in Figures 1A to 1H, and the abnormal embryonic development is indicated in Figures 2A, 2B, and 2C.

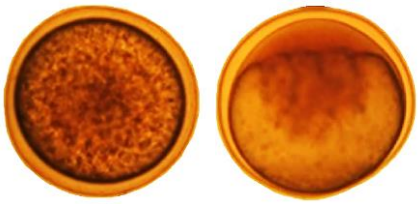


Figure 1.A. Zygote (40x).

Figure 1.B1. 2 cell. Figure 1.B2. 4 cell. Figure 1.B3. 8 cell (40x).

Figure 1.C. Morula stage (40x).

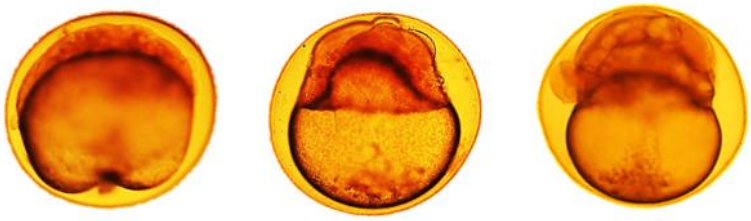
Figure 1.D1. Early blastula (40x). Figure 1.D2. Late blastula (40x).

Figure 1.E. Gastrula stage (40x).

Figure 1.F. Organogensis. (40x).

Figure 1.H. Hatching out (10 x).

Figure 1. Normal development of embryos exposed to different temperatures.



Abnormal morula stage (A) (40x) | Abnormal blastula stage (B) (40x) | Abnormal gastrula stage (C) (40x)

Figure 2. Abnormal development of embryos exposed to different temperatures.

Embryonic development of the striped catfish exposed to different CO_2 concentrations. Fertilization rates of all CO_2 treatments and control had no significant difference (82.3 to 85.0%). However, the time of embryonic development in the three CO_2 treatments was significantly longer than those of the control, whereas hatching rates in CO_2 tested groups had significantly low values (56.1, 53.0 and 47.7% at 7, 14 and 21 mmHg CO_2, respectively) compared to that in control (69.3%) (Table 2).

Table 2

Time spent to complete embryonic development, fertilization rate, and hatching rate of *P. hypophthalmus* embryos at different CO_2 concentrations

Treatments	*Quota*		
	Time of embryo development (hours)	*Fertilization rate (%)*	*Hatching rate (%)*
Control	21.2±0.22^a	85.0±3.46^a	69.3±2.29^a
7 mmHg CO_2	22.6±0.51^b	84.3±1.85^a	56.1±0.88^b
14 mmHg CO_2	22.6±0.44^b	84.3±1.20^a	53.0±0.87^c
21 mmHg CO_2	24.4±0.39^c	82.3±3.18^a	47.7±0.61^c

All data are displayed as mean±standard error; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

Embryonic development of the striped catfish exposed to CO_2 at different temperatures. The time of embryonic development was significantly longer in the treatments of higher CO_2 concentration at the same temperature ($p < 0.05$), but there was no significant difference in fertilization rates for all combinations of CO_2 and temperatures compared to that in control treatments (85.7% at 27°C and 83.0% at 33°C) ($p > 0.05$) (Table 3A, 3B). Nevertheless, hatching rates at elevated temperatures and CO_2 levels were significantly decreased to 47.9% and 43.5% in the treatments of 7 and 14 mmHg at 27°C (Table 3A), and 41.4% and 40.8% in the treatments of 7 mmHg and 14 mmHg at 33°C ($p < 0.05$), respectively (Table 3B).

Table 3A

Time of embryonic development, fertilization rate, hatching rate of *P. hypophthalmus* embryos in CO_2 exposure at 27°C

Treatments	*Quota*		
	Time of embryonic development (hours)	*Fertilization rate (%)*	*Hatching rate (%)*
Control (27°C)	22.5±0.06^a	85.7±0.87^a	53.7±1.07^a
7 mmHg CO_2 at 27°C	24.6±0.33^b	84.3±1.85^a	47.9±1.69^b
14 mmHg CO_2 at 27°C	26.4±0.43^c	83.7±2.33^a	43.5±1.45^b

All data are displayed as mean±standard error; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

Table 3B

Time of embryonic development, fertilization rate, hatching rate of *P. hypophthalmus* embryos in CO_2 exposure at 33°C

Treatments	*Quota*		
	Time of embryonic development (hours)	*Fertilization rate (%)*	*Hatching rate (%)*
Control (33°C)	20.2±0.23[a]	83.0±2.08[a]	49.9±2.35[a]
7 mmHg CO_2 at 33°C	23.4±0.22[b]	82.3±3.18[a]	41.4±1.39[b]
14 mmHg CO_2 at 33°C	25.5±0.20[c]	81.0±1.15[a]	40.8±0.66[b]

All data are displayed as mean±standard error; different subscript letters (a, b, c) in the same column indicate significant differences ($p < 0.05$).

Discussion. The time cost for embryonic development in the control treatment (27°C) was 22.5±0.06 hours, which is the same as those of yellow tail catfish (*Pangasius pangasius*) (Ferosekhan et al 2015). dos Santos et al (2016) reported that the hatching time of *Astyanax altiparanae* at 30°C is 11 hours after fertilization, which is shorter than those of 25 and 16 hours at 22°C or 26°C, respectively. In this study, the embryos of *P. hypophthalmus* died within 2 to 3 hours post-fertilized at 36 and 39°C, which closely resembles the embryos of the Australian strain of *Lates calcarifer* could not develop after two hours of fertilization at 36°C (Thepót & Jerry 2015).

Fish are commonly considered to have high tolerance to increased CO_2 levels and declined water pH because of their well-developed mechanisms for acid-base regulation. Juvenile and adult-stage fish regulate the concentrations of acid-base-relevant ions in the blood and tissues to protect from acidosis in a high CO_2 environment (Heuer & Grosell 2014). Fish are more susceptible to elevated CO_2 levels in the early life stage because their physiological regulatory processes are still developing (Brauner 2008). Some previous studies presented no impacts or only minor impacts of high CO_2 on the growth and survival rate of fish at early life stage or juvenile stage (Munday et al 2009; Franke & Clemmesen 2011; Bignami et al 2014; Pope et al 2014; Huong et al 2021), whereas other studies found adverse effects of high CO_2 on embryonic development (Forsgren et al 2013), yolk provisioning (Chambers et al 2014), tissue and organ development (Frommel et al 2012; Chamber et al 2014), swimming duration (Pimentel et al 2014), growth and survival (Baumann et al 2012).

The embryonic development of fish is significantly affected by high CO_2 concentrations in the water. Extreme environmental conditions seriously injure embryonic development at the detached stage, and the adverse effects of CO_2 depend on fish species. In the present study, isolated and combined high temperatures and high CO_2 concentrations caused adverse effects on the time of embryonic development and hatching rate in *P. hypophthalmus.* In contrast*,* fertilization rates at all treatments remained significantly unchanged during the experimentation. The result is similar to some previous studies on CO_2 and temperatures in aquatic animals; particularly, 50% mortality appeared in red sea bream (*Pagrus major*) and Japanese whiting (*Sillago japonica*) in CO_2 exposures of 1.4 and 2.4 kPa for cell-detached stage and 4.9 and 5.1 kPa for embryonic stage (Kikkawa et al 2003). Similarly, there are significant effects in the cell-detached stage in Japanese flounder (*Paralicthys olivaceus*) with CO_2 exposure of 2.8 kPa and in Eastern little tuna (*Euthynnus affinis*) with CO_2 exposure of 11.8 kPa (Kikkawa et al 2003). Egg mortality in red sea bream (*Pagrus major*) was 85.8% and 97.4% at 5% and 10% CO_2, respectively (Kikkawa et al 2004). After 9 and 19 hours in CO_2 exposures (876, 949, and 957 ppm), the time of embryonic development in Cobia (*Rachycentron canadum*) had no significant difference ($p > 0.05$) (Ebanks 2013).

Conclusions. The elevated temparatures, CO_2 and the combination of these two parameters prolonge the embryonic development time of *P. hypophthalmus*. The hatching rates of fish reduced at high CO_2 and high CO_2 combined with elevated temperatures. These results suggest that the control of water temparature and CO_2 in the

hatchery of *P. hypophthalmus* sould be considered in the context of increased temparature and CO_2 caused by climate change in the Mekong Delta, Vietnam.

Acknowledgements. The ODA-TC2016-3 project at Can Tho University funds this study.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Baumann H., Talmage S. C., Gobler C. J., 2012 Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. Nature Climate Change 2:38-41.

Bignami S., Sponaugle S., Cowen R. K., 2014 Effects of ocean acidification on the larvae of a high-value pelagic fisheries species, mahi-mahi *Coryphaena hippurus*. Aquatic Biology 21:249-260.

Brauner C. J., 2008 Acid-base balance. In: Fish larval physiology. Finn R. N., Kapoor B. G. (eds), Science Publishers, Enfield, pp. 185-198.

Chambers R. C., Candelmo A. C., Habeck E. A., Poach M. E., Wieczorek D., Cooper K. R., Greenfield C. E., Phelan B. A., 2014 Effects of elevated CO_2 in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. Biogeosciences 11(6):1613-1626.

Damsgaard C., Gam L. T. H., Tuong D. D., Thinh P. V., Thanh D. T. H., Wang T., Bayley M., 2015 High capacity for extracellular acid-base regulation in the air-breathing fish *Pangasianodon hypophthalmus*. Journal of Experimental Biology 218(9):1290-1294.

dos Santos M. P., Yasui G. S., Xavier P. L. P., Adamov N. S. de M., do Nascimento N. F., Fujimoto T., Senhorini J. A., Nakaghi L. S. O., 2016 Morphology of gametes, post-fertilization events and the effect of temperature on the embryonic development of *Astyanax altiparanae* (Teleostei, Characidae). Zygote 24(6):795-807.

Ebanks A. D., 2013 The influence of increased CO_2 on early developmental stages of cobia, *Rachycentron canadum*. PhD dissertation, University of Miami, 81 pp.

Ferosekhan S., Sahoo S. K., Giri S. S., Saha A., Paramanik M., 2015 Embryonic and larval development of yellow tail catfish, *Pangasius pangasius*. Journal of Aquaculture Research and Development 6(6):343.

Forsgren E., Dupont S., Jutfelt F., Amundsen T., 2013 Elevated CO_2 affects embryonic development and larval phototaxis in temperate marine fish. Ecology and Evolution 3(11):3637-3646.

Franke A., Clemmesen C., 2011 Effect of ocean acidification on early life stages of Atlantic herring (*Clupea harengus* L.). Biogeosciences 8(12):3697-3707.

Frommel A. Y., Maneja R., Lowe D., Malzahn A. M., Geffen A. J., Folkvord A., Piatkowski U., Reusch T. B. H., Clemmesen C., 2012 Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. Nature Climate Change 2:42-46.

Gam L. T. H., Huong D. T. T., Tuong D. D., Phuong N. T., Jensen F. B., Wang T., Bayley M., 2020 Effects of temperature on acid-base regulation, gill ventilation and air breathing in the clown knifefish, *Chitala ornata*. Journal of Experimental Biology 223(4):jeb216481.

Grottum A. J., Sigholt T., 1996 Acute toxicity of carbon dioxide on European seabass (*Dicentrarchus labrax*): mortality and effects on plasma ions. Comparative Biochemistry and Physiology Part A: Physiology 115(4):323-327.

Heuer R. M., Grosell M., 2014 Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307(9):1061-1084.

Huong D. T. T., Tram C. H. T., Ha N. T. K., Gam L. T. H., Ishimatsu A., Phuong N. T., 2021 Effects of carbon dioxide (CO2) at different temperatures on physiological parameters and growth in striped catfish (*Pangasianodon hypophthalmus*) juveniles. Aquaculture 534:736279.

IPCC, 2007 Climate change 2007: synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Core Writing Team, Pachauri R. K., Reisinger A. (eds), IPCC, Geneva, Switzerland, 104 pp.
Kikkawa T., Ishimatsu A., Kita J., 2003 Acute CO_2 tolerance during the early developmental stages of four marine teleosts. Environmental Toxicology 18(6):375-382.
Kikkawa T., Kita J., Ishimatsu A., 2004 Comparison of the lethal effect of CO_2 and acidification on red sea bream (*Pagrus major*) during the early developmental stages. Marine Pollution Bulletin 48(1-2):108-110.
Law of animal health, 2015 [Article 21 - Vietnam National Assembly Passes Animal Health Law]. Dated on 10/7/2015. Report Number: VM5068. 72 pp.] [in Vietnamese]
MONRE (Ministry of Natural Resources and Environment, Hanoi, Vietnam), 2016 [Climate change, sea level rise scenarios for Vietnam]. NARENCA Publisher, 188 pp. [in Vietnamese]
Munday P. L., Donelson J. M., Dixson D. L., Endo G. G. K., 2009 Effects of ocean acidification on the early life history of a tropical marine fish. Proceedings of the Royal Society B: Biological Sciences 276(1671):3275-3283.
Phuc N. T. H., Mather P. B., Hurwood D. A., 2017 Effects of sublethal salinity and temperature levels and their interaction on growth performance and hematological and hormonal levels in tra catfish (*Pangasianodon hypophthalmus*). Aquaculture International 25:1057-1071.
Phuong L. M., Huong D. T. T., Nyengaard J. R., Bayley M., 2017 Gill remodelling and growth rate of striped catfish *Pangasianodon hypophthalmus* under impacts of hypoxia and temperature. Comparative Biochemistry and Physiology Part A 203: 288-296.
Pimentel M., Pegado M., Repolho T., Rosa R., 2014 Impact of ocean acidification in the metabolism and swimming behavior of the dolphinfish (*Coryphaena hippurus*) early larvae. Marine Biology 161:725-729.
Pope E. C., Ellis R. P., Scolamacchia M., Scolding J. W. S., Keay A., Chingombe P., Shields R. J., Wilcox R., Speirs D. C., Wilson R. W., Lewis C., Flynn K. J., 2014 European sea bass, *Dicentrarchus labrax*, in a changing ocean. Biogeosciences 11(9):2519-2530.
Thépot V., Jerry D. R., 2015 The effect of temperature on the embryonic development of barramundi, the Australian strain of *Lates calcarifer* (Bloch) using current hatchery practices. Aquaculture Reports 2:132-138.
Thinh P. V., Phuong N. T., Brauner C. J., Huong D. T. T., Wood A. T., Kwan G. T., Conner J. L., Bayley M., Wang T., 2018 Acid-base regulation in the air-breathing swamp eel (*Monopterus albus*) at different temperatures. Journal of Experimental Biology 221(10):jeb172551.
Tuan L. A., Chinvanno S., 2011 Climate change in the Mekong River Delta and key concerns on future climate threats. In: Environmental change and agricultural sustainability in the Mekong Delta. Stewart M. A., Coclanis P. A. (eds), Advances in global change research. Vol 45, Springer, Dordrecht, pp. 207-217.

Received: 12 August 2024. Accepted: 22 October 2024. Published online: 23 November 2024.
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How to cite this article:
Huong D. T. T., Phuong N. T., Ha N. T. K., 2024 Effects of carbon dioxide (CO_2) at different temperatures on embryonic development of striped catfish (*Pangasianodon hypophthalmus*). AACL Bioflux 17(6):2549-2556.