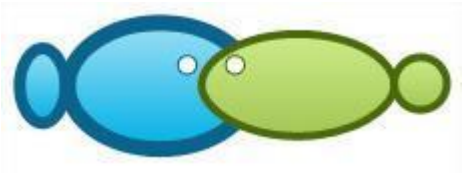

Effects of water temperatures and dissolved oxygen on brain cholinesterase in snakehead fish (*Channa striata*) exposed to quinalphos

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Abstract. Realistic temperatures (24±0.3, 30±0.3, and 34±0.2°C) and dissolved oxygen (DO) levels (1.8±0.2, 6.5±0.7 mg L^{-1}) that can be found in rice fields of the Mekong River Delta were manipulated in a full factorial block design experiments in the laboratory to determine the effects of these parameters on brain cholinesterase activity of juvenile snakehead fish (*Channa striata*) exposed to realistic concentration of quinalphos (2.45 µg L^{-1}). The results show brain cholinesterase inhibition increases significantly with temperature, while no such impact was seen with DO. These parameters did not affect the brain cholinesterase activity in unexposed fish. With the trend of global warming and extreme fluctuation of weather, current rice farming practices in the Mekong River Delta would cause more health risk for snakehead fish in ricefield habitat. Field investigation is recommended to confirm the prediction.
Key Words: global warming, Kinalux 25EC, organophosphate pesticide, striped snakehead, toxicology.

Introduction. The snakehead fish, *Channa striata* (Bloch, 1793), which is indigenous to the Mekong River Delta (MRD) (Ngoc et al 2018) is able to adapt to a wide variety of environmental conditions. Thus it can be found in a wide range of temperature (11-40°C) and pH (4.25-9.4) (Lee & Ng 1994). As a result of being an obligatory air-breather (Vivekanandan 1977), the snakehead can survive in low dissolved oxygen (DO) water. Nevertheless, the snakehead is very sensitive to quinalphos with a 96-h LC_{50} of 49 µg L^{-1}) (Cong N. V., Yen N. T. H., Khanh H. C. – unpublished data) while quinalphos in ricefield water after 1h application Kinalux 25EC was 11.3±0.15 µg L^{-1} and decreased to 5.8±0.6 µg L^{-1} at 1 day and 1.8±0.1 µg L^{-1} at 3 days (Thinh et al 2016). Under laboratory conditions, brain cholinesterase (ChE) of snakehead fish was significantly inhibited at a concentration of 100 times lower than 96-h LC_{50} of diazinon or 1% 96-h LC_{50} of diazinon (Cong et al 2024).

As one of the preferred habitats of this species, approximately 50% of the reproduction of snakehead fish was found to occur in paddy fields during the rainy season (Amilhat & Lorenzen 2005) and experience considerable fluctuations in DO and temperature in rice fields. Temperature increased from approximately 24°C in the early morning to 34°C in the afternoon (Vromant et al 2001) whereas DO levels varied from an early morning 1.8 mg L^{-1} to the afternoon 6.55 mg L^{-1} (Tam et al 2018).

Temperature effects on pesticide toxicity to organisms in general are unclear (Heugens et al 2001). Toxicity of insecticides are found to be both negatively and positively correlated to temperature depending on species and toxin. For example, a negative correlation was found in brown-headed cowbirds (*Molothrus ater*) exposed to the organophosphate dimethoate (Brunet & McDuff 1997). Here, increasing temperature from 5 to 25°C was found to cause a significant reduction in acetylcholinesterase (AChE) inhibition. Similar high toxicity at lower temperatures has also been reported for lizards (*Anolis carolinensis*) exposed to pyrethrins at 20 and 35°C (Talent 2005). However,

increasing the water temperature for *Daphnia magna* from 20 to 27°C caused an increased toxicity and the LC_{50} fell by factors of 1.7 and 9.9 with imidacloprid and aldicarb respectively, whereas as the same treatment of mosquito larvae (*Aedes aegypti*) did not result in changes in LC50 (Song et al 1997). The 96-h EC_{50} values of pesticide chlopyrifos for silver perch (*Bidyanus bidyanus*) were significantly higher at 15°C than other temperatures (20, 25, 30 and 35°C) (Patra et al 2015). Similarly, the 96 h LC_{50} value of pesticide sumithion for zebrafish (*Danio rerio*) was significantly higher at 25°C than at 35°C (Shahjahan et al 2019). It indicates that the toxicity of these pesticides increases at higher temperatures.

High temperature and low DO (30°C, 1.5-3 mg L^{-1} respectively) increased the toxicity of the organophosphate profenofos to the fathead minnows *Pimephales promelas* by 15 times in comparison to normal conditions (20°C, 6-9 mg L^{-1} respectively) (Baer et al 2002). Increased AChE inhibition has also been reported during exposure to the organophosphate dichlorvos under DO depletion in the rainbow trout (*Oncorhynchus mykiss*) (Høy et al 1991).

Since snakehead fish *C. striata* is an obligatory air-breathing fish this animal can change respiratory strategy during exposure to toxins in the water. Natarajan (1981a) has been shown to significantly increase its oxygen uptake from the air and decrease uptake from the water during exposure to the 48-h LC_{50} concentration of metasystox, a concentration where prolonged exposure also resulted in gill damage (Natarajan 1981b). The species has also been found to shift to a greater frequency of air-breathing at a concentration 100 times lower than the 96-h LC_{50} of diazinon (Cong et al 2008).

The sensitivity of brain cholinesterase (ChE) to organophosphates and carbamates has been used as a biomarker for diagnosing exposure and effects from these insecticides (Mayer et al 1992; Peakall 1992). However, it is unclear whether toxicity in the field is greater or smaller than that found in the laboratory (Heugens et al 2001). In any case there are many environmental parameters that can influence the toxicity of a chemical, including physical, chemical and biological as well as interaction among them.

It has been predicted that surface temperatures will increase by 2°C by the end of this century (Jan et al 2017). Across large regions of the world, surface temperatures grew by 1.09°C from 2011 to 2020 – this was greater than the increase in surface temperatures from 1850-1900 (IPCC 2023). In Vietnam, temperature was seen to increase from 0.11 to 0.33°C per decade (Phan & Nguyen 2023). Under intensive rice cultivation (three crops a year) and heavy use of agrochemicals (5-8 applications per crop) in the MRD (Toan & Cong 2018), it is important to understand the effects of these parameters on ChE inhibition during exposure to quinalphos. These environmental factors were studied within the range seen in the rice fields of the MRD (Vromant et al 2001; Tam et al 2018).

Material and Method

Tested animals. Larval snakehead fish were collected in pesticide-free ponds in the MRD. They were raised in the fiberglass tanks as previously mentioned (Cong et al 2024). Larvae were stocked at a density of 200 individuals per tank. They were fed red-worms (*Tubifex* sp.) daily at about five percent of total live fish weight for a month. After which they were fed fish paste until experimentation. The tanks were continuously aerated and cleaned daily before feeding and at about four hours after feeding to remove uneaten food and feces by changing about 50% of the water. Feeding was stopped on the day of experimentation.

Chemicals. Commercial trade name, Kinalux 25EC containing 25% quinalphos by weight was purchased from Loc Troi Joint Stock Company, Vietnam.

Experimental designs. A full factorial randomly blocked design was used to test three temperature levels (24, 30 and 34°C) and two dissolved oxygen levels (1.8-0.2 and 6.5-

0.7 mg L^{-1}) and two treatments, namely with quinalphos at 2.45 µg L^{-1} and without quinalphos. The experiment was conducted in a room at a constant temperature of 24°C. Jager water heaters (Aquarium Regler – Heizer, TSRH 50, Germany) were used to control the water temperature in glass aquaria at 30°C, whereas a water bath (Memmert WB 14, Schutzart DIN, Germany) was used to maintain the temperature at 34°C. Air-pumps (Boss 9500, 4-4.5 W, Guangdong Zhenhua Electrical Appliance, China) were used to aerate water to give the high dissolved oxygen (HDO) (6.5-0.7 mg L^{-1}) and to maintain the low DO water, N_2 was bubbled through the water.

Twelve 1-L glass conical flasks were used to conduct the experiment. The 1-L glass conical flasks were filled with either exactly 700 mL chloride-free tap water for the control group or 700 mL quinalphos solution (2.45 µg L^{-1}) for the group undergoing quinalphos treatment. Afterward, DO was regulated by air pumps or nitrogen gas to meet the required conditions. One juvenile snakehead fish (live weight 5.2-0.8 g) was placed into each flask. Temperature and DO were checked hourly using a DO meter (Orion 830A basic dissolved oxygen meter, Thermo, Beverly, MA, USA). After six hours of exposure, the individual fish was killed on ice and then processed for ChE assay. Briefly, each temperature level has four flasks. Two of them (one for control and one with 4.9 µg L^{-1} of quinalphos) were high dissolved oxygen (HDO) and two of the others were low dissolved oxygen (LDO). Nine replications were conducted for each treatment.

Cholinesterase assay techniques. The whole process was carried out on ice. Before dissecting out the whole brain, the fish was weighed individually (BP410S, Sartorius, Goettingen, Germany) and its total length was measured including the tail fin. The whole brain was dissected out and placed in a pre-weighed Eppendorf tube before measurement of brain weight (BP410S, Sartorius, Goettingen, Germany). The whole brain was then homogenized and diluted in 0.1 M phosphate buffer (pH 7.4, prepared by mixing mono and dibasic sodium hydrogen phosphate) at concentration of approximately 30 mg fresh tissue per milliliter using a glass homogenizer (Uniform, Jencons PLC, Leighton Buzzard, UK). One milliliter of homogenates was transferred into an Eppendorf tube and kept on ice until centrifuging. The homogenizer was rinsed with acetone and distilled water before proceeding to the next brain. The homogenate was centrifuged at 2,000 rpm and 4°C for 20 minutes (Sartorius, Sigma 3-18k, Goettingen, Germany). The supernatant was removed to an Eppendorf tube and kept on ice for ChE analysis within 4 hours.

ChE activity was measured according to the method described by Ellman et al (1961) and modified by Thao et al (2023). For each measurement, a cuvette was prepared containing 2.65 mL of the 0.1 M phosphate buffer (pH 7.4), 0.1 mL of 3 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB; Sigma Aldrich, Germany) then immediately before measurement, 0.05 mL of 10 mM acetylthiocholine iodide (Sigma Aldrich, Germany) and 0.2 mL of the supernatant were added, and the solution was mixed well. Blanks were prepared with 0.2 mL of the 0.1 M phosphate buffer (pH 7.4) instead of supernatant. ChE activity was detected using a spectrophotometer (UV 2900, Hitachi, Japan) for 200 s at a wavelength of 412 nm. The results of these measurements were expressed as a rate (Delta absorbance per minute) and the ChE activity calculated.

Data analysis. A general linear model was applied to determine the sources of effects on brain ChE activity. One way analysis of variance and Tukey pairwise comparisons were used to determine the effect of temperature, whereas Student's T test was used to compare ChE activity between the two levels of DO. Data were checked for normality and variance homogeneity before doing comparisons.

Results. The general linear model showed that DO and the interaction between DO and temperature had no significant effect on the brain ChE activity of the snakehead ($p > 0.7$), whereas temperature, quinalphos and the interaction between the two significantly affected the brain ChE activity ($p < 0.01$) (Table 1).

Table 1

Summary univariate analysis of variance for sources of effects on brain ChE

Source	*Type III SS*	*df*	*MS*	*F*	*Sig.*
Quinalphos	1091.1	1	1092.1	592.6	0.000
DO	0.1	1	0.1	0.1	0.776
Temperature	84.5	2	42.3	23.0	0.000
Quinalphos*DO	14.0	1	14.0	7.5	0.007
Quinalphos*Temperature	71.1	2	35.6	19.1	0.000
DO*Temperature	0.0	2	0.0	0.0	0.990
Quinalphos*Temperature*DO	21.1	2	10.55	5.72	0.004
Error	174.7	95	1.84		

R squared = 0.8.

Further statistical analysis showed that the temperature, DO, and the interaction between them had no effect on ChE activity in control fish ($p > 0.05$) (Figure 1). However, the high temperatures caused significantly more severe ChE inhibition in the fish exposed to 2.45 µg L^{-1} quinalphos. Brain ChE activity was 7.21 µM g^{-1} min^{-1} (35.0% inhibition) and 7.18 µM g^{-1} min^{-1} (37.6% inhibition) at 24°C for LDO and HDO, respectively; and decreased further to 3.05 µM g^{-1} min^{-1} (73.5% inhibition) and 4.2 µM g^{-1} min^{-1} (62.7% inhibition) at 30°C for LDO and HDO, respectively. At 34°C ChE had reduced 2.46 µM g^{-1} min^{-1} (78.7% inhibition) and 2.86 µM g^{-1} min^{-1} (74.8% inhibition) in high and low DO respectively (Figure 1).

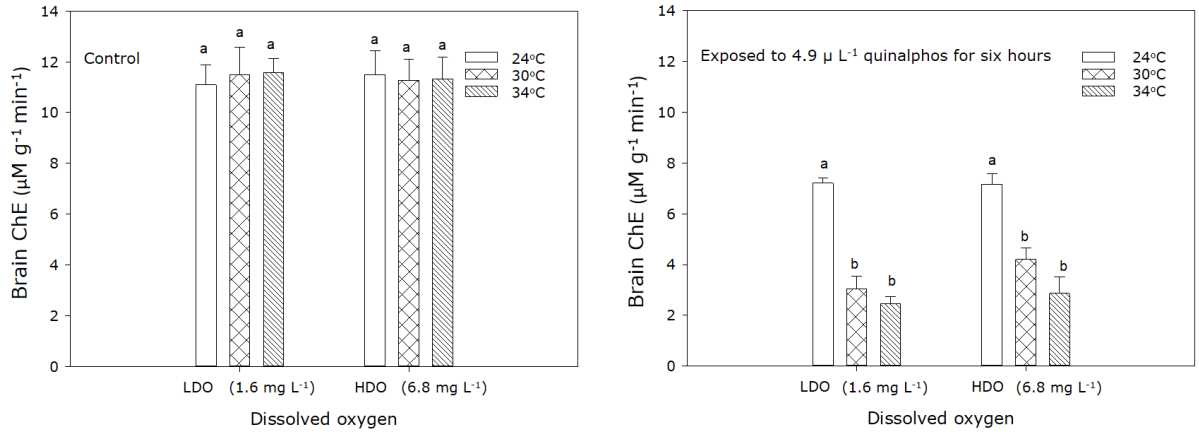

Figure 1. Effects of temperature and dissolved oxygen on brain cholinesterase activity of juvenile snakehead (*Channa striata*) exposed to quinalphos. In each level of dissolved oxygen, the same letter indicates no significant differences among temperatures ($p > 0.05$).

Discussion. We found that the toxicity of quinalphos in terms of its inhibitory effect on ChE activity in the snakehead doubled with a 10°C temperature rise. This suggests that the temperature fluctuations occurring in rice fields of the Delta have a significant impact on the species when the pesticides are applied.

Increasing temperature is usually associated with changes in fish metabolism. The metabolic rate typically increases 2 to 3 times for every 10°C rise of temperature (Jensen et al 1993). Metabolic rates in terms of energy units can be quantified in terms of oxygen consumption (Kutty & Mohamed 1975; Jobling 1993). Therefore, increased metabolism entails increased oxygen consumption. This increased oxygen consumption with increasing temperature has been shown in a variety of aquatic organisms including the air-breathing Chinese snakehead fish *Channa argus* (Liu et al 2000). In the Mekong snakehead *C. striata*, Qin et al (1997) showed that the metabolic rate of snakehead fish also increased continuously to 37°C using a bioenergetics model. Consequently, in contaminated water, toxicants may be absorbed into the body to a greater extent

together with oxygen as the metabolic rate increases. Using ^{14}C, Jimenez et al (1987) showed that the uptake rate, metabolites, and bioconcentration factor (BCF) of polycyclic aromatic hydrocarbon benzo(a)pyrene in bluegill sunfish *Lepomis macrochirus* increased 2.7, 4, and 1.6 times, respectively when temperature was increased from 13°C to 23°C. Similarly, ingestion rate also significantly increased from 14 to 24°C in *D. magna* and resulted in a statistically higher uptake rate of ion mercury (Hg^{2+}) or methyl mercury (MeHg) (Tsui & Wang 2004). In the present study, we did not measure quinalphos uptake into the body of snakehead fish. However, the doubled brain ChE inhibition over the 10°C temperature rise (24 to 34°C) is probably a result of greater quinalphos uptake from the water.

As is the case with a temperature increase, hypoxia also increases the respiration rate or a shift from water-breathing to air-breathing (in air-breathing fish) to obtain enough oxygen for the requirements of the body (Jensen et al 1993). This also leads to greater gill ventilation and greater toxicant absorption in fish without air-breathing capability. This has been found in rainbow trout exposed to organophosphate dichlorvos; AChE inhibition was greater under DO depletion (3 mg L^{-1}) than high DO (15 mg L^{-1}) (Høy et al 1991). However, air-breathing fish can shift from water-breathing to air-breathing in hypoxic and contaminated water. Affonso & Rantin (2005) found that the air-breathing fish *Hoplosternum littorale* significantly increased the air-breathing frequency under hypoxic conditions (5 mm Hg) compared to normal conditions (130 mm Hg). This increase in air-breathing resulted in a reduction in the toxicity of sulfide measured as the 17-18 h LC_{50} from 70 to 87 µM for dissolved oxygen of 130 mm Hg and 5 mm Hg, respectively. In another air-breathing fish, *Clarias macrocephalus*, Kulakkattolickal & Kramer (1998) found that during exposure to a toxic extract from *Croton tiglium* (Euphorbiaceae) seeds, the fish increased their air-breath frequency in both normal (8.1±0.4 mg O_2 L^{-1}) and hypoxic (0.7±0.1 mg O_2 L^{-1}) conditions, increasing their survival times. These data infer that air-breathing fish can avoid exposure to toxicants by altering their respiratory strategy. Indeed a significant increase in surfacing activity was seen in the air-breathing fish *Mystus vittatus* (Arunachalam et al 1980), and *Macropodus cupanus* (Arunachalam & Palanichamy 1982) during long-term exposure to the ChE-inhibiting carbaryl. This increased surfacing frequency was also seen in the air-breathing snakehead fish *C. striata* in 0.008 mg L^{-1} of diazinon during a 5 hours exposure in an aeration system (Cong et al 2008). Snakehead fish have also been shown to significantly increase air oxygen consumption by a factor 1.3 while reducing oxygen uptake from the water by the same factor under a 48-h LC_{50} exposure to the pesticide Metasystox compared to controls (Natarajan 1981a). This concentration actually also caused gill damage (Natarajan 1981b). It indicates that the snakehead can shift to air-breathing during water contamination. These findings may explain why DO had little effect on ChE inhibition in the present study.

Conclusions. Findings from this study indicated that temperature caused significantly more effects of pesticide quinalphos for snakehead fish at 34°C than at 24°C while no such impact was seen with dissolved oxygen. With the trend of global warming and extreme fluctuation of weather, current rice farming practices in the MRD would cause more health risk for snakehead fish in ricefield habitat. Field investigation is recommended to confirm the prediction.

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

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