

Some biological parameters and cholinesterase enzyme profile of *Tilapia* sp. along Maragondon River, Cavite, Philippines

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ABSTRACT

This study evaluated *Tilapia* sp.'s biological parameters and cholinesterase enzyme activity along the Maragondon River. The biological parameters assessed were length-weight relationship and condition factor. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were measured in the brain, muscle, and hepatic tissues of *Tilapia* sp. Enzyme inhibition rates were then calculated at midstream and downstream stations relative to the reference site upstream. Results showed that *Tilapia* sp. exhibited negative allometric growth patterns ($b < 3$), supported by high correlation coefficients (0.86-0.94). The condition factor (K) values across sampling sites ranged from 1.94 to 3.82, indicating the overall fitness of *Tilapia* sp. However, AChE and BChE enzymes above the 20% threshold were observed at midstream and downstream stations of the river. Specifically, 49.03% and 48.41% inhibition in AChE and BChE of muscle tissue in midstream samples, 22.03% inhibition in the liver and 31.53% inhibition in muscle AChE at downstream station. The cholinesterase tissue localisation was also inferred, arranged from highest to lowest activity as follows: liver > brain > muscle. These findings provide valuable insights into the exposure of *Tilapia* sp. to cholinesterase inhibitors in Maragondon River, emphasising the importance of biomarkers in assessing the effect of environmental contaminants on aquatic organisms.

Keywords: Cholinesterase, Biomarkers, Pollution, Tilapia

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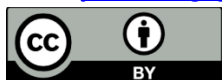
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Introduction

Freshwater ecosystems are sources of various biodiversities; they provide provisioning and regulating services but have been increasingly threatened in recent years due to overwhelming anthropogenic pressures. For instance, the intensified unsustainable agricultural production methods have made agriculture a prominent source of aquatic pollution (Catajan et al., 2023; Harisson et al., 2019; Liu et al., 2021; Zang et al., 2021). Pesticides have emerged as a major concern of all organic pollutants due to their extensive production and utilisation in agricultural countries and their high toxicity to non-target organisms (Neuwirthová et al., 2019). The unintentional release of pesticides into the environment often leads to adverse ecological impacts, affecting unintended organisms, such as fish (Mancini et al., 2019; Neuwirthová et al., 2019; Shah & Parveen, 2022). Some common pesticides are organophosphate (OPs), carbamates, and synthetic pyrethroids.

Organophosphates (OPs) and carbamates are major agrochemicals that strongly affect different neuroenzymes and the growth of various fish species (Ghazala et al., 2014). Fish are directly exposed to these pesticides by absorption through the skin, breathing, and oral intake of pesticide-contaminated water or pesticide-contaminated prey (Stanley & Preetha, 2016). Fish exposed to pesticides often experience alterations in haematological parameters and stress biomarkers (Santana et al., 2022). A notable biomarker in this organophosphate and carbamate exposure is the evaluation of cholinesterase (ChE) enzyme activities (Sepahi et al., 2023; Kaur et al., 2023). The cholinesterases (ChE) group of enzymes has been divided into two types: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Ghazala et al., 2014). AChE is a cholinergic enzyme mostly found in postsynaptic neuromuscular junctions and is important in removing acetylcholine. BChE catalyses the hydrolysis of esters of choline, including acetylcholine. The inhibitory effect of pollutants on cholinesterase may lead to acetylcholine accumulation at the synaptic cleft, disrupting normal neural transmission and causing paralysis and, ultimately, death (Colović et al., 2013).

As an agricultural country, the Philippines has relied on organophosphate (OPs) pesticides, commonly carbamates, and synthetic pyrethroids (Lu, 2022; Manuben et al., 2022). In addition, chlorinated pesticides, including aldrin, dieldrin, endrin, and heptachlor, were prevalent from the 1960s until the early 1980s (Santiago & Kwan, 2016). Following the recognition of the adverse impact of these substances on human health, the country prohibited several Persistent Organic Pollutants (POPs) in agricultural and pest control practices. By

1989, five out of twelve POPs had already been banned. Concurrently, the Department of Environment and Natural Resources (DENR) issued administrative order no. 2004-01 to regulate and eradicate PCBs nationwide. Additionally, the Philippines banned the use, sale, and import of chlorpyrifos and dichlorvos, two types of OPs, in accordance with Republic Act 19711. However, despite these regulations, the widespread utilisation of organophosphates, carbamates, and pyrethroids in agricultural pesticide applications continues to contaminate the environment and pose health risks to humans (Lu, 2010). Multiple studies have corroborated the presence and usage of POPs in various locations in the Philippines (Hallare et al., 2005; Carvalho et al., 2009; Santiago & Kwan, 2016; Villanueva et al., 2010).

The Maragondon River, situated in Cavite, Philippines, receives runoff from diverse agricultural areas throughout Maragondon, a predominantly agricultural town with the largest land area, cultivating a wide range of commodities (CLWUP-Maragondon, 2013). Studies have been conducted to evaluate the Maragondon River. Jalandon (2018) reported heavy metals such as Pb and Cu Maragondon River levels. Another study (Pareja, 2015) highlighted the high nitrogen and phosphorus loads from households and urban runoff due to inadequate sewage treatments. Despite existing reports on contaminants like heavy metals and urban runoff, there is a notable absence of published studies investigating persistent organic pollutants in the Maragondon River. Given that the Maragondon River receives runoff from agricultural sources and considering the lack of research on cholinesterase inhibitors such as organophosphates and carbamates in the river, our study aimed to evaluate the exposure of *Tilapia* sp. to these contaminants using cholinesterase enzyme activity as a biomarker. This study provides baseline data on the aquatic health of the Maragondon River by evaluating the physiological response of fish populations in the ecosystem.

Materials and Methods

Site Description

Tilapia sp. were collected from the Maragondon River, a significant river basin in Cavite, Philippines. This river runs through the upland barangays (administrative districts) of Maragondon, culminating at Ternate, Cavite, where it empties to Manila Bay. This study divided the river into three sampling stations: upstream, midstream, and downstream.

In the Philippines, inland waters are categorised into different classes: AA, A, B, C, and D (DAO, 1994). The upstream station (N14°14'44.9" E120°46'55.6") is classified as Class B. It

features a rocky substrate, abundant vegetation, and a width of 18-20 m. Class B waters have good to excellent water quality, with allowable treated wastewater discharges. The mid-stream station (N14°16'23.3" E120°44'5.3") has an estimated 82-87 m width. Lastly, the downstream station (N14°17'01.6" E120°42'51.1") spans an estimated 132-135 m width. Both midstream and downstream stations are classified as Class C and are used for agriculture and aquaculture, where water quality may be impaired.

The samples from the upstream station were considered reference samples, given that they were collected from Class B water, which is characterised by excellent water quality.

Fish Sampling and Analyses

Juvenile tilapia samples (N=48) were collected from the three stations along the Maragondon River. Haphazard and random seine net collection was conducted, with samples not segregated by sex. This study's use of tilapia fish samples was predicated on its wide distribution and commercial significance in the Philippine waters (Guerrero, 2022). The sampling was conducted during the Philippines' dry season (April 2023). To account for the possibility of interspecific hybridisation and the lack of specificity in the feral fish samples' gene pool, the tilapia samples in this paper were referred to as *Tilapia* sp. Following capture, the fish were anaesthetised and then carefully packed in ice. The specimens were transported to the Fish Diseases and Toxicology Laboratory, Cavite State University Naic, for analysis. In the laboratory, the biometric data, including total length (TL, cm) and wet weight (W, g), were examined and recorded. The TL (cm) measurements were taken from the tip of the mouth to the tip of the longer lobe of the caudal fin using a digital calliper, whereas the W (g) was measured up to 0.0001g accuracy using an analytical weighing balance. Additionally, each fish was dissected, and the hepatic tissues, brain, liver, and cut of muscles were separated. Tissues were then stored in a -18 °C laboratory freezer for subsequent analysis. Samples were analysed within 7 days of storage to avoid enzyme degradation.

Length-Weight Relationship and Condition Factor

The length-weight relationship (LWR) was estimated by using the equation. $W = aL^b$, where W = body weight (g), L = total length (cm), a is a scaling constant, and b is allometric growth. A logarithmic transformation ($\log W = \log a + \log b L$) was used to make the relationship linear. A regression analysis estimated the intercept ($\log a$) and the regression coefficient or slope (b). Meanwhile, condition factor (K) was calculated following a previously defined equation as $K = \frac{W}{L^3} \times 100$. Where

by K = condition factor; W = the weight (g); L = the total length of the fish (cm). Isometric growth in a fish species is identified when the estimated regression coefficient value approaches 3. Conversely, if the coefficient deviates from 3, fish growth is categorised as either negative allometric ($b < 3$) or positive isometric ($b > 3$) (Froese, 2006).

Cholinesterase Enzyme Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity was determined using the rapid colourimetric method following a previously established standard (Ellman et al., 1961). Briefly, *Tilapia* sp.'s brain, liver, and muscle tissues were homogenised in phosphate buffer (pH 8.0) at a concentration of approximately 20 mg/mL. A 0.4 mL aliquot of the homogenate was mixed with 2.6 mL phosphate buffer, followed by 100 μ L of dithiobisnitrobenzoic acid reagent. Absorbance was then recorded at 412 nm. Subsequently, either acetylthiocholine iodide (AChE) or butyryl thiocholine chloride (BChE), in a volume of 20 μ L, was introduced as substrate. The enzyme activity, as indicated by the hydrolysis of acetylthiocholine and S-butyrylthiocholine, was monitored at 412 nm for 7 minutes. The inhibition rate of cholinesterase activity was then calculated using the equation:

$$[(A_{rs} - A_{ss})/100] \times 100$$

Where büyük harf A. alt r s is the absorbance of the reference site (upstream), and A_{ss} is the absorbance of sampling sites midstream and downstream.

Statistical Analyses

All data in this study were presented as means \pm standard deviation unless otherwise specified. One-way analysis of variance (ANOVA) was conducted to determine significant differences in AChE and BChE enzyme activities in different tissues of *Tilapia* sp. Subsequently, Tukey's honest significant difference (HSD) post hoc test was employed to identify statistically significant differences. In all cases, a significance level of $p < 0.05$ was considered. All analyses were conducted using Microsoft 365 software under appropriate licensing.

Results and Discussion

Length-Weight Relationship and Condition Factor

Tilapia sp. samples of larger size were obtained from the mid-stream site, exhibiting a mean length of 19.13 ± 2.63 cm and a mean weight of 135.80 ± 50.46 g. Subsequently, downstream samples displayed a mean length and weight of 94.96 ± 33.53 cm and 16.76 ± 2.09 g, while upstream samples were notably smaller, measuring 10.68 ± 1.61 cm in length and weighing 46.64 ± 10.90 g (Table 1).

In terms of growth patterns, *Tilapia* sp. displayed a negative allometric pattern along the Maragondon River, with *b* values of 2.45 (Upstream), 2.59 (Midstream), and 2.72 (Downstream). Regarding the condition factor, only *Tilapia* sp. samples collected from the upstream fell within the ideal range of *K* values, ranging from 2.90 to 4.80, with a value of 3.82. In contrast, the condition factors of samples from the midstream and downstream were outside the ideal range, with values of 1.94 and 2.01, respectively (Table 1).

The observed allometric growth pattern, where the fish become more rotund as their length increases, is supported by the high correlation coefficients (r^2) ranging from 0.86 to 0.94 across the sampling sites (Figure 1A-1C). This indicates a strong relationship between length and weight, confirming the negative allometric growth pattern.

Table 1. Descriptive statistics and some biological parameters of *Tilapia* sp. were collected from different sampling sites in the Maragondon River, Cavite, Philippines.

Sampling Site	a	b	r^2	Mean Length (cm)	Mean Weight (g)	Condition Factor
Upstream	-2.60	2.45	0.94	10.68 ± 1.61	46.64 ± 10.90	3.82
Midstream	-2.76	2.59	0.86	19.13 ± 2.63	135.80 ± 50.46	1.94
Downstream	-3.13	2.71	0.90	16.76 ± 2.09	94.96 ± 33.53	2.01

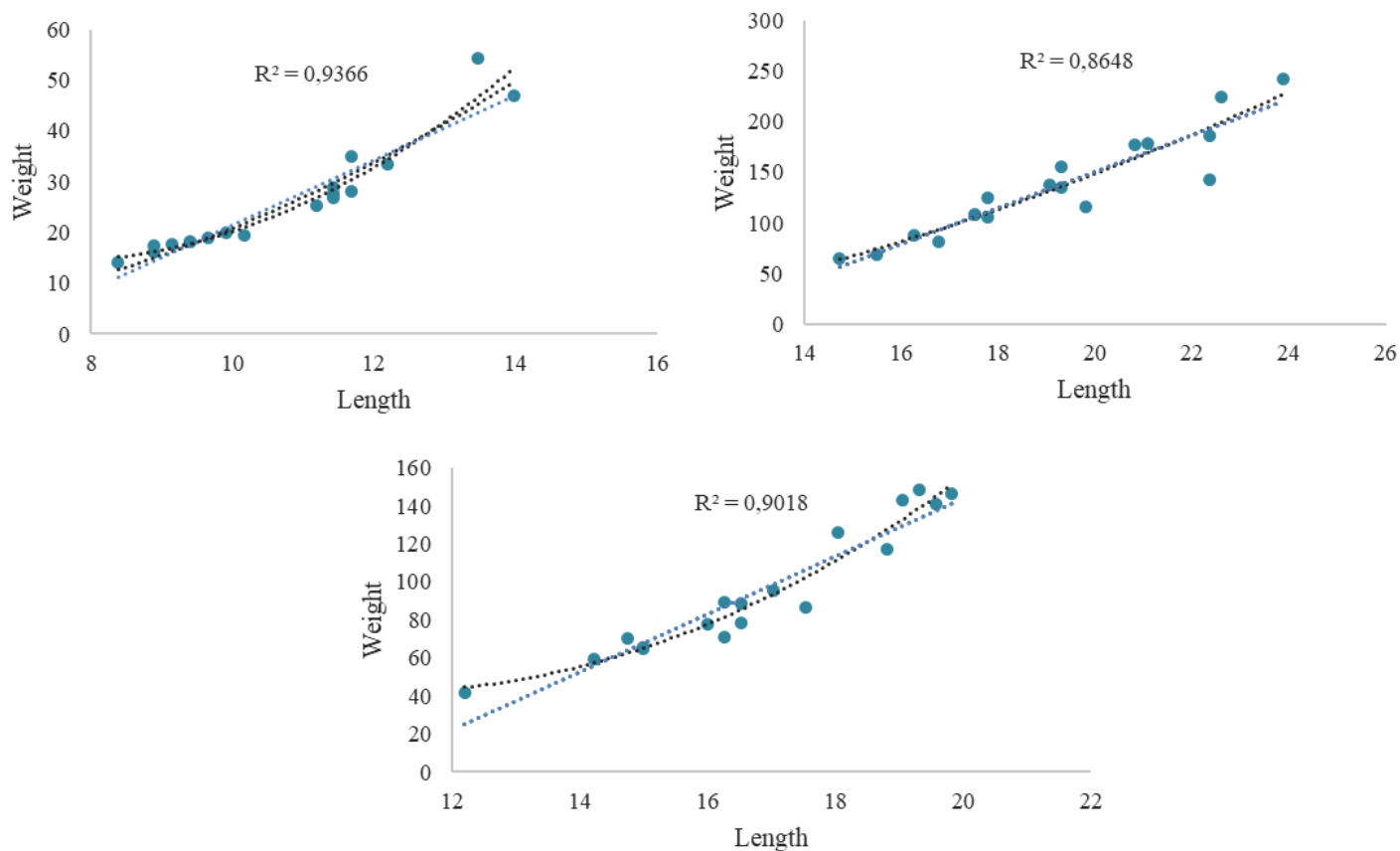


Figure 1. Length-weight relationship of *Tilapia* sp. along Maragondon River during warm months. (a) Upstream (b) Midstream (c) Downstream.

Cholinesterase Enzyme Activities of *Tilapia sp.* along Maragondon River

Brain, liver, and muscle cholinesterase activities expressed in mol min⁻¹ g⁻¹ tissue of *Tilapia sp.* obtained from three sampling sites are presented in Figure 2.

The brain acetylcholinesterase (AChE) activity in *Tilapia sp.* was comparable along the Maragondon River. The mean AChE activity in samples from the upstream was 1.039 ± 0.405 mol min⁻¹ g⁻¹, comparable with mean values of 0.87 ± 0.179 in the midstream station and 0.992 ± 0.402 mol min⁻¹ g⁻¹ downstream. On the other hand, the butyrylcholinesterase (BChE) enzyme activities in the brain of *Tilapia sp.* exhibited a different pattern. The midstream shows higher BChE activity, with a mean value of 1.024 ± 0.327 mol min⁻¹ g⁻¹, compared to both the upstream (0.969 ± 0.418 mol min⁻¹ g⁻¹) and downstream (0.780 ± 0.306 mol min⁻¹ g⁻¹).

In the liver tissue, the enzyme activity of *Tilapia sp.* from the Maragondon River is lower in midstream than upstream and downstream (Figure 3). In midstream, the enzyme activity of AChE is 0.276 ± 0.276, and BChE is 0.712 ± 0.427 mol min⁻¹ g⁻¹, which is lower compared with 0.889 ± 0.351, 0.914 ± 0.364, and 1.312 ± 0.50, 1.022 ± 0.23 mol min⁻¹ g⁻¹ of upstream and downstream.

In muscle tissue, AChE enzyme activity is higher upstream of the river with a mean value of 0.151 ± 0.083 mol min⁻¹ g⁻¹ compared with 0.077 ± 0.038 and 0.078 ± 0.066 mol min⁻¹ g⁻¹ of midstream and downstream, respectively. Meanwhile, for BChE enzyme activity, higher mean values were observed in midstream with a mean value of 0.078 ± 0.085 mol min⁻¹ g⁻¹ compared with 0.055 ± 0.033 and 0.034 ± 0.040 mol min⁻¹ g⁻¹ of upstream and downstream.

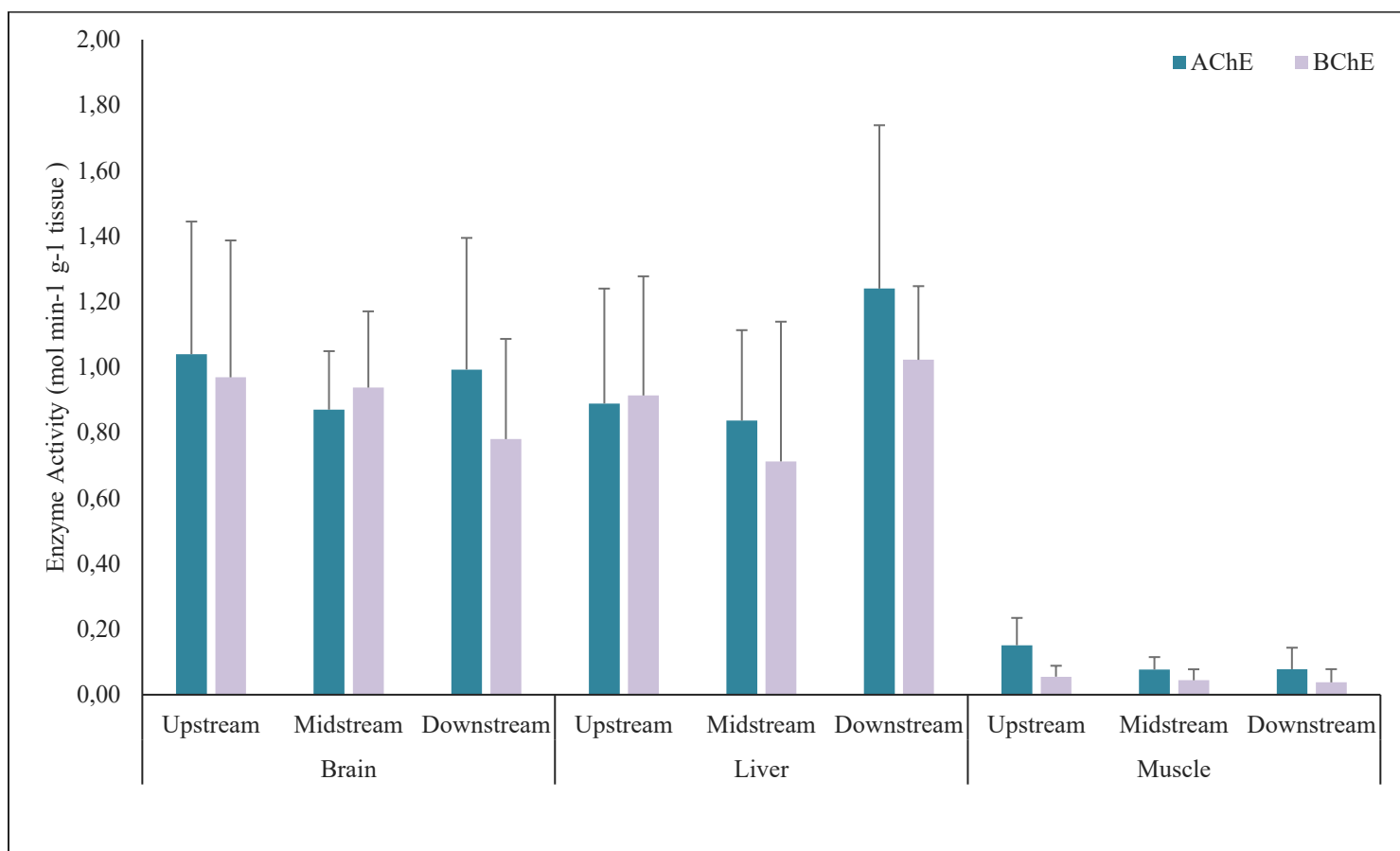


Figure 2. Acetylcholinesterase and butyrylcholinesterase enzyme activities in different tissues of *Tilapia sp.* collected along the Maragondon River.

Inhibition Rates of Cholinesterase Enzyme Activity

The differences in enzyme activities between midstream and downstream samples were expressed as inhibition rates relative to the upstream station (Table 2). The highest inhibition rates for AChE and BChE were found in the muscle of midstream samples, measuring 49.03% and 48.41%, respectively. This was followed by the muscle in the downstream station, exhibiting inhibition rates of 19.96% and 31.53%. Meanwhile, negative inhibition rate values were observed in BChE activities in both midstream (-39.14%) and downstream (-11.91%) samples, indicating that the enzyme activities were higher than those in the upstream station.

Tissue Distribution of Cholinesterase Enzyme in *Tilapia sp.*

The distribution of cholinesterase enzymes in different tissues of *Tilapia sp.* exhibited significant differences. AChE and BChE enzyme activities were significantly higher in the hepatic and brain tissues than in the muscular tissue of *Tilapia sp.* (Figure 3). In the hepatic tissue, the AChE enzyme activity was measured to be $1.01 \pm 0.43 \text{ mol min}^{-1} \text{ g}^{-1}$, while in the brain tissue, it was $0.92 \pm 0.34 \text{ mol min}^{-1} \text{ g}^{-1}$. These values were significantly higher than the AChE activity observed in the muscular tissue, which was only $0.10 \pm 0.07 \text{ mol min}^{-1} \text{ g}^{-1}$. Similar patterns were observed for BChE enzyme activities. The mean values of BChE activity in the hepatic tissue and brain were $0.88 \pm 0.36 \text{ mol min}^{-1} \text{ g}^{-1}$ and $0.92 \pm 0.34 \text{ mol min}^{-1} \text{ g}^{-1}$, respectively. These values were significantly higher than the BChE activity observed in the muscular tissue, which was $0.06 \pm 0.06 \text{ mol min}^{-1} \text{ g}^{-1}$ ($p < 0.01$).

Table 2. Inhibition rate of acetylcholinesterase and butyrylcholinesterase in brain, liver, and muscle of *Tilapia sp.* collected in Maragondon River.

	Midstream		Downstream	
	AChE	BChE	AChE	BChE
Brain	16.29	4.50	3.24	19.47
Liver	5.85	-39.14	22.03	-11.91
Muscle	49.03	48.41	19.96	31.53

* The values represent the inhibition rates (%) for AChE and BChE activities in different tissues of *Tilapia sp.*

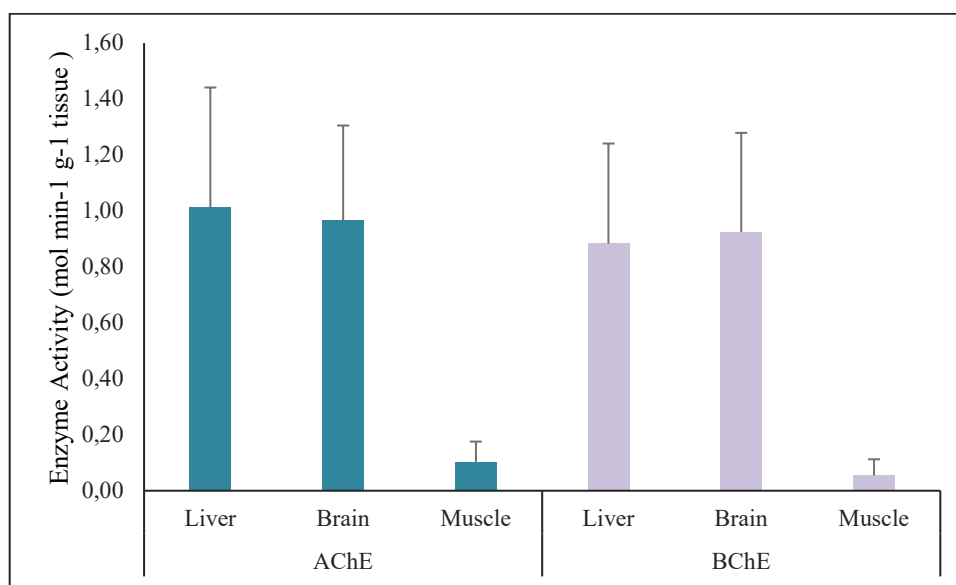


Figure 3. Tissue distribution of cholinesterase enzymes in *Tilapia sp.*

The length-weight relationship (LWR) provides valuable information for studying the growth dynamics of fish populations. Factors affecting differences in LWR among species include variations in environmental conditions, health of fish, food availability, and spawning period (Suquet et al., 2005). Moreover, the growth patterns of fish may be linked to the productivity of the aquatic habitat (Przybylski, 1996; Randall & Minns, 2000; Randall, 2002). In the current study, the LWR regression slope (b) value of *Tilapia* sp. samples along the Maragondon River continuum demonstrated a negative allometric growth pattern; fish grew with weight increasing at a slower rate ($b < 3$) compared to their length. This result is in line with previous studies which also reported negative allometric growth patterns in different cichlid species (Peña Messina et al., 2010; Dalu et al., 2013; Zuh et al., 2019; Abdalla et al., 2023). The negative allometry observed in samples from the Maragondon River, congruent with previous reports, indicates heterogeneity, with body weights varying non-uniformly about the cube of total length (Kwikiriza et al., 2023).

The condition factor (K), on the other hand, reflects a species' robustness and overall health, specifically indicating its feeding status, health, sexual maturity, and adaptability to its environment. Fish species with a K value near or equal to one are generally considered to have a good overall condition (Datta et al., 2013). Moreover, K values above one indicates that fish species are adequately fed and thriving in optimal environmental conditions. In this study, K values were observed to be above the ideal value in the following order: upstream > downstream > midstream. These K values suggest that the Maragondon River provides favourable conditions for the growth of *Tilapia* sp.

Despite the good environmental conditions indicated by both the LWR and K values of *Tilapia* sp. in the Maragondon River, inhibition of cholinesterase enzyme activities was observed. A 20% inhibition of cholinesterase enzymes suggests organismal exposure to anticholinesterase compounds (Menéndez-Helman et al., 2015; Fajardo & Ocampo, 2018). In this study, significant inhibition was observed in midstream and downstream samples. In midstream samples, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were inhibited in the muscle, with inhibition rates of 49.03% and 48.41%, respectively. Meanwhile, in downstream samples, significant AChE inhibition was observed in the liver at 22.03%. AChE and BChE were also inhibited in the muscles of fish collected downstream, with 19.96% and 31.53% rates.

Cholinesterase enzyme activity is used as a biomarker for assessing neurotoxicity caused by organophosphates and carba-

mates (Sepahi et al., 2023; Kaur et al., 2023). Although cholinesterase, including AChE and BChE are considered a specific neurotoxic biomarker of organophosphorus and carbamates exposure, several studies also describe an alteration of their activities by other pesticides in fish including fungicides (Melefa & Nwani, 2021; Kovačević et al., 2023), synthetic pyrethroids (Martins-Gomes et al., 2022) and glyphosate (Bernal-Rey et al., 2020; Thanomsit et al., 2021; Cuzziol Boccioni et al., 2022). Due to the lipophilic characteristics of pesticides from the pyrethroid family, they may interact with the active site, causing inhibition of enzymatic activity (Martins-Gomes et al., 2022).

In Maragondon River, where inhibition of cholinesterase enzyme activity was observed in *Tilapia* sp. samples, there is a high likelihood that some, if not all, of these contaminants are present. Although the concentration of anticholinesterase compounds is still unknown, the midstream station is hypothesised to have the most contaminant levels, as suggested by the results of this study. This is also supported by the results of Jalandoon (2018), who found that midstream stations have the highest amount of nitrates and phosphates. Moreover, the phosphate concentration exceeded the set standards at 0.5-1.0 mg/L limit. The highest cholinesterase enzyme inhibition observed in the midstream station of the river might be attributed to the nearby farmlands, where pesticides and fertilisers might run off into the river.

The tissue distribution of cholinesterase enzymes can also be inferred from the results of this study. AChE and BChE tissue localisation patterns are arranged from highest to lowest enzyme activity as follows: liver > brain > muscle. The significant abundance of cholinesterase enzymes in the liver and brain compared to muscle can be attributed to their physiological functions. The abundance of cholinesterase activity in brain tissues can be attributed to its role in signal termination at cholinergic synapses through the rapid hydrolysis of the neurotransmitter acetylcholine. Cholinesterase activity is also abundant in the liver tissue of *Tilapia* sp. because these enzymes are synthesised in the liver (Weber et al., 1999). Moreover, cholinesterase enzymes play a role in detoxification. Many organophosphates require desulfuration (bioactivation) by cytochrome P450 (CYP) into their active oxon form to effectively inhibit cholinesterase in the liver (Sams et al., 2004; Ellison et al., 2012). Cholinesterase enzyme was also observed in the muscular tissue of *Tilapia* sp., albeit statistically lower compared to liver and brain enzyme activity. The presence of cholinesterase in muscular tissue is primarily due to cholinergic innervation (Lopes et al., 2014)

This study provides baseline information on the exposure of *Tilapia* sp. to anticholinesterase contaminants, such as organophosphates and carbamates, in the Maragondon River. It is noteworthy that although the K value of *Tilapia* sp. along the Maragondon River suggests the overall fitness of fish, the effect of contaminants on cholinesterase enzyme activities was not reflected in these values. This suggests that relying solely on biological parameters to conclude the overall health of fish might not be appropriate. For future research directions, it is recommended that studies be conducted on the levels of organophosphates and carbamates in the Maragondon River and their correlation with cholinesterase enzyme activity. Additionally, future studies should consider sorting and disaggregating the sex of samples to account for the sexual dimorphic characteristics of *Tilapia* sp. Finally, further exploration of other biological endpoints in model organisms should be pursued to gather valuable information for managing the Maragondon River.

Conclusion

Our findings reveal a negative allometric growth pattern ($b < 3$) in the *Tilapia* sp. Additionally, the condition factor (K) indicates that *Tilapia* sp. are generally fit and thriving in optimal conditions for growth. However, inhibition of AChE and BChE enzymes in the river's midstream (muscle tissue) and downstream (muscle and liver) stations was observed. These results underscore the importance of considering multiple biomarkers and indices to assess organismal health comprehensively. The findings from this study provide valuable insights into the ecotoxicological status of *Tilapia* sp. in the Maragondon River and emphasise the significance of biomarker studies in monitoring and managing the impact of environmental contaminants on aquatic ecosystems.

Compliance with Ethical Standards

Conflict of interest: The authors declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The authors affirm that all international, national, and institutional guidelines for the care and use of laboratory animals have been diligently followed and adhered to throughout this study.

Data availability: Data will be made available on request.

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Disclosure: -

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