AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effects of Paraquat on Behavior, Morphology, Mortality and Acetylcholinesterase Expression in Red Tilapia Fingerlings

Chutima Thanomsit[a], Amnuay Wattanakornsiri[b], Witchuda Prasatkaew[c], Jakkaphun Nanuam[d], Tuantong Jutagate[e], Patcharee Mongkolvai[f], Jadsada Saetiew[g], Panomsak Meemon[g, h,†], Phochit Nanthanawat[d,†,*]

[a] *Department of Fisheries, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan Surin Campus, Surin 32000, Thailand*
[b] *Faculty of Science and Technology, Surindra Rajabhat University, Surin 32000 Thailand*
[c] *Faculty of Science and Technology, Dhonburi Rajabhat University, Samutprakan 10540 Thailand*
[d] *Office of Educational Affairs, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[e] *Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand*
[f] *Faculty of Natural Resource, Rajamangala University of Technology Isan Sakon Nakhon Campus, Sakon Nakhon 47160, Thailand*
[g] *School of Physics, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[h] *Center of Excellent in Advanced Functional Material, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*

Article Info

Article history:
Received 17 October 2024
Revised 22 February 2025
Accepted 25 February 2025
Available online 28 April 2025

Keywords:
Acetylcholinesterase,
Morphological alterations,
Neurotoxicity
Optical coherence tomography,
Paraquat toxicity

Abstract

Importance of the work: Fish can be exposed to pesticides, which poses risks and may have cascading effects on consumers. Herbicides used in agriculture can leak into aquatic environments, causing harmful effects on fish. While some effects on tilapia have been documented, insights into aspects such as gene expression remain unreported.

Objectives: To investigate the effects of paraquat exposure on red tilapia (*Oreochromis niloticus* × *O. mossambicus)* fingerlings, focusing on mortality, behavioral and morphological alterations and acetylcholinesterase (AChE) expression.

Materials and Methods: Red tilapia fingerlings were subjected to acute toxicity assessment by exposing them to varying concentrations of paraquat (0 mg/L, 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L) for 24 hr, 48 hr, 72 hr and 96 hr. AChE expression was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting, while the morphological changes were investigated using light microscopy and optical coherence tomography (OCT), to assess the effects of paraquat on the fingerlings.

Results: Cumulative mortality increased with paraquat concentration and exposure duration. Behavioral abnormalities, such as loss of equilibrium and abnormal swimming patterns, were most severe at higher paraquat concentrations and with longer exposure durations. Morphological damage, including wounds and scale loss, worsened with increased exposure. AChE expression in gill tissues decreased over time, indicating neurotoxic effects on the fish's nervous system. OCT was more effective than traditional light microscopy in detecting early-stage morphological changes, providing detailed insights into tissue damage.

Main finding: The urgent need was underscored for stricter herbicide regulations to protect aquatic ecosystems. The findings provided critical insights into paraquat's impact on aquatic life and highlighted the importance of strategies to manage contamination, to mitigate risks to consumers and to reduce the potential health threats posed by paraquat accumulation in the food chain.

† Equal contribution.

* Corresponding author.
E-mail address: phochit@go.buu.ac.th (P. Nanthanawat)

https://doi.org/10.34044/j.anres.2025.59.2.11

Introduction

Agricultural activities release herbicides into the surrounding environment through flooding and runoff. Paraquat, a nitrogen-based herbicide (1-1' dimethyl-4-4'-bipyridinium dichloride), is one of the most used herbicides due to its non-selective and broad-spectrum activity against weeds and grasses (Ogamba et al., 2011). Often, herbicides such as paraquat, enter aquatic ecosystems through runoff after rainfall, posing potential health hazards to aquatic organisms, including arthropods, fish, frogs, birds and mammals, where the level of toxicity depends on the route of exposure, concentration and age of the affected organisms (Akinsorotan et al., 2023).

Many studies have evaluated paraquat's effects to understand and monitor its contamination and exposure. For example, Nualkaw et al. (2022) reported that paraquat exposure caused physiological and behavioral alterations in Nile tilapia (*Oreochromis niloticus*), with the severity depending on exposure duration and concentration. The reported median lethal concentration (LC_{50}) values at 24 hr, 48 hr, 72 hr and 96 hr were 34.25 µL/L (32.78–35.83 µL/L), 25.11 µL/L (23.94–26.29 µL/L), 12.74 µL/L (11.51–13.90 µL/L) and 10.23 µL/L (9.14–11.26) µL/L, respectively. Paraquat exposure for 28 d reduced the activity levels of antioxidant enzymes, including superoxide dismutase, catalase, glutathione-s-transferases (GST)), reduced glutathione and malondialdehyde (MDA) and caused slight shortening of the gill lamellae in Nile tilapia (Aribisala et al., 2022). Similarly, Norhan et al. (2022) studied the effects of paraquat on *Anabas testudineus*, a species highly tolerant to unfavorable water conditions (Froese and Pauly, 2023) and observed tissue changes in the gills, liver and kidneys, with severity increasing at higher concentrations.

Acetylcholinesterase (AChE) biomarkers are increasingly used as indicators of herbicide exposure. AChE, present in the central and peripheral nervous systems, facilitates cholinergic pathways and synaptic transmission in vertebrates and invertebrates by breaking down the neurotransmitter acetylcholine (ACh) into choline and acetate (Ĉolović et al., 2013). Inhibition of AChE leads to ACh accumulation in the synaptic cleft, causing paralysis and death by asphyxiation (Nanthanawat et al., 2022). Therefore, the AChE concentration serves as a specific biomarker of pesticide and herbicide exposure. AChE activity has been used as an indicator in adult fish for assessing herbicide exposure, alongside studies of physiological and behavioral changes, as demonstrated in Nile tilapia and hybrid catfish (Khanchanasal et al., 2022; Nualkaw et al., 2022; Thanomsit et al., 2021).

In Thailand, Nile tilapia [including a hybrid variety, red tilapia, *(Oreochromis niloticus* × *O. mossambicus*)] is the most economically important freshwater fish species, with annual production exceeding 210,000 t and a market value surpassing USD 300 million (Lertwanakarn et al., 2023). Notably, red tilapia is one of the key economic species in Thailand's aquaculture industry, widely farmed across the country and increasingly expanding toward international exports (Sookmanomont et al., 2012).

Given the importance of tilapia farming, the current research aimed to study the effects of paraquat on red tilapia fingerlings by evaluating behavioral responses, morphological changes and AChE expression. The information obtained from this study should provide valuable insights into contamination in sub-adult red tilapia and may be applicable to other fish species.

Materials and Methods

Chemicals

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride, 27.67% w/v) was purchased in commercial form from a local supplier in Thailand. This corresponds to a concentration of 276 g/L of active ingredient. For the test concentrations reported in microliters per liter, the corresponding active ingredient concentrations a weight per volume (w/v) were: 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L). The study used Precision Plus Protein Dual Color Standards (Catalog #161-0394; Bio-Rad; Thailand), along with reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; General Electric Healthcare; Thailand) and Western blotting (Bio-Rad; Thailand). A polyclonal antibody specific to AChE (PAb-electric eel AChE; Raybiotech©; USA) and a goat anti-rabbit HRP conjugate (GAR-HRP; Abcam; UK) were included. All chemicals used were analytical grade.

Animal husbandry

Red tilapia fingerlings (body depth 1.6–1.8 cm, length 5.8–6.2 cm, weight 2.1–2.3 g) were obtained locally from a private farm in Surin province, Thailand and acclimated for 7 d. They were housed in a 250 L tank with flowing fresh water, maintained at 28±1.4°C, with dissolved oxygen levels of 7.2±1.8 mg/L, and a pH of 7.3±0.3. Water quality was

monitored daily during the 0–96 hr study period and no feeding occurred throughout the 4 d of the study. Each experimental group contained 10 fish; experiments were conducted in triplicate.

Bioassays

Preliminary range-finding tests were conducted to determine appropriate paraquat concentrations for acute toxicity assessment. Red tilapia fingerlings were exposed to a broad range of paraquat concentrations (0 mg/L, 2.76 mg/L, 27.6 mg/L, 276 mg/L and 2760 mg/L) to identify sub-lethal and lethal thresholds. Observations covered mortality, behavioral changes (abnormal swimming patterns, loss of equilibrium), and morphological effects. Based on these findings, paraquat concentrations of 0 mg/L, 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L were selected for the definitive toxicity study.

The acute toxicity tests were conducted using a static bioassay system, in accordance accepted guidelines (Organisation for Economic Co-operation and Development, 2019). Fish were exposed to paraquat concentrations of 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L in separate 10 L tanks. Each treatment was performed in triplicate, with 10 fish per replicate, over exposure durations of 24 hr, 48 hr, 72 hr and 96 hr without water renewal. Environmental parameters (temperature, pH, dissolved oxygen and paraquat concentration) were monitored at the start and end of the test to ensure consistent conditions. Mortality was recorded daily and the accumulated percentage mortality of each group was analyzed to determine the median lethal concentrations (LC_{50}) at 24 hr, 48 hr, 72 hr and 96 hr, using probit analysis with the Minitab@17 software; Minitab Inc.; USA). Statistical significance was tested at $p < 0.05$ based on one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Behavioral responses and morphological changes were assessed every 24 hr.

The percentages of fish displaying loss of equilibrium and abnormal swimming behavior were recorded based on the defined criteria: 1) loss of equilibrium defined as (a) abnormal horizontal orientation (the inability to maintain a stable horizontal swimming posture, with fish tilting or rolling to one side) and (b) abnormal vertical orientation (fish adopting a head-up or head-down posture, indicating difficulty in maintaining buoyancy and normal positioning in the water column) and 2) abnormal swimming behavior defined as (a) hyperactivity (rapid, exaggerated and uncoordinated movements of one or more of the fins, trunk or tail, often accompanied by frequent darting across the tank), (b) convulsions (sudden, involuntary muscle spasms or jerky movements that disrupt normal swimming patterns) and (c) abnormal surface distribution (fish swimming excessively near the surface or clustering in unusual patterns, deviating from normal spatial distribution within the tank).

Light microscope and optical coherence tomography analysis

Morphological changes were studied using light microscopy and optical coherence tomography (OCT) analysis. Fish that had been exposed to paraquat were anesthetized using 150 mg/L tricaine methanesulfonate (MS-222) for 5–10 min before imaging. Six regions (eye, dorsal fin, pelvic fin, pectoral fin, caudal fin and muscle) were examined *in vivo* using a 4× microscope and a custom-built, spectrometer-based frequency domain OCT system, operating at 835 nm with a super-luminescent diode (Saetiew et al., 2024). The OCT captured three-dimensional (3D) datasets, consisting of 1,000 cross-sectional images per minute, with a lateral resolution of 11 µm and a depth of 2 mm, as described by Thanomsit et al. (2022).

The OCT imaging allowed for the examination of the internal structure of bulk tissue in 3D without sectioning or staining. The entire body of each red tilapia fingerling was placed in a Petri dish without water for OCT imaging. Each 3D OCT dataset covered a region of interest up to 10 mm x 10 mm laterally and approximately 1–2 mm in depth. The OCT raw data, in the form of depth cross-sectional images, were exported as JPEG files and analyzed using the ImageJ software (version; NIH; USA), according to Schneider et al. (2012). *En face* images at different depth locations were reconstructed and compared with light microscope images. In addition, 3D volumetric rendering was performed on the OCT datasets using the ImageJ software (Schmid et al., 2010).

Acetylcholinesterase extraction and protein quantification

Gill samples were collected from fish exposed to paraquat concentrations of 0 mg/L, 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L for 24–96 hr. The fish were euthanized in ice and the gills were removed using scissors. Gill tissue was ground and mixed with Tris-HCl buffer (pH 7.2) containing 0.1% Triton X-100 and 0.05 M NaCl, followed by homogenization in an ice bath using an Ultra-Turrax homogenizer (IKA, Thailand). The resulting homogenate was used for protein quantification and AChE expression analysis, following the methods described by Thanomsit et al. (2020).

Acetylcholinesterase expression analysis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting)

AChE expression in gill tissues was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting, with slight modifications to the methods described by Thanomsit et al. (2021). Protein samples (each 8 µg/µL) were separated on 10% separating and 4% stacking gels in an SDS-PAGE system and stained with 0.01% Coomassie Brilliant Blue R-250 to visualize protein bands. Protein sizes were compared against a molecular weight marker (Bio-Rad; Thailand).

For Western blot analysis, proteins separated using SDS-PAGE were electro-transferred onto a nitrocellulose membrane using a Trans-Blot® SD Semi-Dry Transfer Cell (Bio-Rad; Thailand). A polyclonal antibody specific to AChE (PAb-electric eel AChE; Raybiotech©; USA) was used as the primary antibody at a 1:200 dilution, followed by incubation with a goat anti-rabbit HRP-conjugated secondary antibody (GAR-HRP; Abcam; Singapore). Protein bands were visualized using an enhanced chemiluminescence detection system, as described by Thanomsit et al. (2022).

Statistical analysis

In the acute toxicity study, the median lethal concentrations (LC_{50}) at 24 , 48 , 72 and 96 hr were determined using probit analysis in the Minitab software. Mortality rates were recorded and analyzed to calculate the cumulative percentage mortality for each paraquat concentration over the exposure durations.

Behavioral responses and morphological changes were analyzed using ANOVA to detect differences between treatment groups. Statistical significance was defined as $p<0.05$. When significant differences were detected, Duncan's multiple range test was applied as a *post-hoc* analysis to identify specific group differences.

For AChE expression and protein quantification, data were expressed as mean ± standard deviation (SD) values. Differences among paraquat concentration groups and exposure durations were evaluated using ANOVA, followed by Duncan's multiple range test for pairwise comparisons, where necessary.

All statistical analyses were conducted using the Minitab software and all graphs and figures were prepared using GraphPad Prism 9.5.0; GraphPad Software Inc.; USA) to visualize the data.

Ethical statement

Procedures involving animals adhered to the guidelines of the Faculty of Agriculture and Technology and were approved by the Committee for Biological Experimentation on Animals at Rajamangala University of Technology Isan (project proposal ID 03-65-001). The animal use license number was U1-03405-2559.

Results

Cumulative mortality percentage and median lethal concentration

The cumulative mortality of red tilapia fingerlings exposed to different paraquat concentrations (2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L) was measured over 24, 48, 72 and 96 hr, with no mortality observed in the control group. Mortality increased with both paraquat concentration and exposure duration. For example, at 24 hr, the cumulative mortality percentages were 11.11±4.81%, 13.89±4.81%, 16.670.00% and 22.22±4.81% for 2.76 mg/L, 5.52 mg/L, 8.25 mg/L and 11.04 mg/L, respectively. By 96 hr, mortality had risen to 22.22±4.81%, 38.8±4.81%, 52.784.81% and 66.67±0.00% at the same concentrations (Fig. 1).

The LC_{50} values for paraquat in red tilapia fingerlings were 18.10 mg/L at 24 hr, 17.08 mg/L at 48 hr, 11.99 mg/L at 72 hr and 7.97 mg/L at 96 hr.

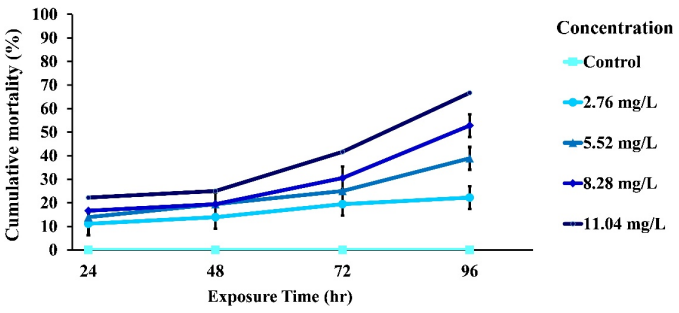

Fig. 1 Cumulative mortality percentages of red tilapia fingerlings exposed to different paraquat concentrations over time, compared to control group

Behavioral response and morphological changes

Behavioral changes in the red tilapia fingerlings exposed to paraquat were assessed at 24 hr, 48 hr, 72 hr and 96 hr. Abnormal behaviors, such as impaired equilibrium, hyperactivity, convulsions and altered surface distribution were observed. The highest concentration (11.04 mg/L) induced the most pronounced behavioral changes, which were significantly different from those in the control group, as shown in Fig. 2.

Fig. 3 presents the morphological changes observed in the red tilapia fingerlings exposed to paraquat at concentrations of 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L, compared to the control group over the same exposure duration. Morphological changes, including wounds and scale loss on the fins, tail and eyes, increased with both paraquat concentration and exposure duration. The severity of these changes was most notable at the highest concentrations and longest exposures (11.04 mg /L and 96 hr, respectively).

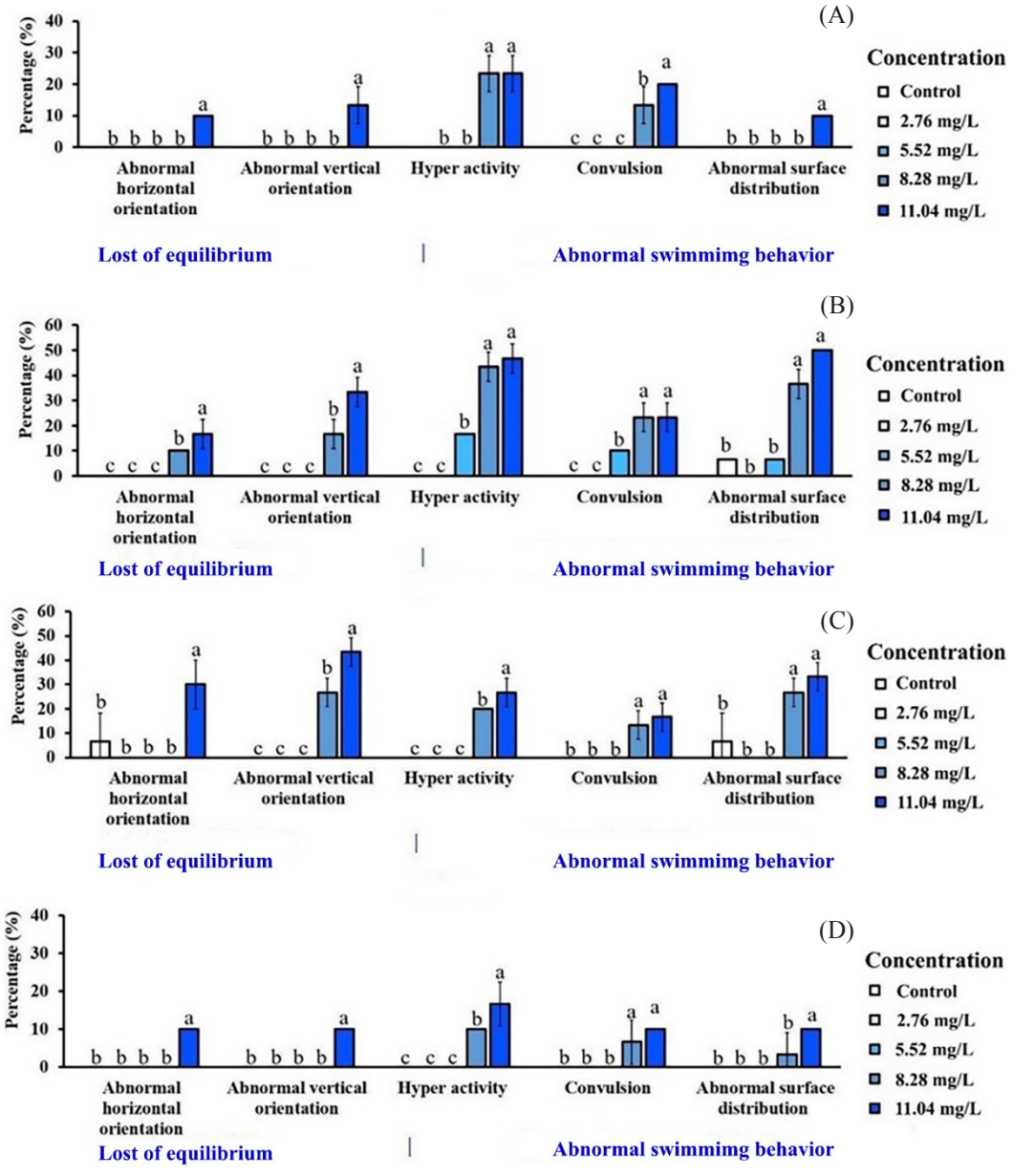

Fig. 2 Percentage behavioral changes in red tilapia fingerlings after exposure to paraquat at different concentrations, compared to the control group, over time: (A) 24 hr; (B) 48 hr; (C) 72 hr; (D) 96 hr. Different lowercase letters above columns indicate significant ($p < 0.05$) differences among treatments within each parameter ($n = 10$, 3 replicates)

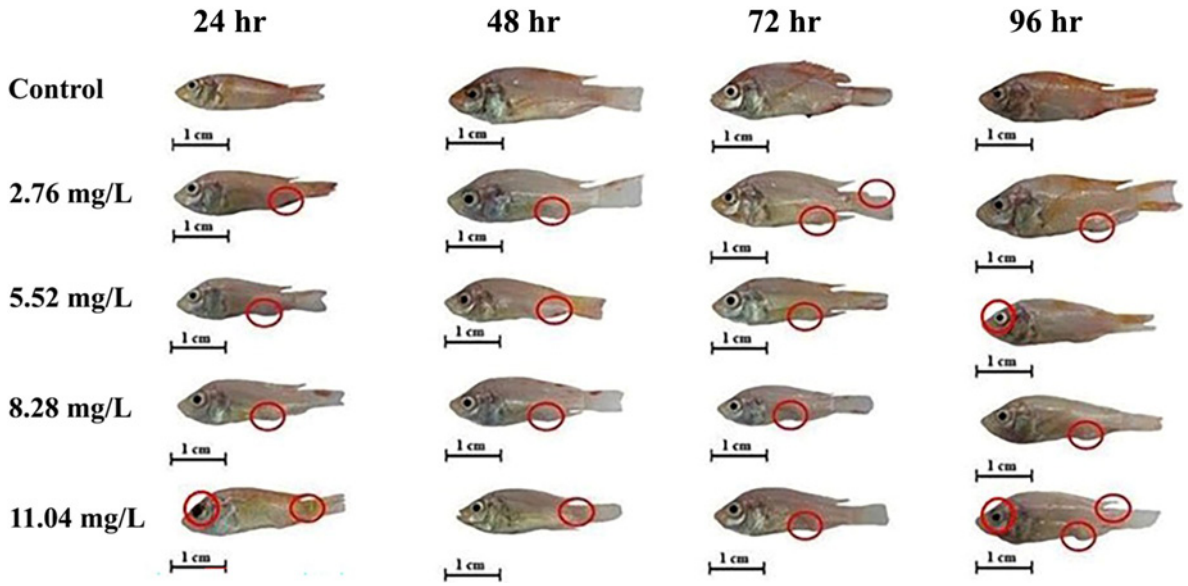

Fig. 3 Morphological changes in red tilapia fingerlings exposed to paraquat at different concentrations, compared to the control group, over time, where red circles highlight unusual appearance, indicating morphological changes

The scale bars are too blurred to see - improve the image quality. Present only the scale bar in each image as unit is provided in the caption.

Morphological changes via light microscopy and optical coherence tomography analysis

Light microscopy (4× magnification) revealed differences in several body parts between the control and paraquat-treated groups. In the treated fish, the eye, pelvic fin, and caudal fin appeared more opaque (Fig. 4). No noticeable changes were observed in the dorsal fin, muscle or pectoral fin.

Fig. 5 illustrates examples of these morphological changes as analyzed using OCT, by comparing the control group (Figs. 5A and 5B) with the group exposed to the highest concentration of paraquat (11.04 mg/L) for 96 hr (Figs. 5C and 5D). The OCT imaging provided digital cross-sectional visualization of biological tissues, as shown in the bottom three sub-images of each column. For example, the green and red boxes on the OCT cross-section images highlight depth-resolved examples of normal and abnormal tissue structures in the control and paraquat-treated groups, respectively.

The OCT analysis revealed that morphological changes became more pronounced with increasing paraquat exposure. For example, at 11.04 mg/L exposure for 96 hr, major structural alterations were observed in multiple tissues (Fig. 5D). The retina appeared deformed, the pectoral fin showed flattened tissue structures, the dorsal fin appeared shriveled and the pelvic fin had rough surfaces. Furthermore, the caudal fin displayed disrupted skin structures, while both superficial and internal muscle layers appeared distorted. These findings highlighted the progressive impact of paraquat toxicity on red tilapia fingerlings.

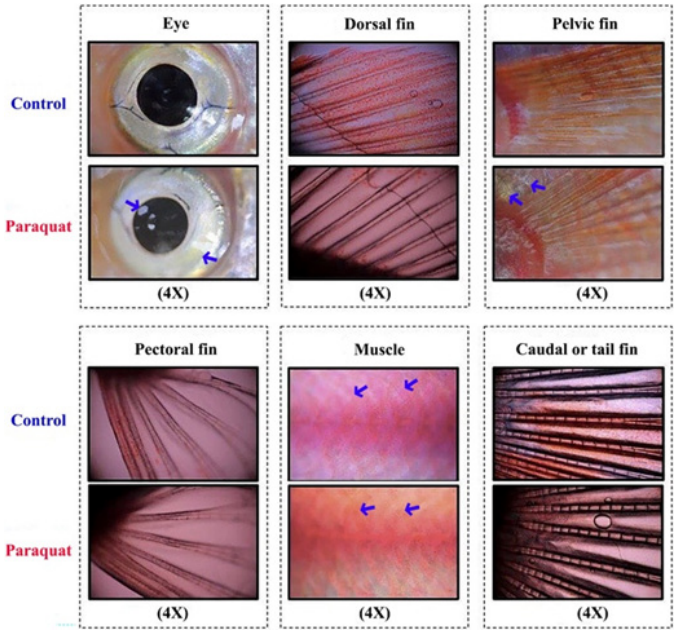

Fig. 4 Tissue-morphological changes in red tilapia fingerlings under light microscopy (4× magnification) in paraquat-treated group (11.04 mg/L) and control group after 96 hr, where blue arrows indicate unusual appearance of tissue-morphological changes in different parts of red tilapia fingerlings

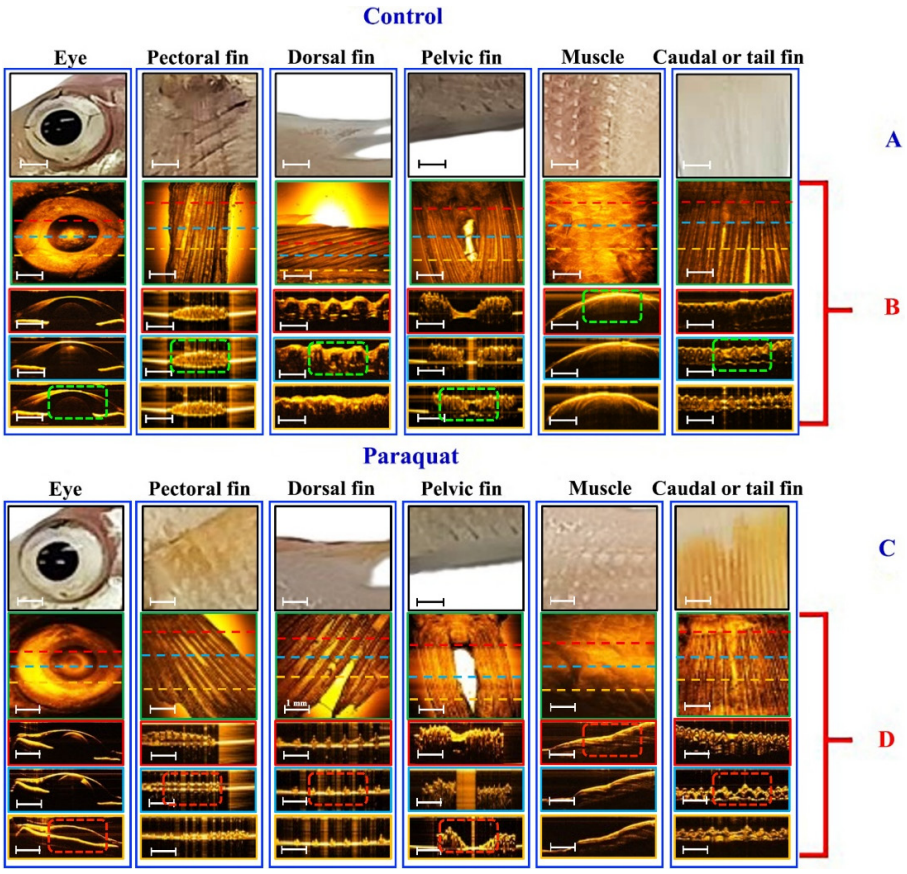

Fig. 5 Comparative morphological analysis of red tilapia fingerlings between control and paraquat-treated groups using optical coherence tomography and light microscopy: top panel (A and B) represents control group, displaying intact and well-organized tissue structures across various regions (eye, pectoral fin, dorsal fin, pelvic fin, muscle and caudal or tail fin); bottom panel (C and D) represents paraquat-treated group (11.04 mg/L for 96 hr), showing structural abnormalities, including disrupted eye morphology, altered fin surfaces and distorted muscle tissues, where green boxes in panel B highlight normal, intact tissue structures in control group, whereas red boxes in panel D indicate areas of structural disruption in paraquat-treated group, such as irregular surface morphology, tissue disorganization and muscle deformation and scale bar = 1 mm.

Protein and acetylcholinesterase expression

AChE expression in gill tissue was evaluated using Western blotting after exposure to paraquat at concentrations of 0 mg/L, 2.76 mg/L, 5.52 mg/L, 8.28 mg/L and 11.04 mg/L, over 24 hr, 48 hr, 72 hr and 96 h The initial protein patterns, analyzed using SDS-PAGE, remained consistent across concentrations and exposure durations.

However, the AChE expression bands revealed a significant decrease in AChE levels with increasing paraquat concentrations and exposure durations. The bands corresponding to AChE (71 kDa) were normalized to β-actin and the statistical analysis confirmed progressive inhibition of AChE expression in the paraquat-treated groups compared to the control group.

At 11.04 mg/L, AChE expression was undetectable after 72 hr, while at 8.28 mg/L and 11.04 mg/L it was absent after 96 hr, indicating that paraquat exposure inhibited AChE expression in concentration-dependent and time-dependent manners, consistent with its neurotoxic effects (Fig. 6).

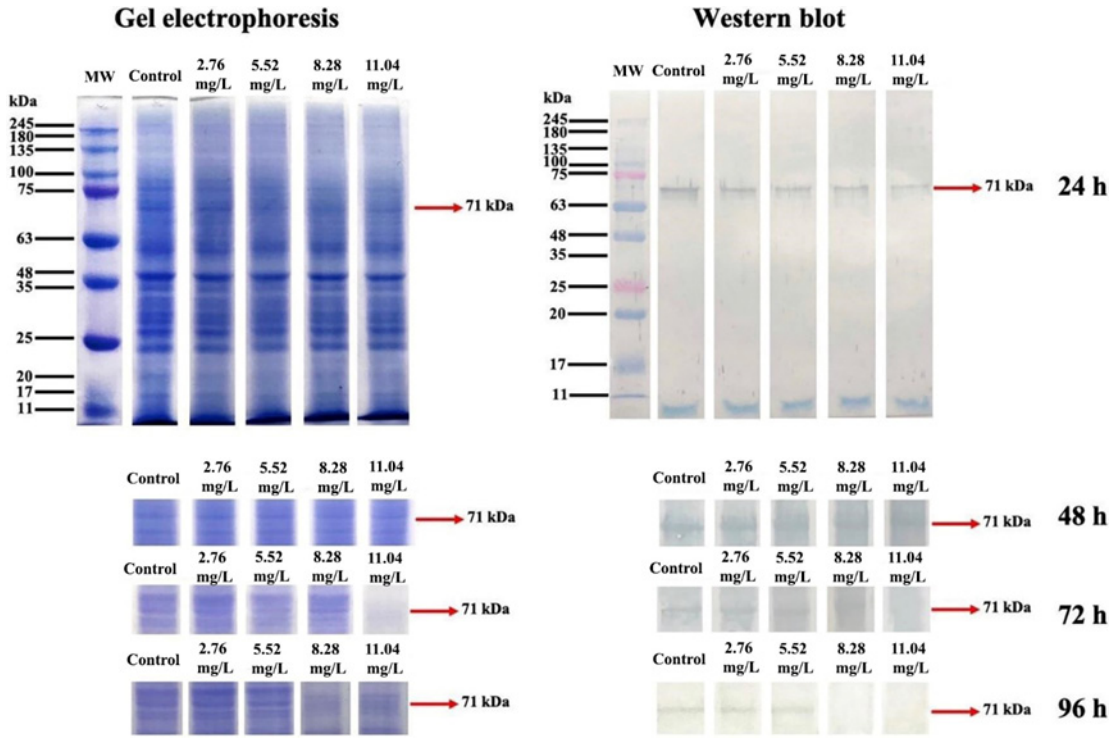

Fig. 6 Protein patterns from gill tissue of red tilapia fingerlings analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (left panel) and acetylcholinesterase expression assessed using Western blot (right panel) in paraquat-treated group compared to control group

Discussion

Herbicides, such as paraquat, pose major risks to aquatic environments, leading to biochemical changes in fish and potentially causing mass mortality, which over time, can impact fish populations in natural water sources (Bojarski et al., 2019). The current study examined the effects of paraquat on red tilapia fingerlings. Initially, paraquat exposure increased cumulative mortality rates with both concentration and duration, consistent with findings in adult fish (Nualkaw et al., 2022) and freshwater fingerlings of *Labeo rohita* (Hamilton) (Arivu et al., 2016). The current values were slightly higher than those in Nualkaw et al. (2022), who reported LC_{50} values of 34.25 µL/L (24 hr), 25.11 µL/L (48 hr), 12.74 µL/L (72 hr) and 10.23 µL/L (96 hr). The differences in LC_{50} values may be attributed to variations in experimental conditions, fish size, environmental factors or the preparation of the paraquat solutions. Nualkaw et al. (2022) used adult-sized fish, which affected both the cumulative mortality rate and the LC_{50} value. In addition, variations in LC_{50} values across studies may have been influenced by species and the form of the substance used (Walker et al., 2006; Arivu et al., 2016). While paraquat is acutely toxic to aquatic animals, its accumulation is generally lower in most species (Leadprathom, 2011). The herbicide's toxicity is attributed primarily to the generation of free radicals, which cause cellular and tissue damage (Leadprathom, 2011; Thanomsit et al., 2021).

The behavioral observations revealed that paraquat exposure led to loss of equilibrium and abnormal swimming behavior, which intensified with increased concentration and duration. These findings were consistent with those of Arivu et al. (2016) and Nualkaw et al. (2022), who reported similar behavioral changes in *Labeo rohita* and Nile tilapia, respectively. Additionally, the current findings aligned with the study by Ogunwole et al. (2018), which demonstrated that pesticides, such as paraquat, cause neurotoxic effects in aquatic organisms, including the inhibition of AChE expression. Furthermore, the current findings aligned with Aribisala et al. (2022), who reported oxidative stress, histological damage and genotoxicity in *O. niloticus* exposed to herbicides. Increased lipid peroxidation (MDA) and GST activity in their study suggest oxidative stress, which may explain the erratic swimming and loss of equilibrium observed in the current study. The increase in micronucleated cells in their study suggested DNA damage, reinforcing paraquat's systemic toxicity. These results emphasized the need for integrated histological, biochemical and molecular analyses to fully assess paraquat's impact on aquatic organisms.

The assessment of morphological changes in red tilapia fingerlings exposed to paraquat involved three methods: (1) digital imaging, (2) light microscopy and (3) OCT imaging. Digital imaging captured general morphological alterations, such as wounds on fins and tails, with severity increasing with exposure time and concentration. Light microscopy revealed noticeable changes primarily in the eyes, likely due to the larger and more rigid structures of other body parts, which limited detection. This limitation underscored the advantages of OCT, which allows for more rapid analysis with minimal sample preparation and provides 3D imaging capabilities (Meemon and Rolland, 2010; Thanomsit et al., 2022; Lichtenegger et al., 2023). OCT's advantages aligned with its proven use in analyzing fish tissues (Yang et al., 2020; Haindl et al., 2020). However, OCT cannot replace histological analysis for cellular-level details. Future studies should combine OCT with histology to validate and enhance these findings.

AChE expression in gill tissues was analyzed following paraquat exposure, as gills are highly sensitive to waterborne toxicants and serve as indicators of systemic pesticide exposure (Fu et al., 2018). Other studies (Thanomsit et al., 2021; Nualkaw et al., 2022) have demonstrated that AChE activity in gills reflects systemic neurotoxic stress caused by pesticides and herbicides. The current results showed a decrease in AChE expression (71 kDa) with increasing paraquat concentrations and prolonged exposure. This aligned with other research indicating that herbicide toxicity inhibits AChE expression, with the extent of inhibition influenced by duration, concentration and the exposure route (Walker et al., 2006; Thanomsit et al., 2020; Nualkaw et al., 2022). Similar reductions in AChE expression have been reported in hybrid catfish, snakehead fish and tilapia (Thanomsit et al., 2020; Thanomsit et al., 2021). Although gill tissues are not directly involved in the central or peripheral nervous systems, where AChE regulates neural transmission, these findings have provided valuable insights into paraquat's systemic effects. However, they do not directly demonstrate its neurotoxic impact on the Central Nervous System or Peripheral Nervous System (CNS or PNS). Future studies should examine nervous tissues, such as the brain or spinal cord, using molecular techniques, such as Western blotting, to better assess AChE expression in these systems.

Immunohistochemistry (IHC) would also be a powerful complementary approach to localize AChE expression at the cellular level in both gill and CNS/PNS tissues. By combining IHC with Western blotting, future studies could identify specific cell types that respond to paraquat exposure, offering a clearer understanding of the herbicide's neurotoxic effects. This approach would further enhance the ability to differentiate immune-related responses in gill cells from neurotoxicity in CNS/PNS tissues.

Conclusion

The toxicological effects were highlighted of paraquat on red tilapia fingerlings, revealing significant impacts on mortality, behavior, morphology, and AChE expression. Acute toxicity testing demonstrated that cumulative mortality increased with both paraquat concentration and exposure duration, with the LC_{50} values aligning with other studies. Behavioral abnormalities, such as impaired equilibrium and abnormal swimming patterns, intensified with higher paraquat concentrations and longer exposure times.

Morphological assessments, conducted via digital imaging, light microscopy, and OCT, confirmed that paraquat exposure caused notable physical damage, including scale loss, wounds, and tissue deformation, particularly at higher concentrations. OCT proved to be a superior technique for detecting early-stage and internal morphological changes compared to traditional light microscopy.

Additionally, the inhibition of AChE expression in gill tissues, observed using SDS-PAGE and Western blotting, confirmed paraquat's neurotoxic effects. The AChE levels decreased progressively with increasing paraquat concentrations and prolonged exposure, highlighting its role as a biomarker for pesticide toxicity.

These findings have emphasized the need for stricter regulations on herbicide use to mitigate environmental contamination and its harmful effects on aquatic ecosystems. The study also underscored the importance of advancing imaging and molecular techniques to better understand the sub-lethal effects of toxicants. Further research is recommended to evaluate the long-term impacts of paraquat on fish populations and aquatic biodiversity, as well as its potential risks to human health through the food chain.

Conflict of Interest

The authors declare that there are no conflicts of interest

Acknowledgements

This work was supported by Suranaree University of Technology, Thailand Science Research and Innovation (TSRI) and the National Science, Research, and Innovation Fund (NSRF) (NRIIS number 195594).

References

Akinsorotan, A.M., Ajisodun, A.F., Izah, S.C., Jimoh, J.O. 2019. Acute toxicity of paraquat dichloride on fingerlings of *Oreochromis niloticus*. Int. J. Res. Stud. Biosci. 7: 29–36.

Aribisala, O.A., Sogbanmu, T.O., Kemabonta, K.A. 2022. Genotoxic, biochemical and histological biomarkers of subacute concentrations of paraquat and glyphosate in Nile Tilapia. Environ. Anal. Health Toxicol. 37: e2022012.

Arivu, I., Muthulingam, M., Jiyavudeen, M. 2016. Toxicity of paraquat on freshwater fingerlings of *Labeo rohita* (Hamilton). Int. J. Sci. Eng. Res. 7: 1965–1971.

Bojarski, B., Jakubiak, M., Witeska, M. 2019. Physiological and histological effects of herbicides in fish. Annals of Warsaw University of Life Sciences - SGGW – Anim. Sci. 57: 207–217. doi.org/10.22630/aas.2018.57.3.20

Ĉolović, M., Krstić, D., Lazarević-Pašti,T., Bondžić, A., Vasić, V. 2013. Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr. Neuropharmacol. 11: 315-335.

Froese, R., Pauly, D. 2023. Fish Base. World Wide Web electronic publication. version (10/2023). www.fishbase.org, 17 October 2024

Fu, H., Xia, Y., Chen,Y., Xu, T., Xu, L., Guo, Z., Xu, H., Xie, H, O., Zhao, B. 2018. Acetylcholinesterase is a potential biomarker for a broad spectrum of organic environmental pollutants; Environ. Sci. Technol. 52: 8065-8074. doi.org/10.1021/ acs.est.7b04004

Haindl, R., Deloria, A.J., Sturtzel, C., et al. 2020. Functional optical coherence tomography and photoacoustic microscopy imaging for zebrafish larvae. Biomed. Opt. Express. 11: 2137. doi.org/10.1364/BOE.390410

Khanchanasal, P., Nuankaew, C., Saowakoon, S., Nanuam, J., Nanthanawat, P., Thanomsit, C. 2022. Toxicity level of 2, 4–D dimethylammonium in Nile tilapia and acetylcholinesterase (AChE) expression (biomarker) to identify exposure in sub-lethal concentration. Buu. Sci. J. 27: 1278–1299.

Lertwanakarn, T., Purimayata, T., Luengyosluechakul, T., Grimalt, P.B., Pedrazzani, A. S., Quintiliano, M.H. Surachetpong, W. 2023. Assessment of Tilapia (*Oreochromis* spp.) Welfare in the Semi-Intensive and Intensive Culture Systems in Thailand. Animals 13: 1–19.

Lichtenegger, A., Baumann, B., Yasuno, Y. 2023. Optical Coherence Tomography Is a Promising Tool for Zebrafish-Based Research–A Review. Bioengineering 10: 5–23. doi.org/10.3390/bioengineering 10010005

Leadprathom N. 2011. Paraquat toxicity in aquatic organisms. King Mongkut's Agric. J. 31: 95–101.

Meemon, P., Rolland, J.P. 2010. Swept-source based, single-shot, multi-detectable velocity range Doppler optical coherence tomography, Biomed. Opt. Express. 1: 955–966.

Nanthanawat, P., Kiatprasert, P., Wattanakornsiri, A., Nanuam, J., Prasatkaew, W., Ruttanakorn, S., Thanomsit, C. 2022. Development of an antibody technique for acetylcholinesterase expression detection in the gill of Nile tilapia (*Oreochromis niloticus*) as a glyphosate-based herbicide biomarker. Toxicol. Rep. 9: 1548–1556. doi.org/10.1016/j.toxrep.2022.07.009

Norhan, N.A.S., Zakariah, M.I., Karim, N.U., Daud, H.M., Melad, A.A.N., Yusoff, N.A.H., Hassan, M. 2022. Paraquat-induced histopathological changes on the gills, kidney and liver tissues of *Anabas testudineus* (BLOCH 1792). J. Sustain. Sci. Manag. 17: 169–178. doi.org/10.46754/jssm.2022.08.010

Nualkaw, C., Wattanakornsiri, A., Nanuam, J., Nanthanawat, P., Prasatkaew, W., Meeprom, C., Saowakoon, S., Thanomsit, C. 2022. Effects of paraquat-based herbicide on acetylcholinesterase (AChE), antioxidant status and histological alterations in freshwater fish: Nile tilapia (*Oreochromis niloticus*). AACL Bioflux 15: 3197–3211.

Ogamba, E.N., I.R. Inyang and Azuma, I.K. 2011. Effect of paraquat dichloride on some metabolic and enzyme parameters of *Clarias gariepinus*. Curr. Res. J. Biol. Sci., 3(3): 186-190.

Organisation for Economic Co-operation and Development. 2019. Test Guidelines for the Chemicals - OECD. 2019. Test guideline No.203 Fish, acute toxicity testing. 1–23.

Ogunwole, G.A., Uju, S., Saliu, J.K. 2018. Paraquat Toxicity on Selected Biomarkers in *Clarias gariepinus*. IOSR J. Environ. Sci. Toxicol. Food Technol. 12: 66–75. doi.org/10. 9790/2402-1205016675

Saetiew, J., Saenjae, J., Thanomsit, C., Nanthanawat, P., Wattanakornsiri, A., Meemon, P. (2024). Development of a field-ready optical coherence tomography system for assessing 2, 4-D dimethylammonium-based herbicide exposure in African catfish. Opt. Eng., 63 024102-024102.

Schmid, B., Schindelin, J., Cardona, A., Longair, M., Heisenberg, M. 2010. A high-level 3D visualization API for Java and ImageJ. BMC bioinformatics. 11: 1–7. doi.org/10. 1186/1471-2105-11-274.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 9: 671–675. doi.org/10.1038/nmeth.2089

Sookmanomon, S., Ratanatrivong, W., Uraiwon, S., Chanin, S. Udlert, T.2012. Growth Improvement of Red Tilapia Uttaradit Strain. Journal of Fisheries Technology Research. 6: 1–12.

Thanomsit, C., Kiatprasert, P., Prasatkaew, W., Khongchareonporn, N., Nanthanawat, P. 2021. Acetylcholinesterase (AChE) monoclonal antibody generation and validation for use as a biomarker of glyphosate-based herbicide exposure in commercial freshwater fish. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 241: 108956. doi.org/10.1016/J. CBPC. 2020.108956

Thanomsit, C., Saetiew, J., Meemon, P. 2022. Optical coherence tomography as an alternative tool for evaluating the effects of glyphosate on hybrid catfish (*Clarias gariepinus* × *Clarias macrocephalus*). Toxicol. Rep. 9: 181–190. doi.org/10.1016/j.toxrep. 2022.01.010

Thanomsit, C., Saowakoon, K., Wattanakornsiri, A., Khongchareonporn, N., Nanuam, J., Prasatkaew, W., Nanthanawat, P. 2020. Acetylcholinesterase (AChE) polyclonal antibody from hybrid catfish (*C. macrocephalus* × *C. gariepinus*): Specification, sensitivity and cross reactivity. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 237: 108837. doi.org/10.1016/j.cbpc.2020.108837

Turchini, G.M. 2023. Aquaculture sustainability: Global challenges need local actions. Rev. Aquac. 15: 402–403. doi.org/10.1111/RAQ.12797

Walker, C.H., Hopkin, S. P., Peakall, D. B. 2006. Principles of Ecotoxicology, Taylor & Francis. USA.

Yang, D., Hu, M., Zhang, M., Liang, Y. 2020. High-resolution polarization-sensitive optical coherence tomography for zebrafish muscle imaging. Biomed Opt. Express. 11: 5618. doi.org/10.1364/boe.402267