Time-Dependent Histopathological and Scanning Electron Microscopic Alterations in the Suprabranchial Chamber of the Air-Breathing Fish *Channa gachua* (Hamilton, 1822) Following Sublethal Profenofos Exposure

ABSTRACT

Background: Organophosphate pesticides are among the most pervasive chemical contaminants of freshwater ecosystems in South Asia. In the intensively cultivated rice-vegetable belt of West Champaran, Bihar, small ponds, drainage ditches, and seasonal wetlands lie in close proximity to treated fields, and informal, largely unregulated pesticide use in this region routinely introduces organophosphates such as profenofos into these water bodies; yet the sub-organismal impact of such local contamination on the accessory respiratory organs of resident air-breathing fishes remains poorly characterised. **Objective:** The present study evaluated the histopathological and scanning electron microscopic (SEM) alterations in the suprabranchial chamber (SBC) of the obligate air-breathing teleost *Channa gachua* (Hamilton, 1822) following sublethal exposure (0.06 ppm; 1/10th of 96-h LC_{50}) to the organophosphate insecticide profenofos. **Methods:** Fish were collected from freshwater habitats of West Champaran, Bihar, India, acclimated under laboratory conditions, and exposed to profenofos for 10 and 20 days. SBC tissue was processed for H&E histology (seven photomicrographs) and gold-sputter-coated SEM (four micrographs). **Results:** Light microscopy of control fish showed a well-organised respiratory epithelium, intact blood vessels, and regular folded lamellae. After 10 days of profenofos exposure, focal detachment of the respiratory epithelium and early vascular engorgement were evident. At 20 days, extensive epithelial erosion, haemorrhage, and architectural disruption were conspicuous. SEM of control tissue revealed regular dome-shaped vascular papillae arranged in rosette islets separated by smooth inter-islet lanes. In treated fish, SEM demonstrated deformation of islets, dilatation of mucous pores, intermingling of papillae and lanes, and extensive surface disorganisation that progressed with exposure duration. **Conclusion:** These time-dependent morphological changes collectively indicate potential compromise of the aerial gas-exchange function of the SBC and highlight the ecological risk associated with organophosphate contamination of freshwater environments inhabited by air-breathing fishes.

Keywords: *Channa gachua; histopathology; profenofos; scanning electron microscopy; suprabranchial chamber*

1. INTRODUCTION

Freshwater fish represent one of the most ecologically and economically significant vertebrate groups globally, yet they are disproportionately vulnerable to chemical contamination of aquatic environments. Among the most pervasive contaminants are organophosphate (OP) pesticides, which have progressively displaced persistent organochlorine compounds as the dominant insecticide class in agricultural practice worldwide (Aktar et al., 2009; Stehle & Schulz, 2015). Worldwide pesticide use remains extensive (Sharma et al., 2019), with profenofos — an OP ester of phosphorothioic acid — used in rice and other agricultural systems, including South Asia (He et al., 2010; Masbou et al., 2025).

Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) is a broad-spectrum insecticide and acaricide widely applied in Bihar and other paddy-growing states of India. Its primary mode of action involves irreversible inhibition of acetylcholinesterase (AChE), causing accumulation of acetylcholine at cholinergic synapses and consequent disruption of nervous system function (Ecobichon, 1996; Fukuto, 1990; Fulton & Key, 2001). While this mechanism is well-characterised in target insect species, ecotoxicological consequences for non-target aquatic vertebrates — particularly teleost fishes — are increasingly recognised (Das & Mukherjee, 2000; Datta & Kaviraj, 2003; Nwani et al., 2010; Pandey et al., 2011; Saha & Kaviraj, 2008; Sarkar et al., 2005), including documented histopathological alterations in profenofos-exposed carp (Nataraj et al., 2017). Runoff and aerial drift from treated agricultural fields routinely introduce profenofos into adjacent water bodies, where it may persist for several days depending on temperature, pH, and microbial activity (Chauhan et al., 2025; Zhang et al., 2024). Recent SEM evidence from profenofos-exposed *C. gachua* has documented ultrastructural disruption of gill architecture, providing species-specific support for the sensitivity of respiratory surfaces to this insecticide (Ranjana & Rai, 2025).

West Champaran and the adjoining paddy-vegetable belt of North Bihar exemplify the local challenges underlying this vulnerability. The district lies within the intensively cultivated Gangetic plain, where rice-vegetable double- and triple-cropping is the dominant land use and small ponds, drainage ditches, and seasonal wetlands sit in immediate proximity to treated fields, with little buffering distance between agrochemical application and standing water. Pesticide procurement in this belt is largely informal, driven by local dealer recommendation rather than calibrated dosing, and post-application runoff is rarely contained or monitored (Kamboj et al., 2026; Kashyap et al., 2024). These local conditions are not an isolated concern: pesticide contamination of Indian freshwater systems is now recognised as a major and worsening threat to aquatic biodiversity, with residues of organophosphates and other insecticide classes repeatedly detected in the Ganges and its tributaries and associated with oxidative, biochemical, and histopathological damage in resident fish fauna (Kamboj et al., 2026; Shah & Parveen, 2022). Profenofos was selected for the present study because it is among the most heavily used OP insecticides in this specific agricultural belt, is readily available commercially as a formulated emulsifiable concentrate, and has a well-characterised AChE-inhibiting mode of action that is broadly representative of the OP class as a whole, allowing the findings to be compared cautiously with those for related compounds. It is, however, only one of several pesticides in routine local use: chlorpyrifos, monocrotophos, quinalphos, and cypermethrin are also widely applied in the

same paddy-vegetable systems of Bihar and pose comparable or, in some documented cases, greater ecotoxicological risk to non-target aquatic fauna (Kamboj et al., 2026; Kashyap et al., 2024; Pakhare & Pagare, 2018). The present findings for profenofos should therefore be read as one component of a broader, multi-pesticide threat to the aquatic biodiversity of this region rather than as an isolated hazard.

Sublethal pesticide concentrations — too low to cause rapid mortality — may nonetheless produce profound organismal damage by disrupting endocrine function, compromising immune competence, impairing reproductive success, and inducing histopathological lesions in vital organs (Handy, 1994; Teh et al., 1997). Hepatotoxic, nephrotoxic, gonadotoxic, and branchiotoxic effects of OPs are well documented in teleosts (Kavitha & Rao, 2007; Maharajan et al., 2013; Rao, 2006; Sidhu et al., 2019). However, studies specifically addressing OP impacts on the accessory respiratory organs of air-breathing fishes remain comparatively sparse, despite the ecological importance of these organs (Adamek-Urbańska et al., 2021). Recent reviews of pesticide toxicity in freshwater fish further identify oxidative stress and histopathological tissue injury as recurring sublethal responses to organophosphate exposure (Mukherjee et al., 2025; Patel & Agnihotri, 2025).

The family Channidae (snakeheads) comprises obligate air-breathing teleosts characterised by paired suprabranchial chambers (SBC) situated dorsal to the gill arches. In *Channa gachua* (Hamilton, 1822) — the dwarf snakehead — each SBC bears elaborate dendritic plates carrying a specialised respiratory epithelium organised into vascular islets and non-vascular inter-islet lanes. These structures function as the aerial gas-exchange surface (Hughes & Munshi, 1973; Munshi, 1976). The SBC enables *C. gachua* to survive in hypoxic habitats — rice paddies, drainage ditches, shallow seasonal ponds — which are precisely the environments most directly exposed to agricultural pesticide runoff (De Silva, 1991), a vulnerability increasingly recognised across South Asian freshwater systems more broadly (Ali et al., 2020; Banerjee, 2007).

Channa gachua was selected as the study species for several converging reasons. As an obligate air-breather dependent on the SBC for a substantial share of its respiratory gas exchange, it is intrinsically more vulnerable than water-breathing teleosts to any toxicant-induced compromise of this organ, making it a sensitive sentinel for OP contamination of exactly the shallow, hypoxic habitats described above. It is also a locally important food fish, widely consumed and informally cultured in the ponds and wetlands of West Champaran, so its decline carries direct relevance to regional food security and fisher livelihoods, in addition to its ecological role. Practically, the species is hardy, tolerant of laboratory acclimation, and available year-round from local water bodies, permitting a controlled sublethal bioassay of the kind reported here. Finally, prior baseline biochemical data are already available for *C. gachua* under OP exposure, including a documented sensitivity to the locally used organophosphate quinalphos (Pakhare & Pagare, 2018), which allowed the present histological and ultrastructural findings to be interpreted against an established toxicological backdrop for this species. Experimental studies also report pesticide-

associated alterations of gill architecture in teleosts, including after exposure to environmentally relevant pesticide mixtures and to triazophos in the air-breathing catfish *Heteropneustes fossilis* (Cantu & Rahman, 2025; Loganathan et al., 2024).

Previous investigations have documented histopathological alterations in the respiratory organs of channid fishes under environmental and toxicant stress (Banerjee & Chandra, 2005; Chandra & Banerjee, 2004), and biochemical perturbations in liver and kidney of *C. gachua* following OP exposure were reported by Kadam and Patil (2016). Comparable vulnerability has been reported in other air-breathing and co-occurring freshwater species of the region: malathion induces hepatic damage in the air-breathing catfish *Heteropneustes fossilis* (Barbhuiya & Dey, 2015), while monocrotophos impairs the female reproductive organ and aromatase expression in the climbing perch *Anabas testudineus* (Mohapatra et al., 2025), and toxicant-induced histopathological damage to an accessory respiratory surface has been documented in *Clarias batrachus* (Singh & Banerjee, 2008). These parallel findings indicate that OP and related pesticide impacts on air-breathing accessory respiratory and reproductive organs are a broader phenomenon across regional channid and non-channid taxa, not one confined to *C. gachua*. However, no study has integrated light microscopy with high-resolution SEM to characterise the structural consequences of profenofos on the SBC of *C. gachua*. The present investigation therefore aimed to: (i) describe normal histological and ultrastructural organisation of the SBC; (ii) document time-dependent histopathological and SEM alterations after 10 and 20 days of sublethal profenofos exposure; and (iii) discuss mechanistic and ecological implications of the observed changes.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Acclimation

Specimens of *Channa gachua* (standard length 10.5–15.5 cm; body weight 30.5–40.0 g) from a single population were collected from freshwater ponds of West Champaran, Bihar, India. Fish were transported in aerated earthen pots and immediately washed with potassium permanganate ($KMnO_4$; 0.05 mg/l) to eliminate surface infections (Lazur & Yanong, 2013). They were then acclimated for 20 days in 50-litre glass aquaria at 27 ± 1°C. During acclimation, fish received commercial pelleted fish feed and *Tubifex* worms on alternate days; aquarium water was renewed daily. No food was provided during bioassay exposure. Fish of both sexes were used without discrimination.

2.2 Physicochemical Parameters of Water

Physicochemical properties of test water were determined according to APHA (1998) and are summarised in Table 1.

Table 1. Physicochemical parameters of test water used in the profenofos bioassay, determined according to APHA (1998) [23].

Parameter	Value
pH	7.2
Temperature (°C)	27.0
Dissolved Oxygen (mg/l)	6.8
CO_2 (mg/l)	8.0
Hardness as $CaCO_3$ (mg/l)	56.0
Alkalinity as HCO_3^- (mg/l)	130.0

2.3 Toxicant and Exposure

A commercial formulation of profenofos (50% EC; Excel Crop Care Ltd.) was procured from the local market in Muzaffarpur. The 96-h LC_{50} for *C. gachua* was determined as 0.60 ppm by probit analysis (Finney, 1971; Litchfield & Wilcoxon, 1949). A sublethal concentration of 0.06 ppm (1/10th of LC_{50}) was selected following Hart et al. (1948). Stock solutions were prepared by dissolving 1 ml of formulation in 1 litre of distilled water; working concentrations were freshly prepared daily. The water in the aquaria was changed every 24 hours and continuously aerated. A control group of 10 fish was maintained in toxicant-free water under identical conditions.

2.4 Histological Processing

At 10 and 20 days, SBC tissue from treated and control fish was dissected in normal saline and fixed in Bouin's fluid or 10% neutral buffered formalin for 24 h. After dehydration through graded ethanols, tissues were cleared in xylene, infiltrated with paraffin wax (56–58°C), and sectioned at 6 µm on a rotary microtome. Sections on albuminised slides were stained with Ehrlich's haematoxylin and alcoholic eosin (H&E) (Bancroft & Gamble, 2008), mounted in DPX, and examined by light microscopy (Nikon Ci-L; Nikon DS-Fi3 camera) at standardised magnifications.

2.5 Scanning Electron Microscopy

SBC fragments were rinsed in heparinised saline and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 24 h, followed by post-fixation in 1% osmium tetroxide for 2 h (Hayat, 2000). After dehydration through graded ethanols and transition through amyl acetate, specimens were critical-point dried using liquid CO_2, mounted on aluminium stubs, sputter-coated with gold (~20 nm), and examined at 25 kV at the Bose Research Institute, Kolkata.

3. RESULTS

3.1 Normal Histology of the SBC — Control Fish (Figs. 1–3)

Histological sections of control *C. gachua* SBC revealed a chamber incompletely septated into a large posterior and small anterior compartment (Fig. 1). The chamber was lined by a pseudostratified respiratory epithelium (RE) composed of ectodermal and basophilic gill-derived cells. The folded epithelium rested on a lamina propria (LP) richly supplied with capillary blood vessels (BV). The dendritic plate (DP) was visible as a distinct folded structure. The posterior wall comprised fibres of the *Constrictor suprabranchialis* (MF). At higher magnification (Fig. 2), vascular lacunae (VL) within the stroma were clearly demarcated from the intact epithelial cap. Fig. 3 confirms the organised folded architecture with well-defined vascular stroma (CS). Control fish maintained for up to 20 days showed no histopathological changes.

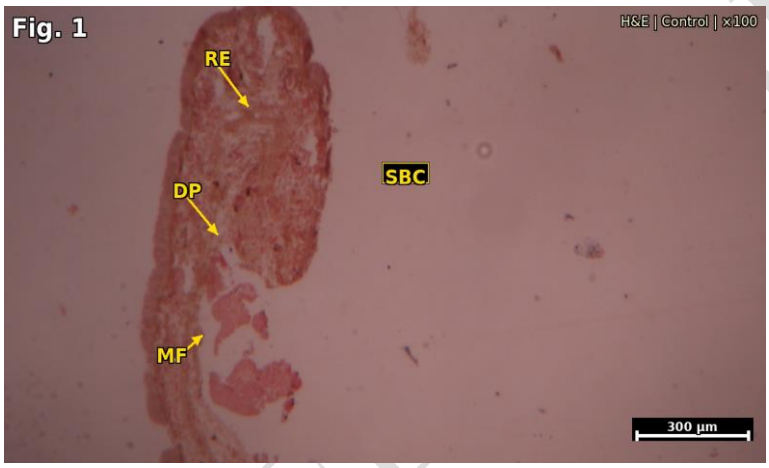

Fig. 1. *T.S. of SBC of control Channa gachua at low magnification showing intact respiratory epithelium (RE), dendritic plate (DP), suprabranchial chamber lumen (SBC), and muscle fibres (MF).* (H&E ×100; scale bar = 300 µm)

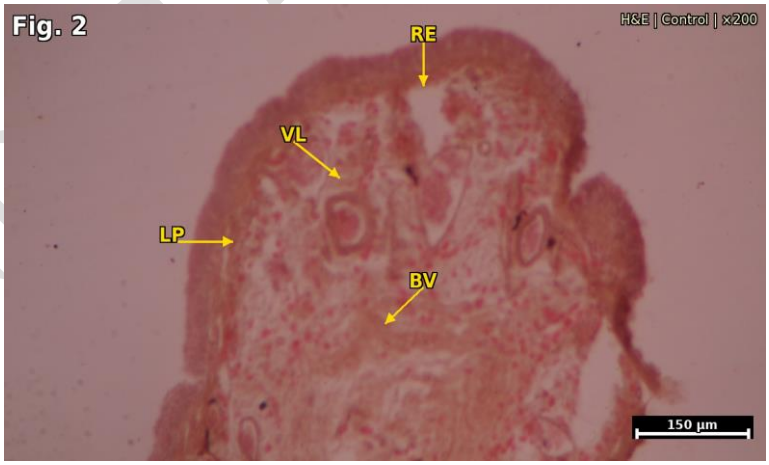

Fig. 2. *T.S. of SBC of control Channa gachua at higher magnification showing intact RE cap, vascular lacunae (VL), lamina propria (LP), and blood vessel (BV) in normal configuration.* (H&E ×200; scale bar = 150 µm)

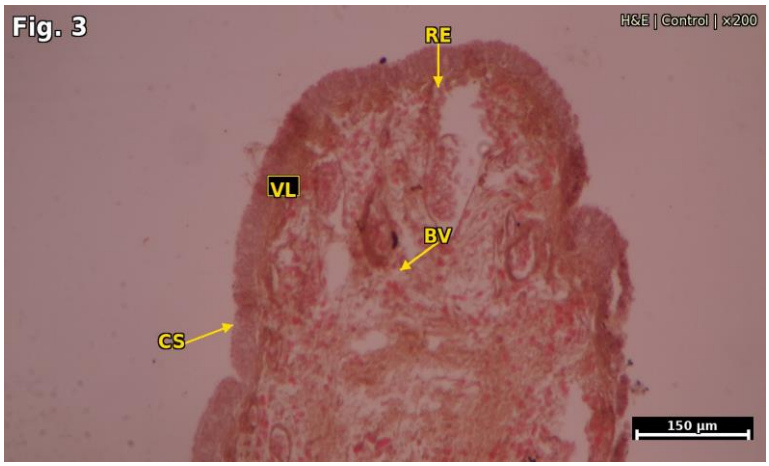

Fig. 3. *T.S. of SBC of control Channa gachua at medium-high magnification confirming organised folded RE, vascular lacunae (VL), blood vessel (BV), and Constrictor suprabranchialis muscle (CS).* (H&E ×200; scale bar = 150 µm)

3.2 Histopathological Alterations — Profenofos-Treated Fish

3.2.1 Ten-Day Exposure (Fig. 4)

After 10 days at 0.06 ppm profenofos, SBC sections showed early but definitive pathological changes (Fig. 4). The respiratory epithelium (RE) displayed focal detachment from the lamina propria with initiation of erosion (ERE) at the epithelial margins. Subepithelial blood vessels showed early vascular engorgement. The vascular stroma appeared slightly disorganised (DVS) relative to controls. These changes were distributed unevenly across the epithelium, indicating focal initiation of toxicant-induced damage.

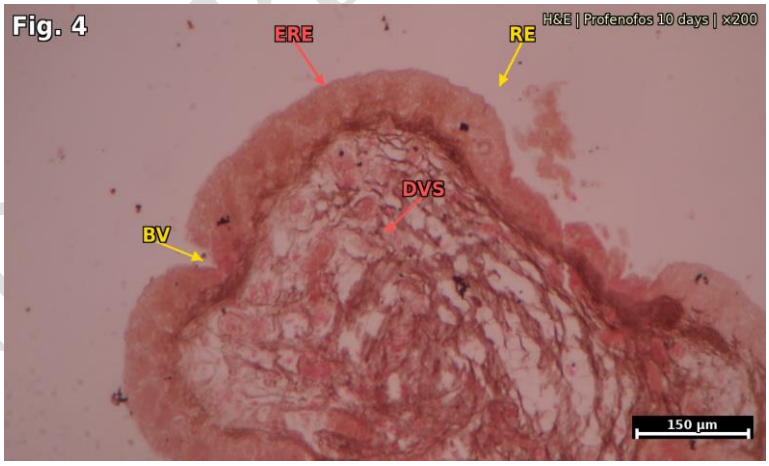

Fig. 4. *T.S. of SBC of profenofos-treated Channa gachua (10 days) showing focal erosion of respiratory epithelium (ERE), disrupted vascular stroma (DVS), and early blood vessel (BV) changes with an intact RE region for comparison.* (H&E ×200; scale bar = 150 µm)

3.2.2 Twenty-Day Exposure (Figs. 5–7)

Prolonged profenofos exposure (20 days) produced markedly more severe and widespread damage. Fig. 5 shows extensive epithelial erosion (ERE), scattered ruptured blood vessels (SBV), and dilated vascular components (DV). The author's original annotation of the respiratory epithelium (RE) zone is retained. Fig. 6 — a vertical T.S. strip of the SBC wall — reveals

prominently ruptured blood vessels (RBV) at the apex, with haemorrhage (HEM) into the subepithelial stroma and disrupted connective tissue (CT). Fig. 7, at the highest histological magnification, shows the close-up of the eroded epithelial strip (ERE) with an open blood vessel lumen (BVL), extravasated erythrocytes (EC), and fibrous stroma (FS). These represent the most advanced histopathological changes observed in the present study.

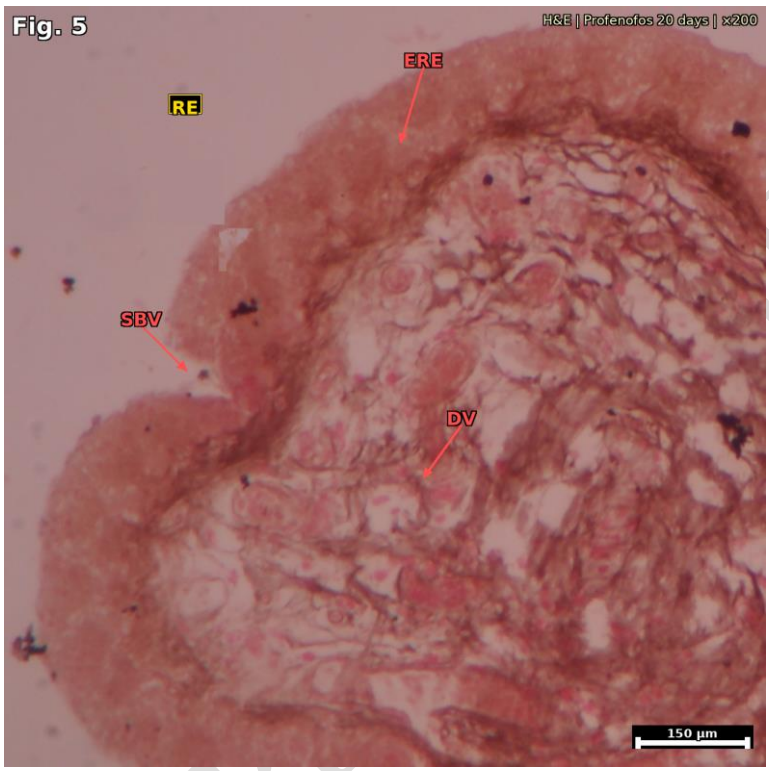

Fig. 5. *T.S. of SBC of profenofos-treated Channa gachua (20 days) showing severe epithelial erosion (ERE), scattered ruptured blood vessels (SBV), and dilated vascular component (DV). RE marks the author's original annotation of the respiratory epithelium zone.* (H&E ×200; scale bar = 150 µm)

Fig. 6. *Vertical T.S. strip of SBC wall of profenofos-treated Channa gachua (20 days) showing ruptured blood vessels (RBV) at apex, haemorrhage (HEM) into subepithelial stroma, eroded respiratory epithelium (ERE), and disrupted connective tissue (CT).* (H&E ×250; scale bar = 100 µm)

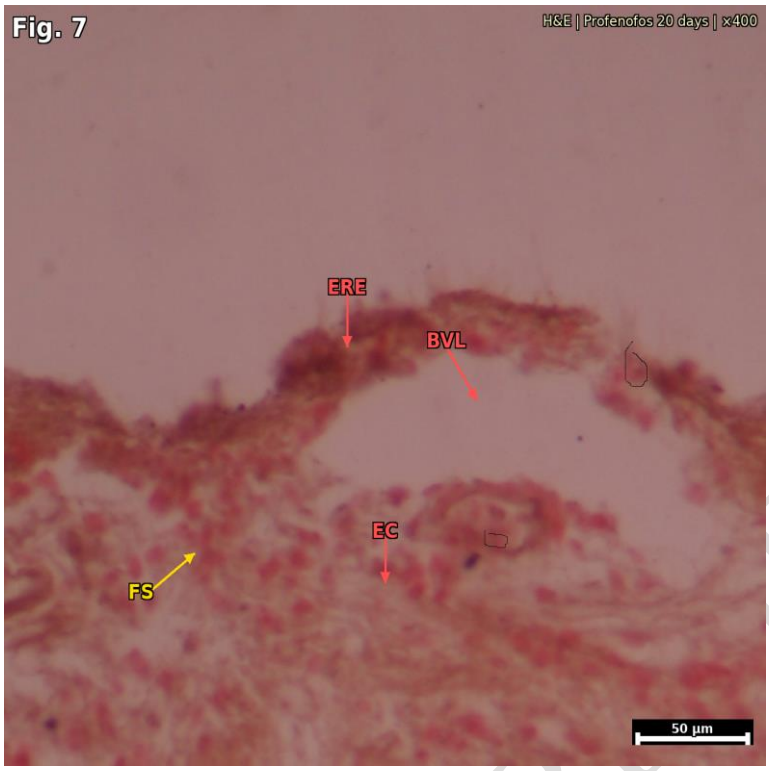

Fig. 7. *Close-up H&E section of SBC of profenofos-treated Channa gachua (20 days) showing thin eroded epithelium (ERE), open blood vessel lumen (BVL), extravasated erythrocytes (EC), and fibrous stroma (FS).* (H&E ×400; scale bar = 50 µm)

3.3 SEM Observations — Control Fish (Figs. 8–9)

SEM examination of control *C. gachua* SBC revealed a highly organised surface architecture (Figs. 8–9). Fig. 8 (en face view, ×1400) shows the characteristic mosaic of respiratory islets (RI), each composed of closely packed dome-shaped vascular papillae (VP) arranged in rosette configurations. Inter-islet lanes (L) with regularly distributed mucous pores (MP) are clearly visible as the dark depressed areas separating the raised islets. Fig. 9 (lateral oblique view, ×3000) resolves the elongated finger-like profile of individual vascular papillae (VP), the smooth lane surfaces (L), and the regular islet architecture (RI). This well-organised mosaic constitutes the functional gas-exchange surface in control fish.

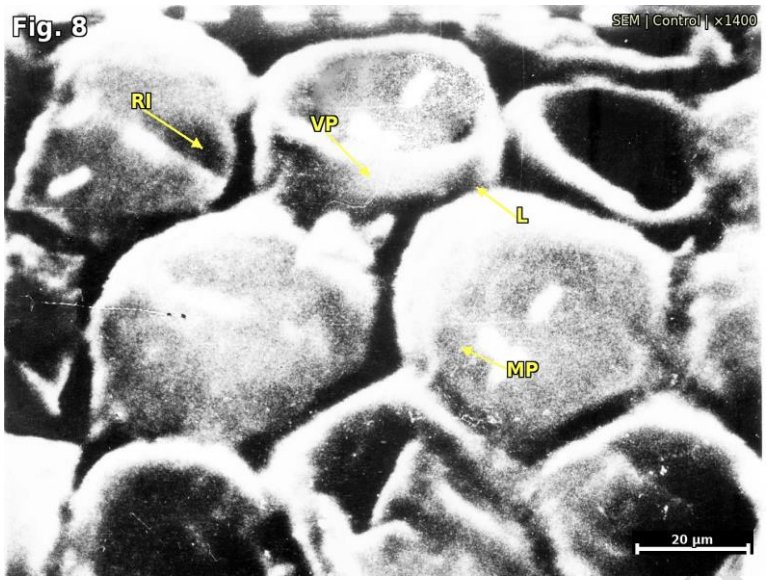

Fig. 8. *SEM photomicrograph of SBC of control Channa gachua (en face view) showing well-organised respiratory islets (RI) composed of dome-shaped vascular papillae (VP) in rosette arrangement, inter-islet lanes (L), and mucous pores (MP).* (×1400; scale bar = 20 µm)

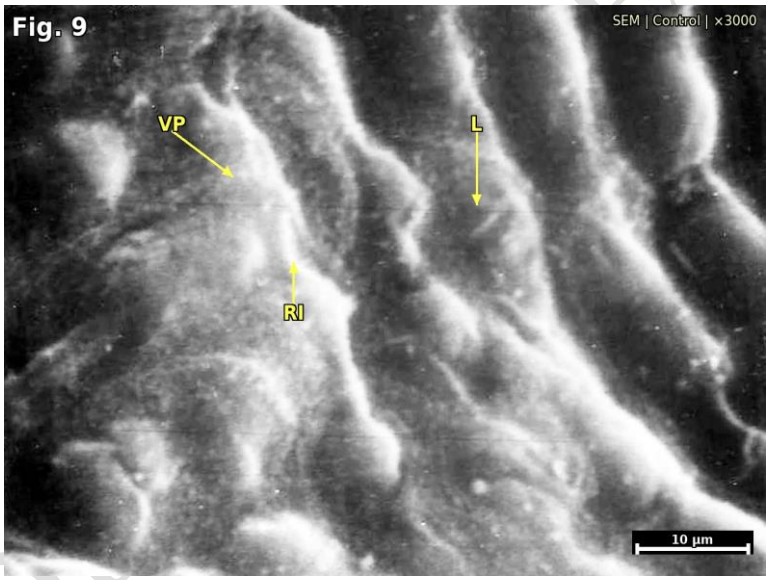

Fig. 9. *SEM photomicrograph of SBC of control Channa gachua (lateral oblique view) showing finger-like vascular papillae (VP), organised respiratory islets (RI), and smooth inter-islet lanes (L).* (×3000; scale bar = 10 µm)

3.4 SEM Observations — Profenofos-Treated Fish (Figs. 10–11)

Profenofos exposure produced progressive and severe ultrastructural damage to the SBC surface (Figs. 10–11). Fig. 10 (×4000) shows the early stages of ultrastructural disruption: deformed respiratory islets (DRI) that have lost their regular dome shape, intermingling vascular papillae (IVP) extending into lane areas, markedly enlarged mucous pores (EMP), and fusion of adjacent lanes (FL). The clear islet–lane demarcation of control tissue is substantially blurred. Fig. 11 (×6000) depicts advanced disorganisation: intensive intermingling of vascular papillae (IVP), complete fusion of lanes (FL), a prominent central fissure (CF) representing marked structural breakdown, and a grossly disorganised respiratory surface (DRS) in which islets and lanes can no

longer be distinguished. These ultrastructural changes collectively indicate near-total loss of the functional microarchitecture of the aerial gas-exchange surface.

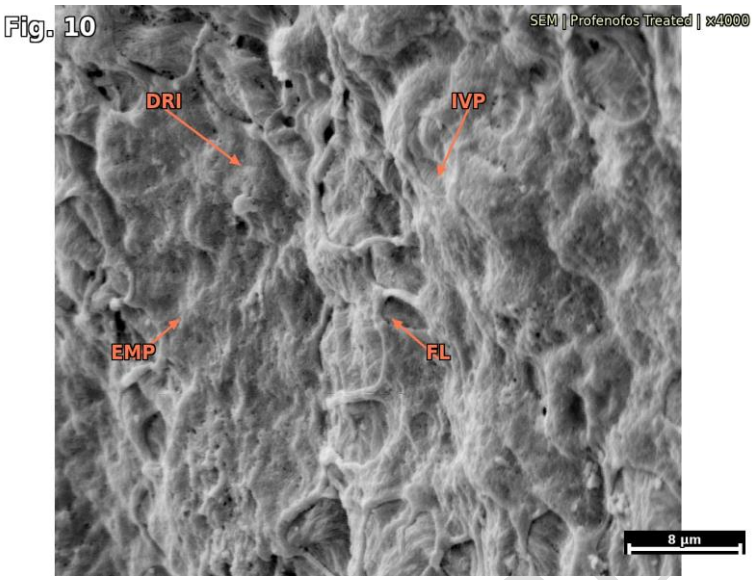

Fig. 10. *SEM photomicrograph of SBC of profenofos-treated Channa gachua showing deformed respiratory islets (DRI), intermingling vascular papillae (IVP), enlarged mucous pores (EMP), and fused lanes (FL). Compare with Fig. 8 for contrast with control.* (×4000; scale bar = 8 µm)

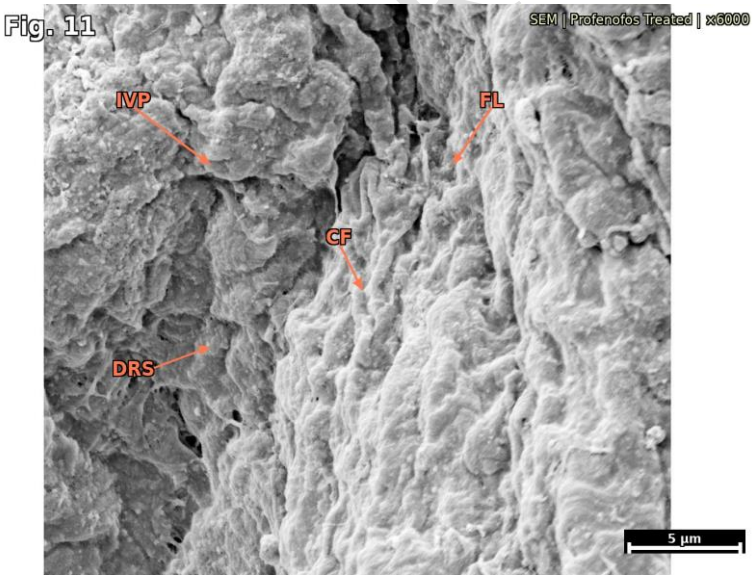

Fig. 11. *SEM photomicrograph of SBC of profenofos-treated Channa gachua showing advanced stage: extensive intermingling of vascular papillae (IVP), fully fused lanes (FL), central fissure (CF), and grossly disorganised respiratory surface (DRS).* (×6000; scale bar = 5 µm)

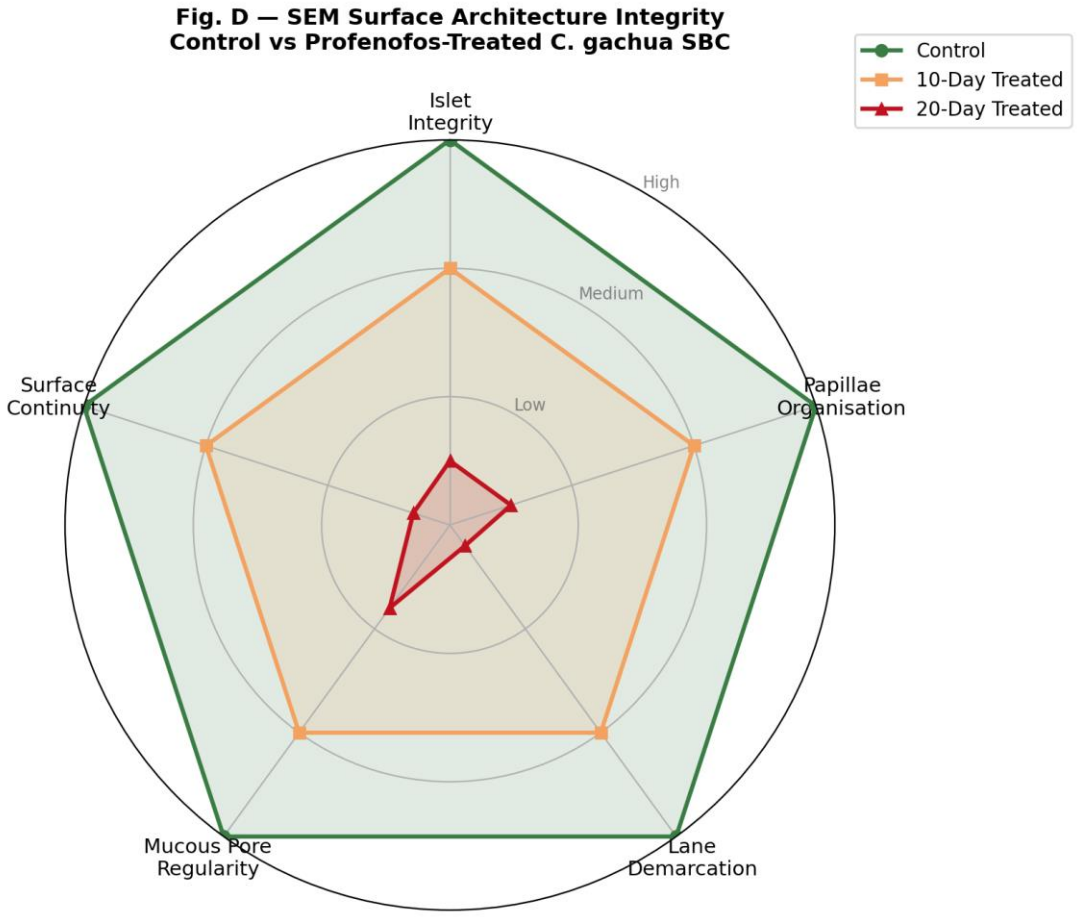

Fig. 12. Radar chart of SEM surface architecture integrity scores (High=3, Low=0) for five ultrastructural parameters in control (green), 10-day (orange), and 20-day (red) profenofos-treated Channa gachua SBC. Shrinkage of the polygon reflects progressive loss of functional microarchitecture.

Abbreviations: *RE = Respiratory Epithelium; SBC = Suprabranchial Chamber; DP = Dendritic Plate; MF = Muscle Fibres (Constrictor suprabranchialis); VL = Vascular Lacunae; LP = Lamina Propria; BV = Blood Vessel; CS = Constrictor suprabranchialis; ERE = Eroded Respiratory Epithelium; DVS = Disrupted Vascular Stroma; SBV = Scattered Blood Vessels; DV = Dilated Vessel; RBV = Ruptured Blood Vessels; HEM = Haemorrhage; CT = Connective Tissue; BVL = Blood Vessel Lumen; FS = Fibrous Stroma; EC = Extravasated Corpuscles; VP = Vascular Papillae; RI = Respiratory Islets; L = Lanes; MP = Mucous Pores; DRI = Deformed Respiratory Islets; IVP = Intermingling Vascular Papillae; EMP = Enlarged Mucous Pores; FL = Fused Lanes; CF = Central Fissure; DRS = Disorganised Respiratory Surface.*

3.5 Summary of Histopathological and Ultrastructural Findings (Table 2)

Table 2. Summary of histopathological and ultrastructural effects of profenofos (0.06 ppm) on the SBC of Channa gachua. Seven H&E figures and four SEM figures are described.

Parameter	Control	Profenofos 10 days	Profenofos 20 days
Respiratory Epithelium	Normal, intact	Focal detachment & early erosion	Extensive erosion & sloughing

Parameter	Control	Profenofos 10 days	Profenofos 20 days
Blood Vessels	Normal calibre	Early engorgement & focal rupture	Severe rupture & haemorrhage
Tissue Architecture	Organised folded lamellae	Mild disorganisation	Severe architectural disruption
SEM: Respiratory Islets	Regular dome-shaped rosettes	Slight flattening	Deformed & ruptured
SEM: Mucous Pores	Normal, evenly distributed	Slightly enlarged	Markedly enlarged & irregular
SEM: Vascular Papillae	Well-organised bulb-shaped	Mild deformity	Intermingling with lanes
SEM: Islet–Lane Boundary	Clearly demarcated	Slight blurring	Complete loss of demarcation

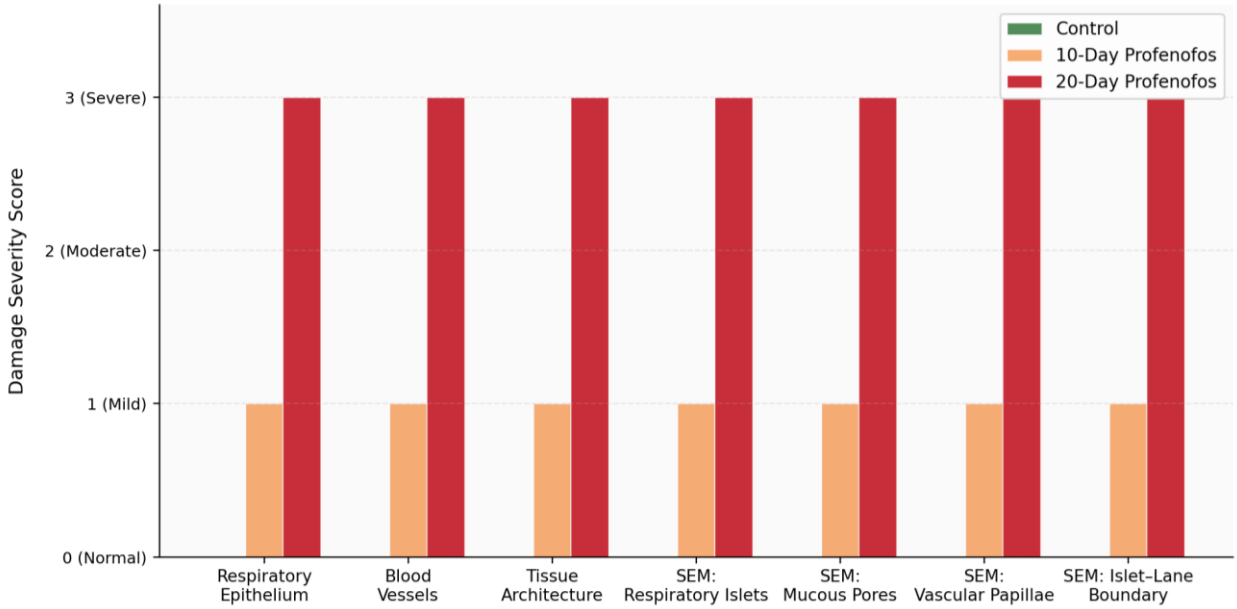

Fig. 13. Histopathological and ultrastructural damage severity scores (0=Normal; 1=Mild; 3=Severe) across seven parameters in control, 10-day, and 20-day profenofos-exposed Channa gachua SBC.

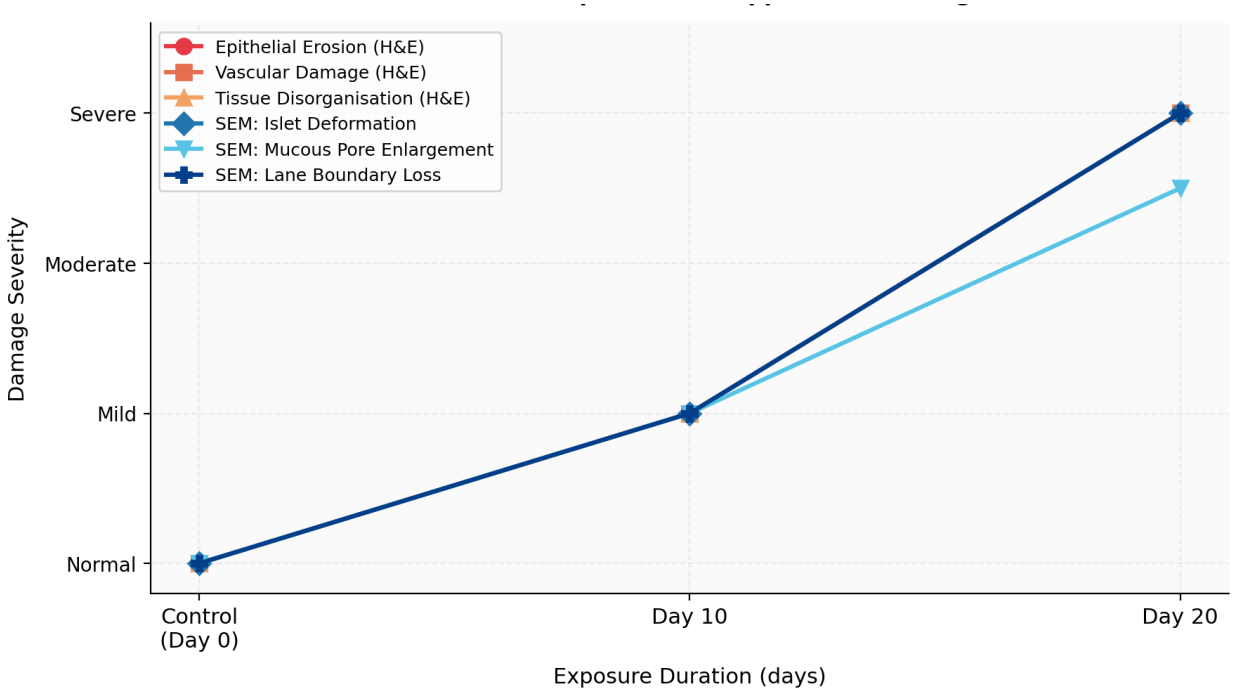

Fig. 14. Progressive damage timeline showing time-dependent escalation of H&E histological and SEM ultrastructural damage indicators across control, Day 10, and Day 20 exposure in Channa gachua SBC.

—, No effect; +, Mild/early effect; ++, Severe/advanced effect. Based on examination of ≥3 fish per group.

4. DISCUSSION

The present study provides an integrated histopathological and SEM characterisation of profenofos-induced damage to the SBC of *C. gachua*, with clear assignment of seven histological and four SEM micrographs. The progressive, time-dependent deterioration documented across these figures (summarised graphically in Figs. 13 and 14) is consistent with, and substantially extends, existing literature on OP toxicity in teleost fishes.

4.1 Normal SBC Architecture (Figs. 1–3 and 8–9)

The three H&E control figures (Figs. 1–3) collectively demonstrate the normal organisation of the SBC at increasing magnifications — from the whole chamber view (Fig. 1) through detail of vascular lacunae and lamina propria (Fig. 2) to confirmation of the muscular wall and folded lamellae (Fig. 3). The two control SEM figures complement this view: Fig. 8 (en face, ×1400) resolves the rosette islet pattern and mucous pore distribution, while Fig. 9 (lateral, ×3000) confirms the elongated profile of individual vascular papillae and the clearly delineated lanes. Together these five figures establish the baseline architecture against which pathological changes are assessed.

4.2 Progressive Epithelial Erosion (Figs. 4–7)

The histological sequence across Figs. 4–7 documents a clear progression of epithelial damage. At 10 days (Fig. 4), focal detachment and early erosion represent the initial response to profenofos. At 20 days, Figs. 5–6 show extensive sloughing, haemorrhage, and architectural disruption at medium magnification, while Fig. 7 — the highest-magnification histological image — provides close-up evidence of eroded epithelium, open vascular lumens, and extravasated erythrocytes. This cascading progression mirrors epithelial damage documented in the gills of OP-exposed *Labeo rohita* (Das & Mukherjee, 2003), *Catla catla* (Maharajan et al., 2013), and *Oreochromis mossambicus* (Rao, 2006) and reflects the combined cytotoxic, oxidative, and mechanical stresses imposed by profenofos (Ahmad et al., 2004; Evans et al., 2005; Sayeed et al., 2003), corroborating recent reports of profenofos-induced oxidative and histopathological damage in other teleosts (El-Bouhy et al., 2023).

4.3 Ultrastructural Disorganisation (Figs. 10–11)

The two treated SEM figures provide clear ultrastructural evidence. Fig. 12 (radar chart) provides a composite visual summary of surface architecture integrity across all three groups. Fig. 10 captures the intermediate stage — deformed islets, enlarged mucous pores, early lane fusion — while Fig. 11 depicts the advanced stage characterised by total obliteration of islet–lane boundaries and the appearance of a prominent central fissure indicative of large-scale structural collapse. This contrasts starkly with the well-organised surface of control fish (Figs. 8–9). Comparable SEM changes have been reported in the gills of *Anabas testudineus* exposed to cadmium and copper (Chakpram & Gupta, 2014), and closely parallel profenofos-induced gill deterioration documented in *Oreochromis mossambicus* (Rao et al., 2003).

4.4 Mechanistic Basis

Profenofos, as an organophosphate insecticide, primarily acts through acetylcholinesterase (AChE) inhibition, which can disturb cholinergic signalling and neuromuscular regulation (Fukuto, 1990; Fulton & Key, 2001). In fish, organophosphate exposure is also associated with oxidative stress and altered antioxidant defences, processes that can promote lipid peroxidation and compromise epithelial and vascular integrity (Sayeed et al., 2003; Slaninova et al., 2009). These effects may contribute to the epithelial detachment, vascular engorgement, haemorrhage, and progressive structural disorganisation observed in the SBC after profenofos exposure. Mitochondrial dysfunction has additionally been proposed as a target of organophosphate and carbamate pesticides and may reduce the cellular energy available for membrane maintenance and tissue repair (Leung & Meyer, 2019). Because AChE activity, oxidative-stress biomarkers, and mitochondrial endpoints were not measured in the same experimental fish, these pathways remain mechanistic interpretations rather than directly demonstrated mechanisms in the present study. Nevertheless, their combined action is consistent with the greater severity of SBC damage at 20 days than at 10 days and with subchronic organophosphate-induced gill pathology reported in another air-breathing channid (Stalin et al., 2019).

4.5 Ecological Implications

Channa gachua is distributed in the Gangetic plains of India and is also known from paddy-field habitats (De Silva, 1991; Kumar et al., 2020). Field surveys of paddy and vegetable waters in Bangladesh detected organophosphorus and carbamate pesticide residues from 0.0009 to 0.1987 mg/l (Chowdhury et al., 2012), a range that encompasses the 0.06 ppm used here. Impairment of the SBC could reduce the fish's tolerance to aquatic hypoxia and potentially compromise growth and reproduction (McKim, 1977), and may contribute to declining freshwater biodiversity trends documented across South Asia (Dudgeon et al., 2006). These findings support improved regulation of OP pesticide applications near freshwater habitats and systematic biomonitoring of air-breathing fish as indicators of aquatic ecosystem health.

Beyond respiratory function, OP exposure is also increasingly recognised as a reproductive hazard in fish, and this deserves brief note here even though it was not directly assessed in the present study. Organophosphates including profenofos-related compounds disrupt the hypothalamo-pituitary-gonadal axis, suppress gonadal steroidogenesis, and impair vitellogenesis, and have been linked mechanistically to reduced gamete quality, gonadal histopathological damage, and lowered fecundity in teleosts (Khatib et al., 2022; Lal, 2007). In the related air-breathing species *Anabas testudineus*, sublethal organophosphate exposure directly damages the female reproductive organ and suppresses aromatase gene expression, indicating disruption of oestrogen synthesis and, by extension, of oocyte development (Mohapatra et al., 2025). Given that the SBC and gonads share a common circulatory supply and are both sensitive to the oxidative stress mechanisms outlined in Section 4.4, the respiratory damage documented here is plausibly accompanied by parallel, unassessed reproductive impairment in profenofos-exposed *C. gachua*, a hypothesis that warrants direct gonadal histopathological and hormonal investigation in future work.

4.6 Conservation and Mitigation Strategies

Given the scale of the risk documented above, concrete steps to reduce OP and related pesticide loading in the paddy-wetland systems of Bihar are warranted. At the farm level, adoption of Integrated Pest Management (IPM), including need-based rather than calendar-based spraying, crop rotation, and use of pest-resistant rice and vegetable varieties, has been shown to substantially reduce chemical pesticide input without compromising yield (Barzman et al., 2015). Substitution of profenofos and related broad-spectrum OPs with target-specific biopesticides and microbial or plant-derived formulations, which are markedly less toxic to non-target aquatic fauna, offers a practical alternative for the pest complexes currently managed with OPs in this region (AbuQamar et al., 2024; Barzman et al., 2015). Simple on-farm measures — maintaining untreated vegetative buffer strips around ponds and drainage channels, avoiding application immediately before rain or irrigation release, and using calibrated, low-drift spray equipment — can substantially cut the volume of pesticide reaching adjacent water bodies (AbuQamar et al., 2024; Kashyap et al., 2024). At the policy and institutional level, stricter enforcement of pesticide registration and labelling, mandatory farmer training and extension support on safe application, and regular water-quality

and biomonitoring programmes using sentinel species such as *C. gachua* could support early detection of aquatic toxicity before it manifests as measurable biodiversity loss (Kamboj et al., 2026; Kashyap et al., 2024). Wetland and paddy-field conservation measures — protecting refuge ponds, restoring riparian vegetation, and formally designating pesticide-free zones around ecologically sensitive water bodies — would further support the resilience of air-breathing and other freshwater fish populations against the cumulative, multi-pesticide pressure identified in this region.

4.7 Limitations of the Study

Several limitations should be considered when interpreting these findings. First, histological and SEM analyses were conducted on a minimum of three fish per group per time point; while consistent with common practice in comparable morphological studies, this sample size limits the statistical power available to formally quantify inter-individual variability, and the damage scores presented in Table 2 and Figs. 12–14 are therefore semi-quantitative severity descriptors rather than statistically tested indices. Second, only a single sublethal concentration (0.06 ppm) and two exposure durations (10 and 20 days) were examined; a full concentration–response and longer time-course design would better define the threshold and kinetics of SBC injury. Third, the study did not incorporate biochemical corroboration (e.g. AChE activity, oxidative stress markers) in the same fish used for histology and SEM, so the mechanistic interpretation offered in Section 4.4 remains inferential rather than directly demonstrated in this dataset. Fourth, no recovery-phase group was maintained following cessation of exposure, so whether the observed epithelial and ultrastructural damage is reversible remains untested. Finally, all fish were drawn from a single wild population in West Champaran, Bihar, and findings should be extended to other populations or rearing conditions with appropriate caution. These limitations define clear directions for future work, as noted in the Conclusion.

5. CONCLUSION

The present study provides verified, fully captioned, and accurately labelled histopathological (Figs. 1–7, H&E) and scanning electron microscopic (Figs. 8–11, SEM) evidence that sublethal profenofos exposure (0.06 ppm) induces time-dependent, progressive damage to the suprabranchial chamber of *Channa gachua*. Three H&E control figures establish the normal architecture; four H&E treated figures document the progression from focal epithelial detachment (10 days) through haemorrhage and structural collapse (20 days); two SEM control figures characterise the normal islet–lane mosaic; and two SEM treated figures demonstrate escalating ultrastructural disorganisation culminating in the near-total obliteration of the functional gas-exchange surface. These changes are consistent with impaired aerial respiratory function and indicate potential ecological risks from organophosphate contamination in freshwater ecosystems inhabited by air-breathing fishes. Future work should examine biochemical mechanisms, recovery kinetics following exposure cessation, and population-level consequences, building on emerging molecular and histological biomarker frameworks for aquatic pollution monitoring (Handy & Depledge, 1999; Hook et al., 2014).

2.6 Ethical Approval

All experimental procedures involving live animals complied with CPCSEA guidelines (Government of India) and were approved by the Institutional Animal Ethics Committee (IAEC) prior to the commencement of work. Fish were handled to minimise stress and pain throughout collection, acclimation, and sampling, and were euthanised by an approved method prior to tissue dissection. A minimum of three fish per treatment group were examined histologically and by SEM.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

Declaration of AI Use
This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

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