



## Research Article

# Occurrence of neoplastic lesion in spotted catfish (*Arius maculatus* Thunberg, 1792) from the estuarine environment, southeast coast of India

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Present study reports the neoplastic lesions in *Arius maculatus* (Spotted catfish), widely distributed species across the coastal waters of India, a phenomenon previously underreported for this species. A wild adult female *Arius maculatus* from the Vellar estuary, India, was found with a significant tumor. The gross morphology revealed a mass of fleshy, tumorous outgrowth located ventrally near the mandibular region, measuring approximately 2.5 cm × 2.6 cm × 2 cm, and presenting as an irregular, reddish-white. Comprehensive pathological examination, including histological analysis, revealed that the tumour exhibited aggregated eosinophils and anucleated inflammatory cells in areas of necrosis. Notably, no metastasis was detected, and no internal organs showed abnormalities. This report concludes that this is the first case documenting neoplastic lesions in *Arius maculatus*, histopathologically diagnosed as eosinophilic coagulative necrosis that may arise from tissue damage, contributing to the understanding of fish pathology and highlighting the need for further research to elucidate the environmental or genetic factors influencing tumour development in this species.

[**Keywords:** Necrosis, Neoplasm, Spotted catfish, Tamil Nadu]

## Introduction

The spotted catfish, *Arius maculatus* (Thunberg, 1792), is a species within the family Aridae, known for its extensive distribution across the Indian Ocean. This species is commonly encountered along both the eastern and western coastlines of the Indian subcontinent, inhabiting a range of environments from marine to estuarine zones. The ecological versatility of *Arius maculatus* enables it to thrive across diverse aquatic habitats within this geographical region, as documented by De Bruin *et al.*<sup>1</sup>.

The marine environment is home to a diverse variety of physico-chemical and biological characteristics. Previous reports state that neoplasms are frequent in fish and are categorised similarly to those in higher animals<sup>2</sup>. Tumours are an increasing problem that can arise from infectious viruses, inherited genetic factors, or chemical carcinogens. The disease causes cells to become aggressive, invasive, and even metastatic<sup>3</sup>. Research has demonstrated that tumours are present in nearly all tissue systems of natural fish populations. Despite the widespread occurrence of these tumours, the majority remain etiologically uncharacterised, with their underlying causes largely unidentified. This

observation highlights a significant gap in understanding of tumorigenesis in aquatic species<sup>4</sup>.

The absence of an established etiology underscores the need for further investigation into the potential environmental, genetic, or biological factors contributing to tumour development in these fish populations. The most abundant class of vertebrates is the fishes. A large number of species are caught in large quantities due to their economic significance. Any neoplastic lesion that may be detected will likely be discovered when the catch is being marketed and processed for human consumption.

Fishes have been reported to be carrying all the major tumour types found in birds and mammals, including humans. However, there is a dearth of information on the prevalence of fish neoplasms in India<sup>5-7</sup>. Recent studies have documented a notable incidence of tumours in both cultured and wild fish species in India. Tumour occurrences have been reported in a variety of species, including *Mugil cephalus*<sup>8,9</sup>, *Sardinella longiceps*<sup>10-13</sup>, *Sphyræna barracuda*<sup>10</sup>, *Chanos chanos*<sup>14</sup>, *Sphyræna jello*<sup>15-17</sup>, *Lates calcarifer*<sup>18</sup>, *Arius jello*<sup>19</sup>, *Rastrelliger kanagurta*<sup>20</sup>, *Carassius auratus*<sup>21</sup>, and *Rhabdosargus sarba*<sup>22</sup> (Table 1). More recently, tumour incidence was reported in *Planiliza macrolepis*<sup>23</sup>. Understanding

Table 1 — Notable previous reports of fish tumour from Indian waters

S. No	Scientific name	Common name	Commercial importance	Species availability	References
1	<i>Mugil cephalus</i>	Flathead grey mullet	High-value food fish; aquaculture	Marine, Estuarine	Jithendran <i>et al.</i> , 2011; Singaravel <i>et al.</i> , 2016
2	<i>Sardinella longiceps</i>	Indian oil sardine	Major commercial fishery	Marine	Gopalakrishnan <i>et al.</i> , 2011; Sinduja <i>et al.</i> , 2014; Singaravel <i>et al.</i> , 2015, 2016
3	<i>Sphyraena barracuda</i>	Great barracuda	Game fish; local consumption	Marine	Gopalakrishnan <i>et al.</i> , 2011
4	<i>Chanos chanos</i>	Milkfish	Important aquaculture species	Marine, Estuarine	Ananda Raja <i>et al.</i> , 2012
5	<i>Puntius sarana</i>	Olive barb	Inland fishery; local markets	Freshwater	Sahoo <i>et al.</i> , 2012
6	<i>Sphyraena jello</i>	Pickhandle barracuda	Commercial; recreational fishing	Marine	Vijayakumar <i>et al.</i> , 2014; Singaravel <i>et al.</i> , 2017
7	<i>Lates calcarifer</i>	Asian seabass/ Barramundi	High-value aquaculture species	Marine, Estuarine	Vijayakumar <i>et al.</i> , 2015
8	<i>Arius jella</i>	Blackfin sea catfish	Coastal fishery; local markets	Marine, Estuarine	Singaravel <i>et al.</i> , 2016
9	<i>Rastrelliger kanagurta</i>	Indian mackerel	Important pelagic fishery	Marine	Singaravel <i>et al.</i> , 2016
10	<i>Carassius auratus</i>	Goldfish	Ornamental fish	Freshwater	Sood <i>et al.</i> , 2017
11	<i>Planiliza macrolepis</i>	Large-scaled mullet	Emerging commercial value	Marine, Estuarine	Yosuva Mariasingarayan <i>et al.</i> , 2024
12	<i>Arius maculatus</i>	Spotted sea catfish	Coastal fishery; local markets	Marine, Estuarine	Current study

the incidence and characteristics of these lesions may provide valuable insights into the health of aquatic ecosystems and the potential risks posed to fisheries and human consumers. This study aimed to investigate neoplastic lesions in wild-caught marine fish, *Arius maculatus*, from the estuarine environment of the southeast coast of India.

## Materials and Methods

### Collection of specimen

The specimen was collected during routine biological sampling conducted under the project, entitled “Taxonomy and Epidemiology of Pathogenic Virus and Fungi of Commercially Important Fin and Shell Fishes of the Coromandel Coast,” financially supported by the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India, New Delhi. The specimen was collected from the Vellar Estuary 11°29'51.17" N, 79°46'29.63" E (Fig. 1) in June 2024. The fish, a wild-caught *Arius maculatus*, weighed 560 g, measured 44 cm in length, and exhibited a notable neoplastic lesion. Following collection, the specimen was carefully transported to the laboratory for detailed pathological examination. Species identification was performed using the FAO fish identification guide<sup>1</sup>.

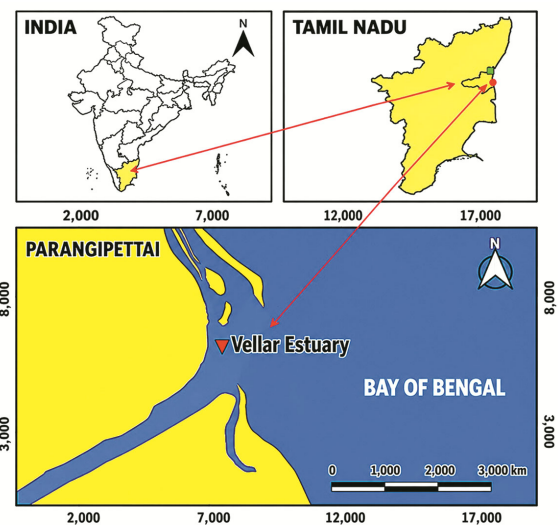


Fig. 1 — Map showing the location of Vellar estuary, Parangipettai, Southeast Coast of India

### Tumour gross observation

The tumour size, texture, colour, and morphology were all thoroughly documented. The fish was then subjected to an autopsy.

### Histopathology

The tumour lesions were removed gently from the tumour-affected portion of the specimen and

immediately fixed in 10 % Neutral-Buffered Formalin (NBF) for 48 h for proper fixation. This fixation was done to preserve the tissue integrity for further histological examination. After the period of fixation, the removed tumour tissues were prepared for histological study (Fig. 2). The tissues were placed in paraffin wax, and thin sections of 5  $\mu\text{m}$  were cut using a rotary microtome (LEICA RM2125 RTS). The tissue sections were mounted on glass slides, and stained for microscopic study. The tissue sections were stained with Hematoxylin & Eosin (H&E) according to the procedure described by Coolidge & Howard<sup>33</sup>. The sections were examined under a light microscope to observe the cellular features, tissue architecture, and tumour morphology.

#### Transmission electron microscopy

Glutaraldehyde-fixed neoplastic lesions were post-fixed in 1 % osmium tetroxide after being fixed in 0.1 M sodium cacodylate buffer containing 2 % glutaraldehyde. They were subsequently dried using an escalating ethanol series (50 – 100 %) and embedded in Epon plastic in accordance with standard histological protocols. To observe the sections under a light microscope, semi-thin (1  $\mu\text{m}$ ) sections were cut using an Ultratome-Nova microtome and stained with toluidine blue solution (to screen for orientation and lesion presence). Ultrathin sections (less than 100 nm) were cut with a Leica Ultracut UC7 ultramicrotome, stained with methylene blue, and then double-stained with lead citrate and Uranyl acetate. The stained

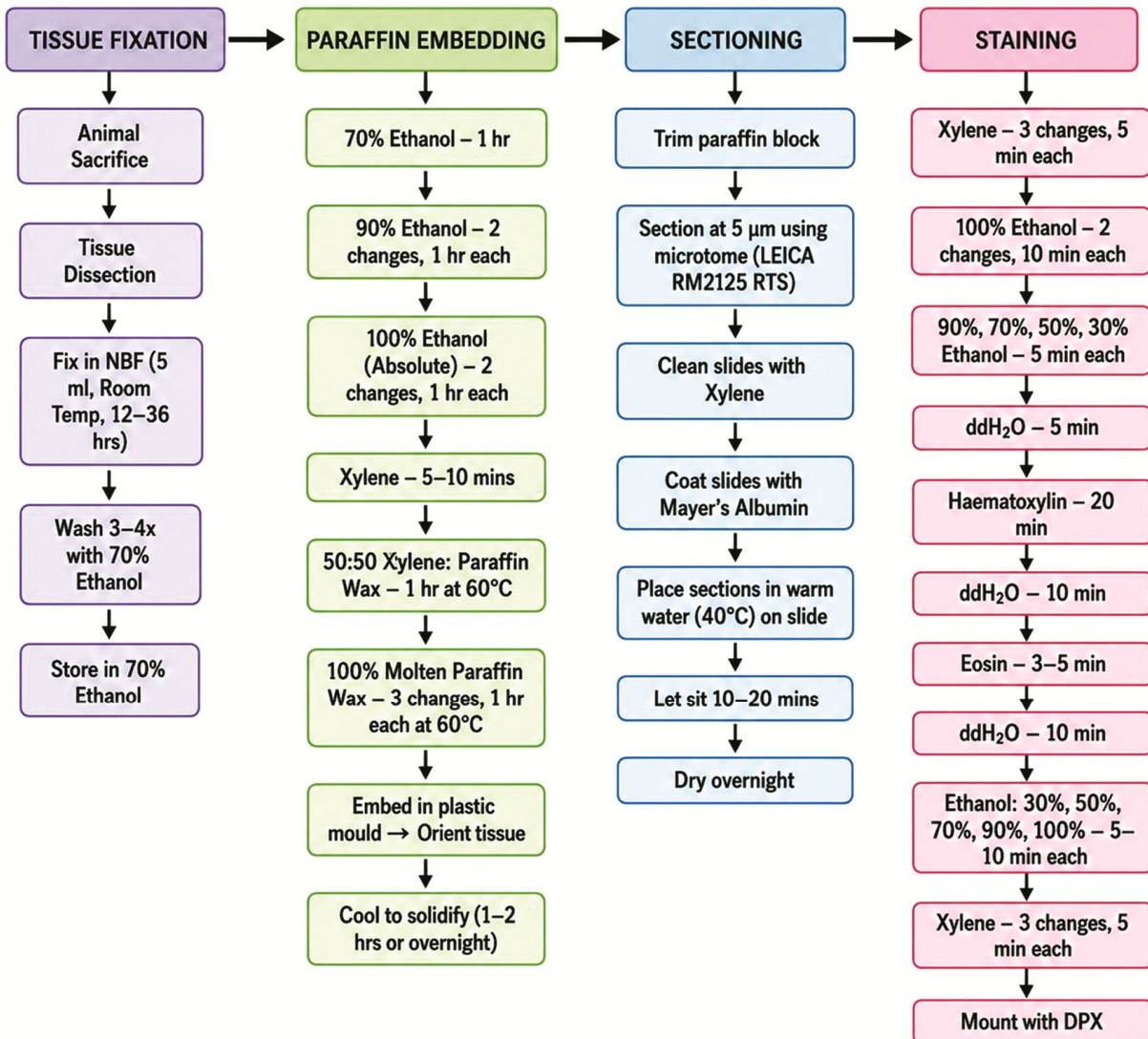


Fig. 2 — Workflow of tissue processing for histological analysis showing sequential steps of tissue fixation, paraffin embedding, sectioning, and Hematoxylin - Eosin (H&E) staining

lesions were examined at 80 kV using a Philips Tecnai T12 transmission electron microscope.

## Results

### Tumor observation

The specimen in question exhibited a notable tumour-like mass characterized by significant tissue

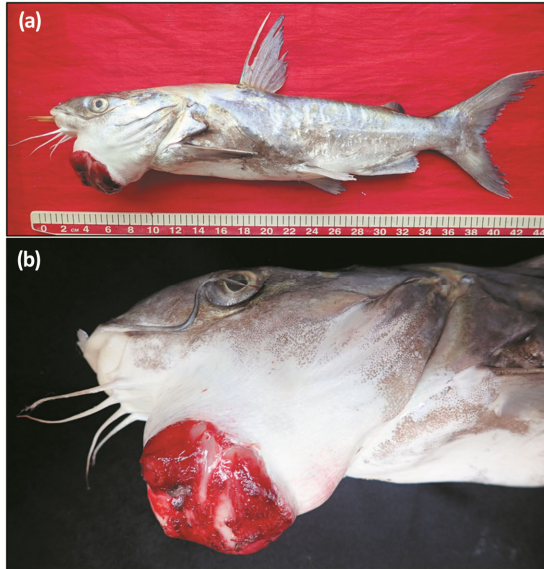


Fig. 3 — (a) *Arius maculatus* tumor lesion on ventral side of the mandible region; and (b) Close up view of the tumor lesion

proliferation. This mass was prominently located ventrally on the lateral side of the fish, emerging from the muscular tissues near the mandibular region. The tumour presented as an irregularly round, reddish-white, fleshy growth measuring approximately 2.5 cm × 2.6 cm × 2 cm (Fig. 3). Upon dissection, the surface of the mass revealed a homogeneous, hard texture with a whitish colour. Comprehensive examination of the remaining organs in the fish showed no visible lesions or abnormalities. Furthermore, necropsy findings indicated the absence of gross evidence of local invasion or metastatic spread to other visceral organs of *Arius maculatus*. This thorough assessment suggests that the observed tumour is localized and does not appear to affect other internal tissues or organs.

### Histopathology

Histopathological examination of H&E-stained tissue sections from the reddish tumour lesion revealed distinct pathological alterations in both the affected lesion and adjacent muscle tissue (Fig. 4). The peripheral region of the neoplastic lesion showed aggregation of eosinophilic material associated with anucleated cells, indicating areas of coagulative necrosis and tissue degeneration (Fig. 4a). Higher magnification of the inner region demonstrated active eosinophilic inclusions beneath the affected tissue

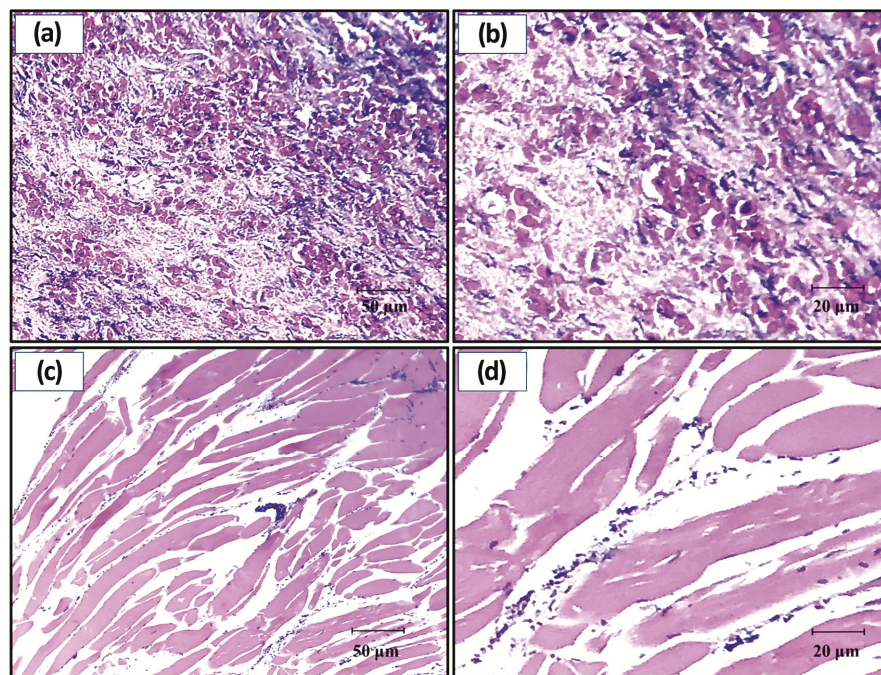


Fig. 4 — Histopathological micrographs of tissue sections showing neoplastic lesions at different magnifications: (a) Peripheral region of the neoplastic tissue showing accumulation of eosinophilic material with anucleated structures (50 µm scale bar); (b) Inner region of the neoplastic tissue showing active eosinophilic inclusions (20 µm scale bar); (c) Low-magnification view of muscle tissue architecture (50 µm scale bar); and (d) High-magnification view of muscle tissue displaying structural details (20 µm scale bar)

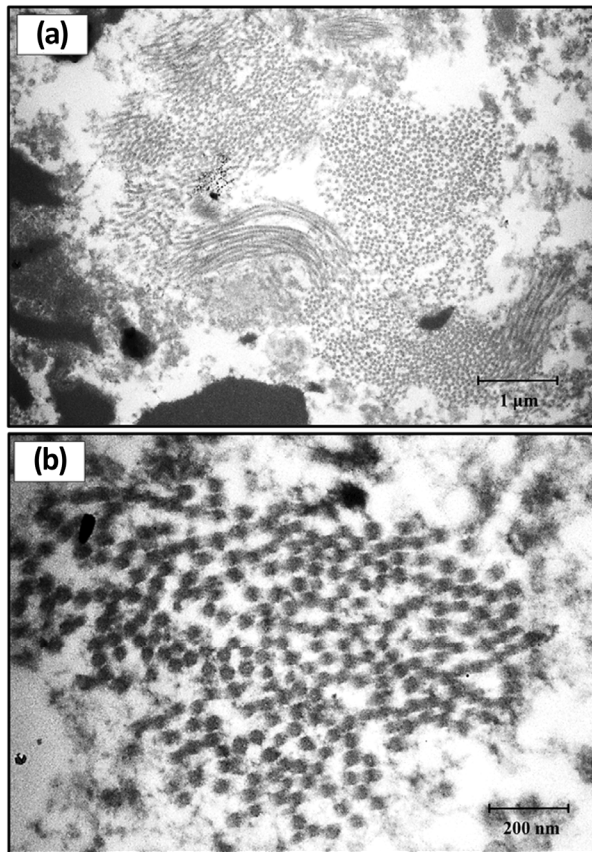


Fig. 5 — Transmission electron micrographs of ultra-thin sections of neoplastic lesion: (a) Aggregation of viral particles; and (b) Cell showing the electron dense viral particles

area, suggesting ongoing cellular damage and inflammatory response (Fig. 4b). In contrast, the muscle tissue sections displayed relatively organized muscle fibers with preserved architecture at both low and high magnifications (Fig. 4c, d). The histological findings collectively indicate localized tissue injury associated with eosinophilic infiltration and degenerative changes within the neoplastic lesion.

#### Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) of ultra-thin sections of neoplastic lesions from infected fish shows viral particle aggregation within cells, suggesting active viral replication (Fig. 5). The viral particles are electron-dense structures. The aggregation indicates a high viral load in the infected tissue, which may indicate viral causation of the neoplastic lesion. The finding of these electron-dense viral particles within the tumour cells shows the potential that the virus may play a critical role in the pathogenesis of the neoplasia, resulting in tumour formation.

#### Discussion

Fish tumours have been documented in nearly every organ, with skin tumours among the most identifiable due to their distinct appearance and clear pathological characteristics<sup>24</sup>. The skin is affected in approximately 50 % of fish tumours<sup>25</sup>. In this study, it is important to note that hyperplastic growths represent early stages of abnormal tissue proliferation and may not always immediately exhibit dysplastic or anaplastic changes. In the present case, histopathological features indicated abnormal tissue growth, significant eosinophilic infiltration, and coagulative necrosis. Hyperplastic lesions can sometimes be precursors to neoplastic transformation, especially under conditions of chronic injury or persistent inflammation. Therefore, the observed lesion can reasonably be classified as a neoplastic lesion, especially since it represents an abnormal, uncontrolled proliferation of tissue distinct from normal architecture. One of the significant pathological features observed in fish tumour studies is the presence of *eosinophils coagulative necrosis*, a form of cell death characterised by a marked infiltration of eosinophils in the affected tissue<sup>26-30</sup>.

The term eosinophils coagulative necrosis refers to the appearance of tissue undergoing coagulative necrosis, where eosinophils, white blood cells that stain pink when treated with hematoxylin and eosin, accumulate at the site of injury. This accumulation causes the affected tissue to appear intensely pink, or eosinophilic, under a microscope. Coagulative necrosis occurs when cellular proteins denature due to ischemia, preserving the cell's structure despite their death. This phenomenon is commonly seen in conditions with elevated eosinophil counts, such as allergic reactions, parasitic infections, and specific malignancies<sup>31</sup>. The presence of eosinophils in the necrotic tissue suggests a tissue injury that has attracted these cells to the site, leading to the characteristic bright pink staining and anucleated, cell-shaped remnants observed histologically<sup>32</sup>. In the present study, the neoplastic lesion observed in the affected fish displayed eosinophils coagulative necrosis, with features consistent with previous reports<sup>26-30</sup>. These features included eosinophilic cytoplasm, anucleated cells, and large hyperchromatic nuclei with prominent nucleoli. Based on these characteristics, the diagnosis of *eosinophils coagulative necrosis* was established. The eosinophils coagulative necrosis observed in this case likely resulted from harmful stimuli, either exogenous or

endogenous, leading to neoplastic transformation in the affected tissue. The presence of eosinophilic necrosis may not only indicate tissue damage but could also play a role in triggering or coinciding with neoplastic transformation, which may ultimately lead to tumour formation.

In the present investigation, repeated attempts were made to amplify viral sequences by PCR; however, successful molecular detection was hindered by several factors. The virus can be a new strain or carry mutated genomic segments, leading to primer mismatches and consequently, PCR failure. Furthermore, the viral load in the lesion may have been very low, below the detection limit of standard PCR procedures. RNA degradation, a frequent problem due to the delicate nature of viral RNA and the environmental exposure inherent to wild-caught specimens, may also have contributed to detection problems. In addition, natural PCR inhibitors, including lipids and proteins native to fish tissues, may have disrupted amplification efficiency. Despite these technical challenges, the demonstration of electron-dense viral-like particles by TEM is very suggestive of a viral infection, if not necessarily the sole cause of the neoplastic lesion. More detailed molecular analyses are to be performed to ascertain the viral etiology. It is important to note that field-sampled wild fish often pose greater challenges for precise molecular diagnostics than laboratory-reared animals.

In line with these findings, electron microscopy revealed viral-like particles in the affected tissue, which may offer insight into the etiology of this neoplasm. These particles could represent the primary cause of the neoplastic transformation, suggesting that a viral infection may have triggered both the necrosis and the neoplastic process<sup>13,15,22</sup>. Alternatively, the viral-like particles might represent a secondary infestation or an opportunistic infection that occurred following physical injury or tissue inflammation, which in turn could have contributed to the development of the neoplasm.

In conclusion, the findings of eosinophils coagulative necrosis in combination with viral-like particles, suggest a multifactorial process involving both tissue damage and potential viral involvement. Further investigation is needed to determine the exact role of these viral-like particles in the pathogenesis of the neoplasm and their potential as a primary etiological agent or as a secondary consequence of tissue injury.

## Conclusion

The present study documents the occurrence of a neoplastic lesion in the spotted catfish, *Arius maculatus*, collected from the Vellar estuary, southeast coast of India. Gross examination revealed a localized tumour-like growth in the mandibular region, while histopathological analysis demonstrated eosinophilic infiltration, coagulative necrosis, and degenerative tissue changes associated with the lesion. Furthermore, transmission electron microscopy revealed the presence of electron-dense viral-like particles within the neoplastic tissue, suggesting a possible association between viral infection and tumour development. Although the exact etiology remains uncertain, the findings indicate that the lesion may result from a multifactorial process involving tissue injury and potential viral involvement. This study represents the first report of such a neoplastic lesion in *A. maculatus* and contributes to the understanding of fish pathology in estuarine environments. Further molecular and epidemiological investigations are required to clarify the causative factors and determine the role of viral agents in the pathogenesis of these lesions.

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## Conflict of Interest

The authors declare that they have no conflict of interests.

## Ethics Statement

This article does not contain any studies involving human participants or live animals performed by any of the authors.

### Author Contributions

KV: Conceptualization, formal analysis, methodology, data curation, and writing-original draft preparation. AG: Supervision.

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