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Histopathological evaluation of *Wallago attu* (Bloch & Schneider, 1801) infested by ectoparasites

Anu prasanna Vankara^{1*} , Chandrasekhar Thummala², Riazunnisa Khateef³ and Rafeeq Ahammed Peddinti¹

Abstract

Background: Host-parasite interaction studies represent the dependency of the parasites on the host through various models. Gill infesting copepods, monogeneans and isopods served as excellent models to study the details of host-parasite interaction. In the present investigation, *Wallago attu* (Bloch & Schneider, 1801) was extensively studied for histopathological damage caused by ectoparasites like monogeneans (*Thaparocleidus indicus*, *Thaparocleidus wallagonius* and *Mizelleus indicus*), copepod (*Ergasilus malnadensis*) and an isopod (*Alitropus typus*) during August 2017 to February 2018. Both the control (least infested) and severely infested gills were examined through light microscope to approximate the intensity of damage caused by above ectoparasites.

Results: Pathological effects of ectoparasites displayed a fusion of secondary lamella, hyperplasia of the gill filaments and the epithelial cells, propagation of bronchial tips, thinning of the central axis, deshaping, shortening and fusion of secondary gill lamellae epithelium, severe degenerative and necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucous cells proliferation.

Conclusions: Ectoparasites cause severe damage to gills of *W. attu*, thereby diminishing the gill breathing capacity of the fish. Timely pathological studies can reveal the extent of damage caused by the parasites and thus various diagnostic programs and best management practices can be implemented in aquaculture for the better yield.

Keywords: Copepods, Gills, Histopathology, Host-parasite interactions, Isopods, Microscopy, Monogeneans

Background

Ectoparasitic infestation of wild and farmed freshwater and marine fishes caused by crustaceans, monogeneans, protozoans and isopods may severely affect the host health (Johnson et al., 2004; Boxshall, 2005; Shinn et al., 2015; Modi and Vankara, 2021). Branchiuran copepod parasites (*Argulus*) and ergasilids can cause severe impact on the freshwater fish production (Møller, 2009). Ergasilids are found mainly on the gills of fish which are specialized to perform various

functions like gaseous-exchange, hormone production, circulation, ionic and acid–base balance and excretion (Pelster and Bagatto, 2010; Lima et al., 2013; Pádua et al., 2015). The infested gill tissues hinder the water flow and diminish the capability of the fish to sustain normal oxygen uptake (Ojha and Hughes, 2001). Monogeneans do not require any piercing mouthparts to feed on gill blood and need much effort to damage thin walls of the secondary lamellae, perhaps by suction or by applying of digestive secretions. Till date commendable work on gill histopathology of wild and farmed freshwater and marine fish was carried out by Roubal (1999), Campos et al. (2001), Dezfuli et al. (2003), Avenant-Oldewage et al. (2005), Vinobada (2010), Mohammedi et al. (2012), Kaur and Srivastava (2014), Singh and Kaur (2014), Weli et al. (2017), Mathews et al.

*Correspondence: annuprasanna@gmail.com; dr.anu@yogivemanauniversity.ac.in

¹ Department of Zoology, Yogi Vemana University, YSR District, Kadapa 516005, Andhra Pradesh, India

Full list of author information is available at the end of the article

(2018), and Suliman et al. (2021). Histopathological changes can be extensively used as biomarkers in the appraisal of health condition of fish (Roberts, 2001). The exploitation of these histopathological biomarkers in environmental monitoring permits the inspection of specific organs (lungs, gills, liver, kidney, etc.) accountable for vital functions such as respiration, excretion, accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001). The thorough information of histology gives a crystal clear idea to differentiate the pathological changes induced by the parasites in infested and control tissues. The type and intensity of mechanical activity exerted by the ectoparasites are the main causes of the tissue damage and severity of the effect, but the secondary infections through bacteria, virus and fungi in the wounds produced by crustacean parasites are considered to be more dangerous. The freshwater shark, *Wallago attu* (Bloch & Schneider, 1801) is one among the 10 largest catfish species on earth and are commercially important species harvested across tropical Asia owing to its high nutritional quality and consumer demand (Thella et al., 2018). In the present study, the control gill tissue (least infested)

and the infested gill tissue (with severe infection) of the *W. attu* were considered to note the pathological changes induced by the ectoparasites.

Methods

About 95 freshwater shark (*W. attu*) (11.52 ± 1.95 cm, mean total length \pm standard deviation; total length ranged from 7 to 15 cm and 293.15 ± 100.9 gms mean total weight \pm standard deviation; total weight ranged from 150 to 500 g) were obtained from the two different sampling stations of YSR Kadapa District (Lat. $14^{\circ}28'N$ $78^{\circ}49'E$, 137 m altitude), located in Andhra Pradesh State during August 2017 to February 2018. The two sampling sites include Adinimmayapalli Dam across the river Penna in Chennur Village (Lat. $14^{\circ}34'0.12''N$, $78^{\circ}48'0''E$ longitude), YSR Kadapa District (Site-I, $n=34$) and backwaters of Somasila reservoir across the river Penna in Somasila Village ($14^{\circ}29'22'' N$ $79^{\circ}18'19''E$) Nellore District that reach near Vontimitta Village in YSR Kadapa District (Site-II, $n=61$) (Fig. 1). Fish were examined for parasitic diagnosis and gross pathology under the stereo zoom microscope (LM-52-3621 Elegant) and light microscope Lynx trinocular microscope (N-800M) at parasitology laboratory,

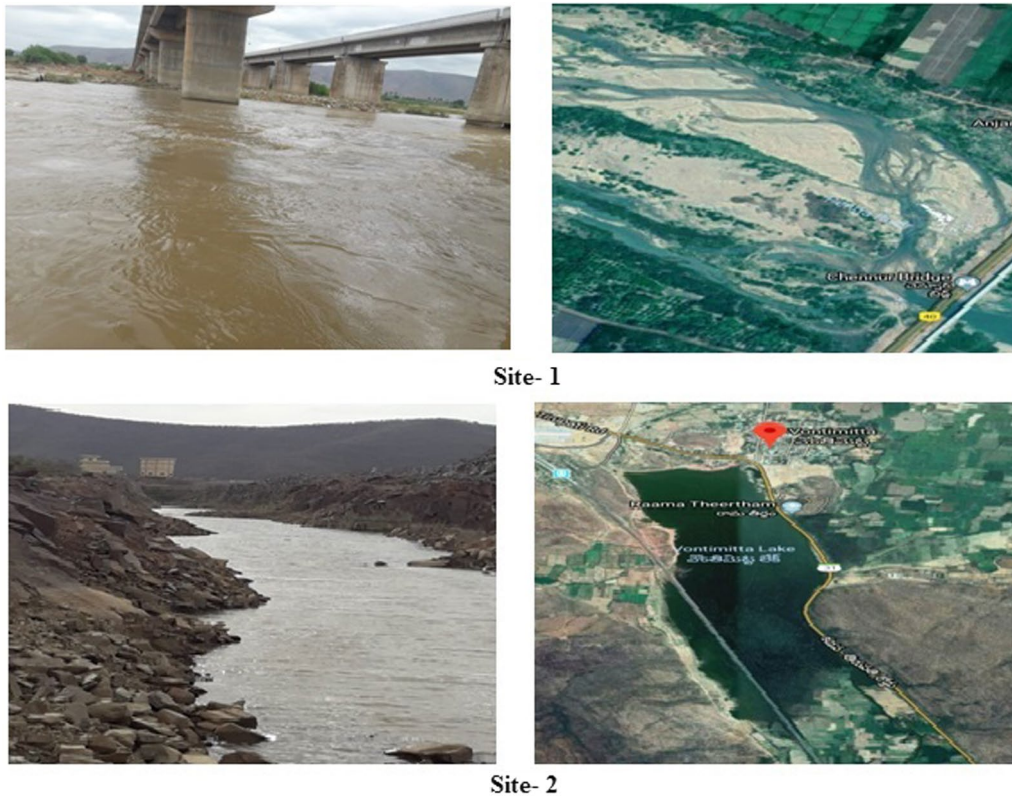


Fig. 1 Geographical location of the two fish sampling sites of YSR Kadapa District, Andhra Pradesh. Site-1: Adinimmayapalli Dam across the Penna River in Chennur Village, YSR Kadapa District. Site-2: Backwaters of Somasila reservoir across the river Penna in Somasila Village, Nellore District, Andhra Pradesh reach near Vontimitta Village, Kadapa

Department of Zoology, Yogi Vemana University, India. The temporary slides of monogenetic trematodes were prepared using neutral red ($C_{15}H_{17}N_4Cl$) and ammonium picrate-glycerine mixture by the method of Malmberg (1970) and were identified using the taxonomic volumes of Gusev (1976a, b) and Pandey and Agrawal (2008). The copepod and isopod parasites were fixed in 10% formalin solution and processed according to the method of Pillai (1985). The fragments of both infested and uninfested gill arches were carefully removed and processed according to usual histopathological techniques, embedded in paraffin at 58 °C, sectioned at 4–8 μm with Yorco YSI 115 rotary microtome, and stained in hematoxylin-eosin. The slides were investigated and photomicrographs were obtained using drawing tube. Ocular micrometer measurements were depicted in micrometers (μm) using Image J software (<https://imagej.nih.gov/ij/download.html>) unless indicated.

Results

Of the 95 examined fish, 94 fish ($P = 98.6\%$) were found be infected with one or more parasites. The diseased fish showed distinct alterations such as focal and multifocal paleness and necrosis, hemorrhages, and congestion signs on the gill filaments. In the current work, gills of *W. attu* were found infested with three monogeneans, i.e. *Thaparocleidus indicus* (Kulkarni, 1969) Lim, 1996 ($n = 688$, $P = 55.8\%$, $MI = 13.0$), *Thaparocleidus wallagonius* Jain, 1952, ($n = 405$, $P = 56.8\%$, $MI = 7.5$) and *Mizelleus indicus* Jain, 1957 ($n = 3$, $P = 2.1\%$, $MI = 1.5$), one parasitic copepod *Ergasilus malnadensis* Venkateshappa, Seenappa & Manohar, 1998 ($n = 2096$, $P = 98.6\%$, $MI = 22.8$) and an isopod, *Alitropus typus* Milne-Edwards, 1840 ($n = 10$, $P = 9.5\%$, $MI = 1.11$) was also found in *W. attu* (Figs. 2a–d, 3a–e). These parasites attach to all the parts of gill and lead to the damage of the tissue. Normal gill histology revealed the intact nature of both primary and secondary lamellae whereas

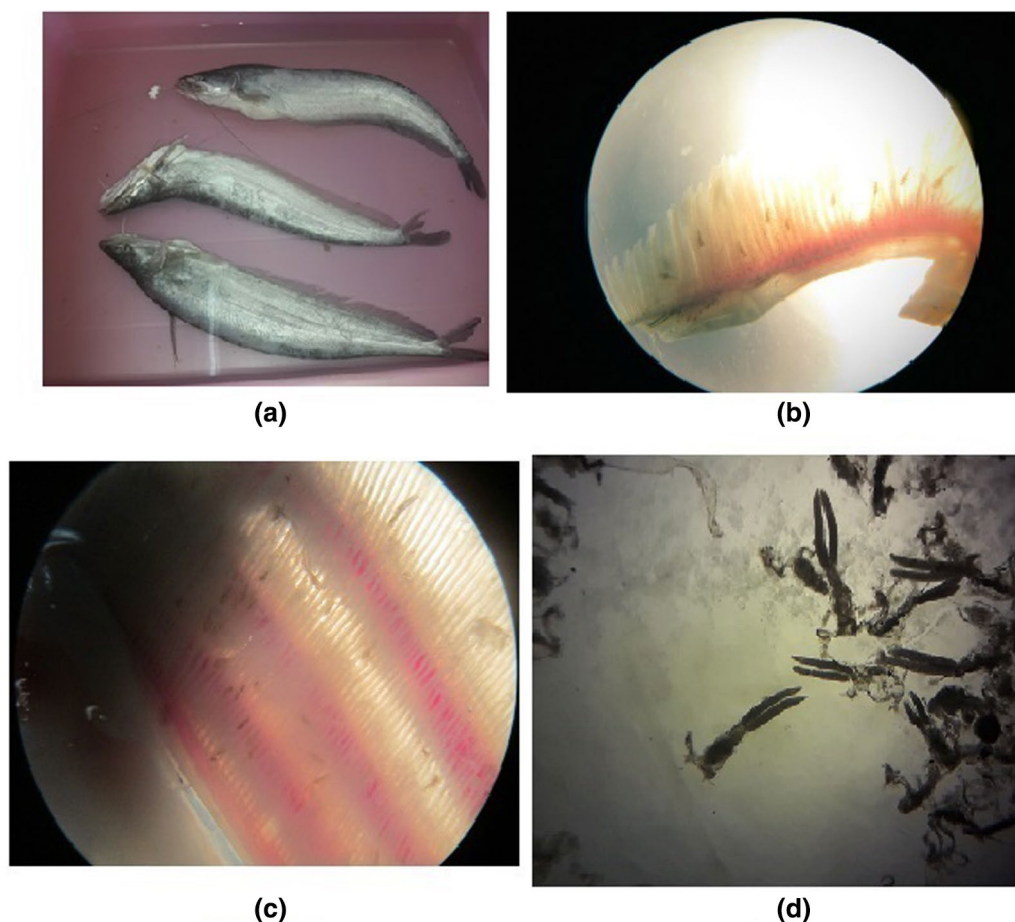


Fig. 2 a *Wallago attu* infested with ectoparasites, b Gill rakers of *W. attu* showing adult and larval stages of *Ergasilus malnadensis* and monogeneans *Thaparocleidus indicus* and *T. wallagonius*, c adult copepod, *E. malnadensis* attached to the gill rakers of *W. attu*, d adult copepod, *E. malnadensis* in embryo cup

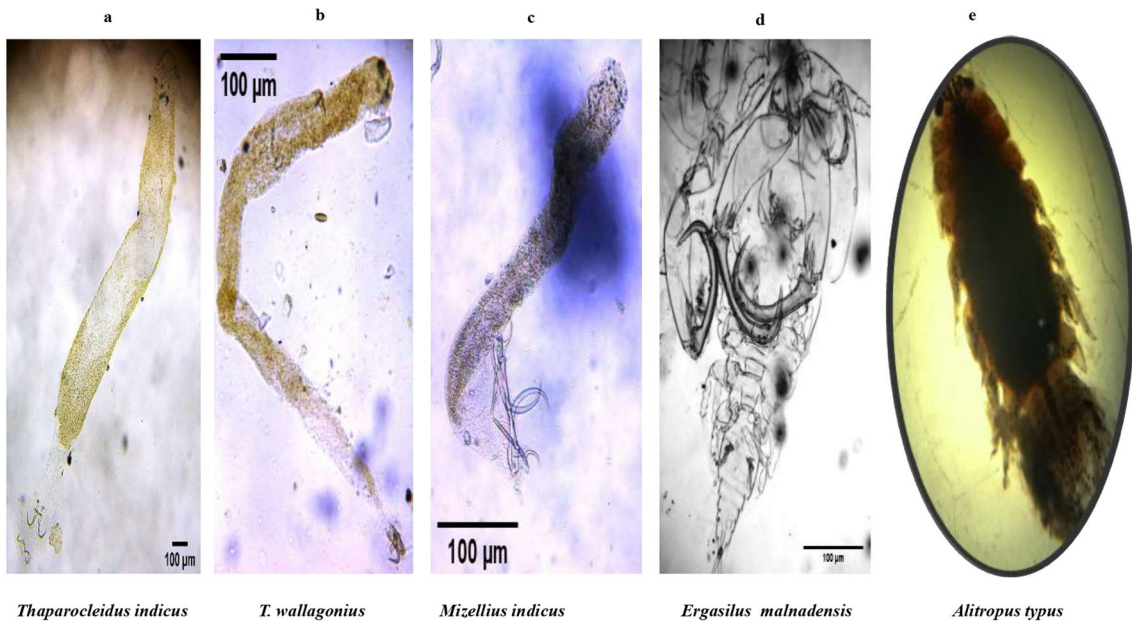


Fig. 3 a–e Microphotographs of ectoparasites of *W. attu*, **a** *Thaparocleidus indicus*; **b** *Thaparocleidus wallagonius*; **c** *Mizellius indicus*, **d** *Ergasilus malnadensis*, **e** *Alitropus typus*

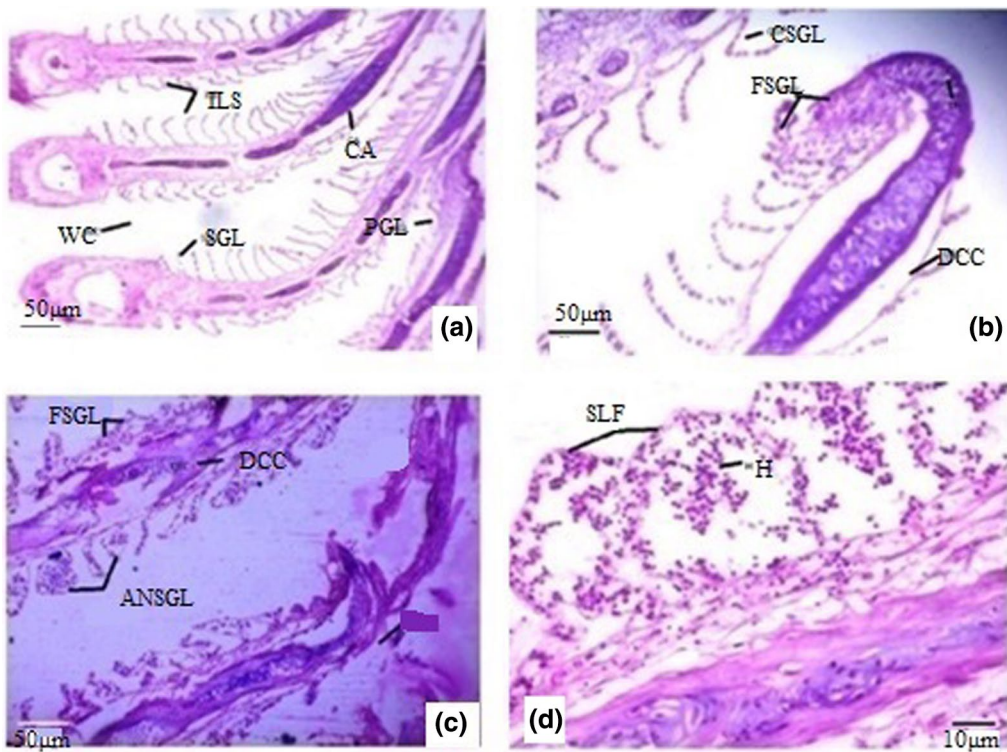


Fig. 4 **a** Normal gill with inter lamellar spaces (ILS), secondary gill lamella (SGL), water channels (WC). **b** Infested gill with curling of secondary gill lamella (CSGL), fusion of secondary gill lamella (FSGL), damaged central core (DCC). **c** Infested gill with fusion of secondary gill lamella (FSGL), damaged central core (DCC) and aneurysm in secondary gill lamella (ANSGL). **d** Infested gill with hyperplasia (H)

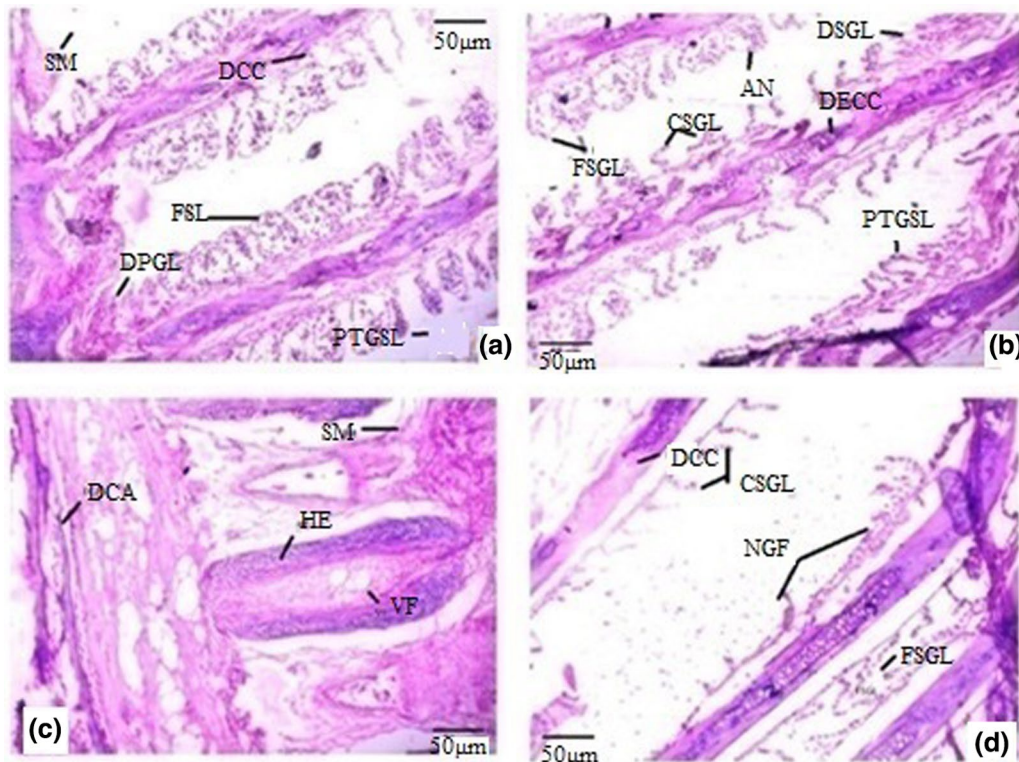


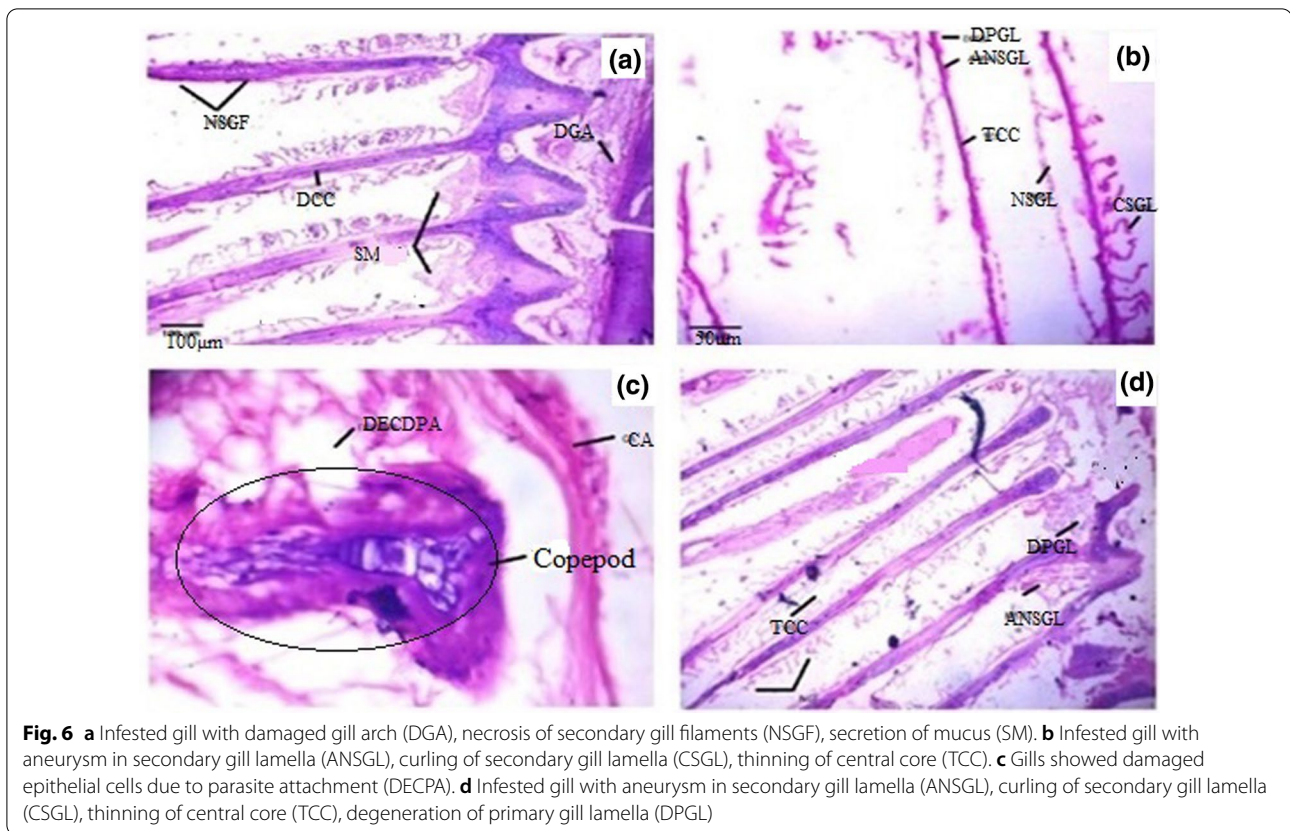
Fig. 5 **a** Infested gill showing secretions of mucus (SM), fusion of secondary gill lamella (FSL), degeneration of primary gill lamella (DPGL), proliferated tips of secondary gill lamella (PTGSL). **b** Infested gill with curling of secondary gill lamella (CSGL), fusion of secondary gill lamella (FSGL), damaged central core (DCC), proliferated tips of secondary gill lamella (PTGSL). **c** Infested gill showing degeneration of central axis (DCA), secretion of mucus (SM), vacuole formation (VF), hyperplasia of epithelial cells (HE). **d** Infested gill showing necrosis of gill filaments (NGF)

diseased fish had marked pathological changes in its gills. Histological section of the normal gills of *W. attu* revealed an intact structure with primary gill lamellae (PGL), secondary gill lamellae (SGL), interlamellar region (ILR) and intact water channels (WC) (Fig. 4a). Histopathological observation revealed the severe damage to primary gill lamellae and secondary gill lamellae, due to attachment and feeding by the parasite. In the present study, all infested *W. attu* showed color spots. Pathological effects of ectoparasites on the gills include curling of secondary gill lamellae (CSGL), a fusion of secondary lamellae (FSL), damaged central core (DCC) and aneurysm in secondary gill lamellae (ANSGL) (Fig. 4b, c). In a severe infestation, epithelial hyperplasia (EH) of different cell types was observed in both primary and secondary lamellae (Fig. 4d). Also there is proliferated tips of secondary gill lamellae (PTSGSL) (Fig. 5a), curling of secondary gill lamellae (CSGL) (Fig. 5b), secretion of mucus (SM), vacuole formation (VF), hyperplasia of epithelial cells (HE) (Fig. 5c), necrosis of gill filaments (NGF) (Fig. 5d), secretions of mucus from gill arch (SM) (Fig. 6a), mucus secretions

from epithelial cells (Fig. 6b) and copepod attachment to the epithelial cells (Fig. 6c) resulted thinning of a central core (TCC) (Fig. 6d). The section also exhibited the attachment of the copepod parasite near the gill arches.

Discussion

W. attu infested with ectoparasites (copepods, isopods and monogeneans) demonstrated serious lesions in the gill filaments such as necrosis, hemorrhages, congestion, epithelial and mucus cells proliferation. These alterations may impact the hydromineral balance, gaseous exchange, hormonal production, circulation, nitrogenous waste secretion and making the fish hypoxic or anoxic and finally lead to death and similar pathological results were also observed by Mohammadi et al., (2012) who worked on parasitic infestation of skin and gill on Oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*) and Dias et al. (2021) who worked on *Colossoma macropomum* (Serrasalmidae), respectively. The severity of respiratory damage is directly related to the number of parasites on the gills. The higher intensity, the more serious the gill damage



which can decrease the body weight and condition factor, severe changes in osmoregulation or respiratory dysfunction and ultimately lead to death. Ectoparasites such as monogeneans and copepods append to the primary lamellae with their anchors and legs resulted in cell damage and hemorrhages at the site of adherence. The oozed blood from the hemorrhagic site is fed by the parasite while some parasites secrete exogenous enzymes to digest the gill filaments of the host fish which can be witnessed in the gill histological sections with a high number of cell nuclei within the gut of gill parasites (Vinobada, 2010; Noga, 2010; Seenappa and Venkateshappa, 2000; Pádua et al., 2015). Though the gill epithelial cells are mechanically injured by the hooks of monogenean parasites and copepods (Purivirojkul, 2012), the greatest histopathological damage is induced by scraping and sucking activities of the parasites on host tissues as per the works of Derwa (1995), Endrawes (2001) and Hanna (2001).

The histological sections of the infested gills by the parasitic infection revealed the functional impairment of the gill lamellae, attachment of parasite at the tips of the gill filament, and swelling of the host tissue at the site of attachment. Pathological alterations observed in this study were similar to the works of Campos et al. (2001), Fadaei et al. (2001), Barzegar and Jalali (2004), Shamsi

et al. (2009), Kaur and Shrivastav (2014), Pádua et al. (2015) and Arya and Singh (2020) who also recorded hyperplasia of the epithelial cells and succeeding lamellar fusion to be the distinctive feature of the parasitic infection. Hyperplasia of epithelial cells and the fusion of some secondary lamella are the patterns of the defense mechanisms of gills. Severe stress in fish may result in some alterations in blood vessels and damaged cells cause blood congestion or even an aneurysm (Rosety-Rodriguez et al., 2002). The impact of the diseases caused by ectoparasites especially monogeneans and copepods are not only linked to health status of fish but also to economic and societal impacts in case of culture fish, due to diminished respiratory capacity obstructing the growth leading to high mortality rates (Dezfuli et al., 2011; Shinn et al., 2015). The imbalance in the environment can be detected through host-parasite relationships and the consequences caused by the parasites to its hosts. The present findings stress on the fact that diseases are serious threats to cultivable and wild fishes which can be visualized with naked eyes. Hence, regular diagnostic programs together with exceptional management practices need to be executed from time to time to avoid sudden parasitic outbreaks and economic losses in aquaculture.

Conclusions

In present study we have observed with the histopathological alterations induced by the ectoparasites on the gill tissues. The greatest histopathological damage in the gills of the *W. attu* was induced by scraping and sucking activities of the parasites on host tissues. Histopathological observations disclose the health status of the host fish, i.e., stunted growth, susceptibility to various diseases, resulting in hypoxia or anoxia and thereby leading to the death. Thus, the findings of the present work accomplished that ectoparasitic infestations are serious threats for fish and can cause extensive damage and yield.

Abbreviations

PGL: Primary gill lamellae; SGL: Secondary gill lamellae; ILR: Interlamellar region; WC: Water channels; CSG: Curling of secondary gill lamellae; FSL: Fusion of secondary lamellae; DCC: Damaged central core; ANSGL: Aneurysm in secondary gill lamellae; HE: Epithelial hyperplasia; PTSG: Proliferated tips of secondary gill lamellae; CSGL: Curling of secondary gill lamellae; SM: Secretion of mucus; VF: Vacuole formation; HE: Hyperplasia of epithelial cells; NGF: Necrosis of gill filaments; DECPA: Degeneration of epithelial cells due to copepod parasite attachment; TTC: Thinning of central core.

Acknowledgements

The corresponding author commemorates this work to late Dr. Asha Kiran Modi, who worked under the financial assistance of UGC Faculty improvement programme (FIP)-Award No.APSC021/001(TF)ZOOLOGY/PH.DXII PLAN/2016-17 dt. July 2016.

Author contributions

APV designed the work and RAP assisted late Dr. Asha Kiran Modi for the fish sample collection to carry out the pathological work. The second and third authors CST and RK helped in framing the manuscript and correction part. All the authors have read and approved the manuscript.

Funding

The work was funded to Late, Mrs. Asha Kiran Modi under UGC Faculty improvement programme (FIP)- Award No.APSC021/001(TF)ZOOLOGY/PH.DXII PLAN/2016-17 dt. July 2016.

Availability of data and materials

The raw data used to support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval and consent to participate

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by IAEC (Institution of Animal Ethics Committee-Regd. No.1460/PO/a/11/CPCSEA, dt. 20.05.2011), Zoology Department in Faculty of Life Sciences, Yogi Vemana University, Andhra Pradesh.

Consent for publication

Not applicable.

Competing interests

The authors declare that no competing interests exists.

Author details

¹Department of Zoology, Yogi Vemana University, YSR District, Kadapa 516005, Andhra Pradesh, India. ²Department of Environmental Science, Yogi Vemana University, YSR District, Kadapa 516005, Andhra Pradesh, India. ³Department

of Biotechnology and Bioinformatics, Yogi Vemana University, YSR District, Kadapa 516005, Andhra Pradesh, India.

Received: 30 November 2021 Accepted: 24 May 2022

Published online: 23 June 2022

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