

## **Ectoparasite-infested *Wallago attu* (Bloch & Schneider, 1801) histopathological examination**

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### **Abstract**

**Background:** Studies of the connection between the host and the parasite use a variety of models to illustrate how the parasites are dependent on the host. Host-parasite interactions may be studied in great depth using gill-infesting copepods, monogeneans, and isopods as ideal models. It was from August 2017 to February 2018 that the histopathological damage caused by monogeneans (*Thaparocleidus indicus*, *Thaparocleidus wallagonius*, and *Mizelleus indicus*), copepods (*Ergasilus malnadensis*), an isopod, and other ectoparasites on *Wallago attu* (Bloch & Schneider, 1801) was thoroughly examined in this study. The light microscope was used to examine both the control (the least infested) and the seriously infested gills to determine the extent of damage produced by the aforesaid ectoparasites.

**Results:** Secondary gill lamellae epithelium was reshaped, curled, and fused as a result of the pathological effects of ectoparasites, which included a fusion of secondary lamellae, hyperplasia of gill filaments and epithelial cells, bronchial tip propagation, a narrowing of the central axis, severe degenerative and necrotic changes in gill filaments and secondary lamellae, curled

**Conclusions:** The gills of *W. attu* are severely damaged by ectoparasites, which reduces the fish's gill breathing capacity. To improve aquaculture productivity, timely pathology examinations can disclose the full degree of parasite damage, allowing different diagnostic programs and optimal management methods to be followed.

**Keywords:** Anatomy and physiology of the host-parasite relationship, as well as histopathology and microscopic examination of the parasite.

### **Background**

Creatures like crustaceans, monogeneans, protozoans, and isopods may all have a significant impact on the health of the fish they infest, including wild and farmed fish as well as marine fish (Johnson et al., 2004; Boxshall, 2005; Shinn et al., 2015; Modi and Vankara, 2021). *Argulus* and *Ergasilida* parasites (*Argulus*) may have a significant influence on the production of freshwater fish (Miller, 2009). For

example, the gaseous exchange, hormone synthesis, circulation, acid–base balance and excretion all take place on the gills of fish (Pelster and Bagatto, 2010; Lima et al., 2013; Pádua et al., 2015). There is an effect on the waterfowl's capacity to consume oxygen because of infected gill tissues (Ojha and Hughes, 2001). To feed on gill blood, monogeneans don't need piercing mouthparts, and it takes a lot of effort to destroy the thin secondary lamellae walls by suction or digestive secretion application. On gill histopathology of wild and farmed freshwater and marine fish, Roubal (1999), Campos et al. (2001) and Dezfuli and Oldewage (2003), Vinobada (2010), Kaur and Srivastava (2014), Singh and Kaur (2014), Weli and Mathews (2017, 2018), and Suliman and Suliman et al. (2019) have all done commendable work (2021). Biomarkers based on histopathological alterations may be employed extensively in the evaluation of fish health (Roberts, 2001). It is possible to examine specific organs (lungs, gills, liver, kidney, etc.) that are responsible for vital functions like respiration and excretion as well as the accumulation and biotransformation of xenobiotics in the human body thanks to the use of these histopathological biomarkers in environmental monitoring (Gernhofer et al., 2001). The pathological alterations caused by parasites in infested and control tissues may be distinguished with clarity because to the in-depth knowledge of histology. Primary causes of tissue damage and severity of effect are mechanical activity exerted by ectoparasites, while secondary infections via bacteria, viruses and fungal fungus in the wounds generated by crustacean parasites are regarded more hazardous. Sharks like the freshwater *Wallago attu* (Bloch & Schneider, 1801) are economically valuable because of their excellent nutritional value and consumer demand in tropical Asia (Tella et al., 2018). The degenerative alterations generated by the ectoparasites were seen in the *W. attu*'s gill tissue that was least infested and most infested, respectively.

### **Methods**

Between August 2017 and February 2018, researchers in Andhra Pradesh collected 95 freshwater sharks (*W. attu*) from two different sampling stations in the YSR Kadapa District (Lat. 14°28'N 78°49'E, 137 m altitude) (mean total length standard deviation: 11.521.95 cm; total length: 7 to 15 cm; mean total weight standard deviation: 293.15100.9gms; total weight: 150 to

500g). Adinimayapalli Dam across the river Penna in Chennur Village (Lat.14°34'0.12 "N, 78°48'0 "E longitude), YSR Kadapa District (Site-I, n=34) and the backwaters of Somasila reservoir across the river Penna in Somasila Vil lage (14°29'22" N 79°18'19"E) Nellore District that reach near Vontimitta Village in YSR Kadapa District (Site-II, n=61) (Fig. 1). Using a stereo zoom microscope (LM-52-3621 Elegant) and a light microscope (N800M), fish were examined for parasite diagnosis and gross disease at the parasitology laboratory.

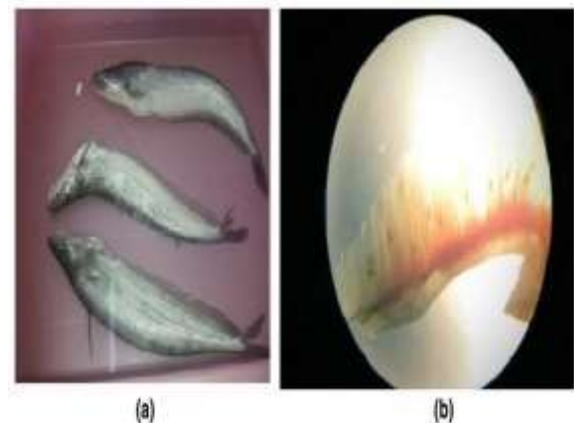


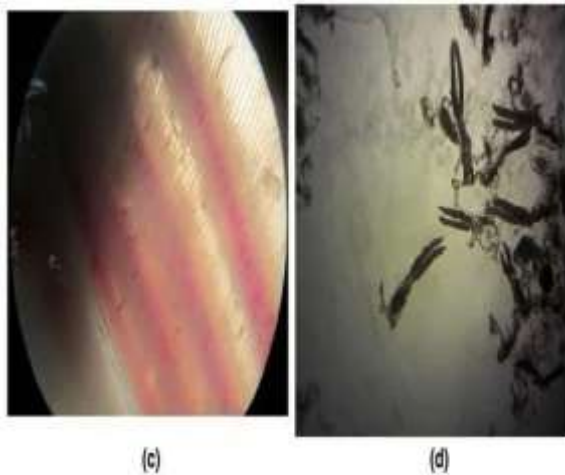
**Fig. 1**Andhra Pradesh, YSR Kadapa District has two fish sampling locations. Adinimayapalli Dam at Chennur Village, YSR Kadapa District, over the Penna River. On the other side of the Nellore River in Somasila Village, Andhra Pradesh's Nellore District, is the Vontimitta Village, home to the Kadapa Department of Zoology at Yogi Vemana University. The Malmberg (1970) technique was used to make the temporary slides of monogenetic trematodes, which were then identified using the taxonomic volumes of Gusev (1976a, b) and Pandey and Agrawal (Pandey and Agrawal and Pandey and Agrawal, respectively) (2008). The copepod and isopod parasites were fixed in 10% formalin solution and processed in accordance with Pillai's procedure (1985). After careful removal of both infected and

uninfested gill arches, they were gently embedded in parafn at 58 °C, sectioned using the Yorco YSI-115 rotary microtome, and stained in HematoxylinEosin. Using a drawing tube, we examined the slides and took photomicrographs. Micrometer measurements were made using Image J software ([www.imagej.nih.gov/ij](http://www.imagej.nih.gov/ij)). Unless otherwise stated, all measurements were made using ocular micrometers (m).

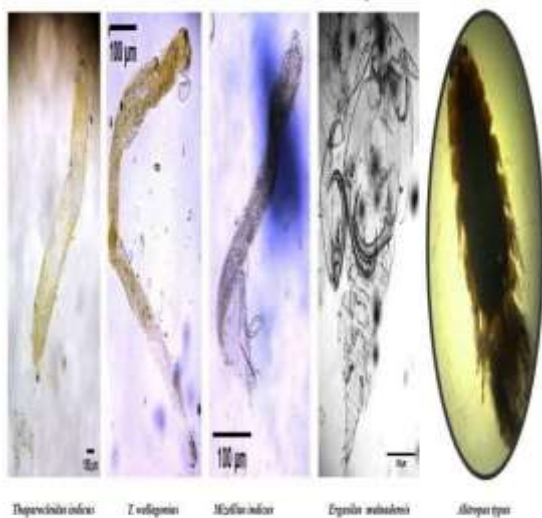
## Results

One or more parasites were found to be present in 94 of the 95 fish tested (P=98.6%). Anatomical changes in the afflicted fish included paleness, necrosis, hemorrhages, and congestive changes to the fish's gill flaps. *Taparocleidus indicus* (Kulkarni, 1969), *Taparocleidus wallagonius* (Jain, 1952), and *Mizelleus indicus* (Jain, 1957) were found infesting the gills of *W. attu* in the current study (n=688, P=55.8%, MI=13.0), while *Ergasilus malnadensis* (Venkateshappa, Seenappa & Manohar, 1998, n=2096, P=98.6%, MI=22.8) was found to be a parasitic copepod. The tissue of the gills is damaged by these parasites, which attach themselves to every portion of the gill. The main and secondary lamellae of a healthy fish's gills were found to be intact, however

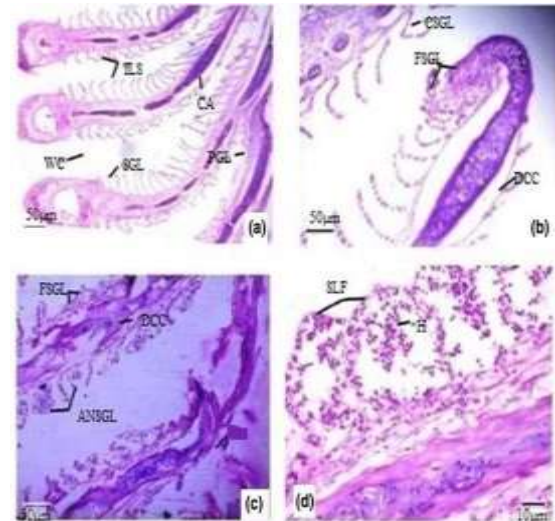




**Fig. 2** Ectoparasite-infested *Wallago attu*, b Gill rakers of *W. attu* displaying adult and larval *Ergasilus malnadensis* and monogeneans *Thaparocleidus indicus* and *T. wallagonius*, c adult copepod *E. malnadensis* clinging to *W. attu* gill rakers, d adult copepod *E. malnadensis* in an embryo cup, d



**Fig. 3** aAttu, *Thaparocleidus wallagonius*, *Mizelleus indicus*, *Ergasilus malnadensis*, and *Alitropaus typus* have all been photographed in their natural habitats as microparasites.



**Fig. 4** a Normal gill with interlamellar gaps (ILS), secondary gill lamella (SGL), and water passages (WC). Coiled secondary lamella (CSGL), fused secondary lamella (FSGL), and damaged central core in an infested gill (b) (DCC). secondary gill lamella fusion, damaged central core, and aneurysm in the secondary gill lamella of infested gills (ANSGL). d Hyperplasia-infested gill (H) Gills of a sick fish showed significant pathological alterations. Histological examination of *W. attu* demonstrated an intact structure with primary and secondary gill lamellae (PGL), interlamellar region (ILR), and intact water channels (WC) in the normal gills of the fish (Fig. 4a). As a result of parasite attachment and feeding, histopathological examination showed severely damaged primary and secondary gill lamellae. Color spots were seen on every *W. attu* that had been infected in this investigation. Pathological effects of ectoparasites include curled secondary gill lamellae (CSGL), fusion of secondary lamellae (FSL), damaged central cores (DCC) and aneurysm in the secondary gill lamellae (ANSGL) (Fig. 4b, c). Epithelial hyperplasia (EH) of several cell types was found in main and secondary lamellae after a severe infestation (Fig. 4d). Additionally, there is an increase in the number of secondary gill lamellae (PTSGL) (Fig. 5a), a curling of the secondary gill lamellae (CSGL) (Fig. 5b), a mucus secretion (SM), a vacuole formation (VF), an increase in the number of epithelial cells (HE) (Fig. 5c), a decrease in the number of gillfilaments (NGF) (Fig. 6d). The copepod parasite was also found attached to the gill arc in this area.

## Discussion

Ectoparasite infected *W. attu* showed necrosis, hemorrhages, congestion, epithelium and mucus

cell growth in the gill filaments of the fish. Similar pathological results were observed by Mohammadi et al. (2012) who studied parasitic infestation of skin and gill on Oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*) and Dias et al. (2021) who studied *Colossomamacropomu* in the presence of hypoxic or anoxic fish and similar changes to hydromineral balance, gaseous exchange, hormonal production, circulation, nitrogenous waste secret. The more parasites a fish has on its gills, the more likely it is to suffer respiratory harm. Gill injury may lead to a drop in body weight and condition factor, significant alterations in osmotic control or respiratory dysfunction, and finally mortality. With their legs and anchors, ectoparasites such as monogeneans and copepods attached themselves to the major lamellae, resulting in cell damage and bleeding. In some cases, exogenous enzymes are secreted by the parasite to digest the gill filaments of the host fish, which can be seen in the gill histological sections with a high number of cell nuclei within the gut of gill parasites (Vinobad, 2010, Noga, 2010, Seenappa and Venkateshappa, 2000; Pádua et al., 2015). Although the hooks of monogenean parasites and copepods may mechanically hurt gill epithelial cells, the greatest histopathological damage is generated by parasites scraping and sucking on host tissues, according to the studies of Derwa (1995), Endrawes (2001) and Hanna (2002). (2001). It was found that the gill lamellae were functionally impaired, parasites were attached to gill filament tips, and host tissue was swollen at the location of parasite attachment. Researchers in previous studies, including Campos et al. (2001), Fadai et al. (2001), Barzegar and Jalali (2004), Shamsi et al. (2009), Kaur and Shrivastav (2014), Pádua et al. (2015), and Arya and Singh (2020) have found that parasitic infection is characterized by hyperplasia of epithelial cells and subsequent lamellar fusion. Anti-invasion strategies in fish gills include hyperplasia and the fusing of certain secondary lamella. Damaged cells and changes in blood arteries may induce blood congestion or possibly an aneurysm in the event of severe stress in fish (Rosety Rodriguez et al., 2002). Diseases transmitted by ectoparasites, particularly monogeneans and copepods, have consequences not only for the fish's health but also for the economy and society as a whole. This is especially true for cultured fish, whose respiratory ability is impaired, resulting in high death rates (Dezfuli et al., 2011; Shinn et al., 2015). Host-parasite associations and parasite consequences may be utilized to identify environmental imbalances.

Diseases pose a severe danger to both cultivable and wild fish that can be seen with the naked eye, according to the findings. To minimize abrupt parasite outbreaks and economic losses in aquaculture, frequent diagnostic programs and extraordinary management methods must be used from time to time.

## 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.

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