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Prevalence of *Argulus indicus*, histopathology and hematological properties of infected wild fish in Lake Towuti, Indonesia

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Abstract. *Amriana, Sari DK, Sriwulan, Anshary H. 2021. Prevalence of* Argulus indicus, *histopathology and hematological properties of infected wild fish in Lake Towuti, Indonesia. Biodiversitas 22: 3578-3584.* Parasites are disease agents that can threaten the health and survival of wild fish as individuals and at a population level. This study aimed to improve knowledge on the prevalence of *Argulus indicus*, infestation rates and the pathological effects of parasite infestation on hosts as well as hematological properties of infected fish. This study can inform future studies on the prevention and control of the cases of *Argulus indicus* infestation. Fish from Lake Towuti (2^0 45′ 0″ S, 121^0 30′ 0″ E) were caught from February to May 2019 using traps and gill nets. The total of 373 specimens obtained comprised 102 climbing perch (*Anabas testudineus*), 74 three-spot cichlids (*Cichlasoma trimaculatum*), 84 Nile tilapia (*Oreochromis niloticus*), and 113 striped snakeheads (*Channa striata*). The prevalence, mean intensity and abundance of *A. indicus* were highest in *C. striata* with a prevalence of 81%, mean intensity of 5.17 parasites/fish and abundance of 4.06 parasites/fish. The lowest parasite infection level was seen in *C. trimaculatum* with a prevalence of 4.1%, mean intensity 1.66 and mean abundance of 0.06. Histological analysis showed inflammatory responses in the skin of snakeheads (hemorrhage, increased spread of melanomacrophage and leukocyte cells), melanomacrophage and the spread of leukocyte cells were observed in climbing perch. In contrast, melanomacrophage was often observed in tilapia and trimac cichlid. Blood imaging analysis showed significant differences ($P < 0.05$) in the number of leukocytes and percentage of monocyte cells between uninfested fish and those infested with *A. indicus.*

Keywords: Fish blood, infestation rate, parasitism, South Sulawesi, skin inflammation

INTRODUCTION

Lake Towuti is part of the Malili ancient lake complex formed through tectonic activity, with an area of just over 50000 hectares and a depth of more than 200 m (Nasution et al. 2015). Lake Towuti provides valuable services to the local community, including fresh water and electricity generation, while the aquatic biodiversity supports livelihoods based on ecotourism and fishing. Fishes reported from Lake Towuti include endemic species and introduced species, several of which are fished to provide food or as a source of broodstock for freshwater aquaculture around the lake. The size of Lake Towuti and the abundance of fish with economic value can positively impact local incomes from capture fisheries and aquaculture. However, the disease is a threat to wild fish populations, and of course, can also affect the supply of wild broodstock for aquaculture. Parasites are disease agents that can infect both wild and farmed fish (Alsarakibi et al. 2014). Parasitic infections are common in fish, especially in wild fish populations from various aquatic environments where the ecological requirements are met for both the host organism and parasite transmission (McPherson et al. 2012).

Fish lice of the genus *Argulus* comprise ectoparasites reported to infect fish in the wild and aquaculture, causing epidemics in many regions worldwide (Alsarakibi et al. 2012). They can adapt to environmental change to survive even in extreme conditions (Alsarakibi et al. 2014). *Argulus* can attach to the body of a fish using a stylet and sucker, extracting nutrients from host blood through a proboscis; this feeding activity can decrease the number of red blood cells and lower hematocrit levels, causing anemia in the host *Argulus* can also cause the formation of wounds, stimulating increased white blood cell production in the fish (Walker et al. 2011). Histologically, *Argulus* infestation can also cause pathological changes and damage to the skin of the fish. Parida et al. (2018) report an inflammatory reaction in the skin of *Labeo rohita* infested with *Argulus siamensis.* The formation of wounds on the skin of the fish can directly reduce endurance and interfere with osmoregulation mechanisms, and can increase the susceptibility of fish to fungal, bacterial and viral diseases (Kumar et al. 2016).

Argulus infestation is rarely reported to be the leading cause of host death; however, it does cause the host to be more susceptible to pathogenic infections, and there are several reports of fish mortality due to severe *Argulus* infestation (Pekmezci et al. 2011). Infestations by several *Argulus* species have been reported in Indonesia, one of which is *Argulus indicus*, first discovered in Indonesia in 1892 (Weber 1892). However, there have been no further reports of *A. indicus* infestation in Indonesia since then, even though *A. indicus* infestations have been reported

from many regions worldwide (Neethling and Avenant-Oldewage 2016; Sriwongpuk 2020). The cases of *A. indicus* infestation in Indonesia are still very minimal, with no recent reports from aquaculture facilities or in the wild. However, the haplotype of this species was first obtained from Indonesia (Weber 1892). This indicates that there is a high probability of *A. indicus* spread across various regions in Indonesia, requiring further research to investigate the distribution of *A. indicus* within Indonesia. This study aimed to improve knowledge regarding the prevalence of *A. indicus*, the rate of infestation and the pathological effects of this infestation on the host. This information can inform future in-depth studies on cases of *A. indicus* infestation.

MATERIALS AND METHODS

Ethics statement

This research was conducted with the approval of the Hasanuddin University Health Research Ethics Committee for the use of experimental animals in research (Protocol No. 13720093017). All stages in this research followed the Animal Ethics Guidelines issued by the Ministry of Health of the Republic of Indonesia.

Study site and sample collection

Fish were collected from Lake Towuti (2° 45′ 0″ S, 121° 30′ 0″ E, Figure 1) from February to May 2019 using traps and gill nets. The total of 373 fish used in this study comprised 102 climbing perch (*Anabas testudineus*), 74 three-spot cichlids (*Cichlasoma trimaculatum*), 84 Nile tilapia (*Oreochromis niloticus*), and 113 striped snakeheads (*Channa striata*). Each fish was measured (total length in cm) and weighed (g). The body surface, fins, head, and gills of each fish were examined for parasites. Each parasite was removed from the body of the fish using a pair of tweezers which was rinsed in 70% alcohol between fish. Then, the samples were taken to the Fish.

Parasite and Disease Laboratory of Universitas Hasanuddin in Makassar, Indonesia. Species identification of *A. indicus* based on morphological and genetic analysis according to the previous research of Amriana et al. (2021).

Histology

Fish infected with *A. indicus* were anesthetized before dissection to remove the skin from each *Argulus* attachment site. Each tissue sample was placed in a sample bottle filled with a fixative solution (10% neutral formalin buffer) and washed using 70% alcohol. The tissue sample was dehydrated through the graded alcohol (70-100%), cleaned by xylol, impregnated using paraffin and xylol, and embedded in paraffin. The skin sections were cut with a thickness of 3-5 µm, deparaffinized, rehydrated, and stained using hematoxylin-eosin (Parida et al. 2018).

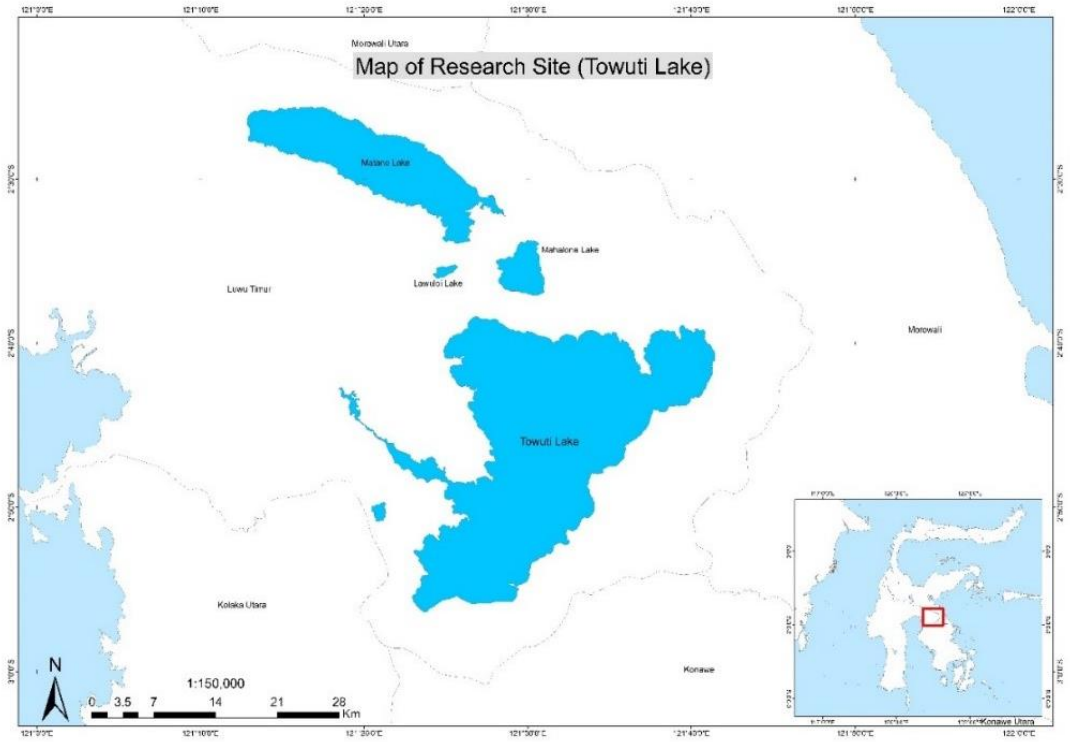

Figure 1. Lake Towuti in Luwu Timur District, South Sulawesi Province, Indonesia

Blood collection

For each of the species found to be infested with *A. indicus*, three infested and three non-infested specimens were anesthetized using clove oil at a concentration of 1 mL/L. Blood was then drawn from each anesthetized specimen using a 1 mL Terumo medical syringe. The needle was inserted into the muscle along the lateral line behind the anal fin until it reached the spine. The syringe plunger was then slowly withdrawn until the syringe was filled with blood. The blood obtained was placed in a vacuum tube containing an anticoagulant and a paper label. Blood samples were analyzed to determine the number of erythrocytes and leukocytes as well as to observe leukocyte cell differentiation, according to the methods of Blaxhall and Daisley (1973).

Erythrocyte count

The number of erythrocytes was determined by aspirating blood into a special pipette for measuring the number of red blood cells, with red stirring grains in it until the scale of 1 was reached. The blood was then mixed with Hayem's solution to the 101 marks on the pipette. The pipette was shaken by making a figure of eight movements to ensure the contents were thoroughly mixed. The first two drops of the solution were discarded before transferring the liquid to a hemocytometer and covering it with a glass cover. Counts were performed under a binocular microscope with a magnification of 40X. The number of erythrocytes were counted in 10 small compartments in the hemocytometer and converted to obtain the number of blood cells per mm3. Erythrocyte count equation:

Number of Red Blood Cells = No. cells counted x dilution factor/area counted (mm^2) x depth (Blaxhall and Daisley 1973).

Leukocyte count

The number of leucocytes was determined by aspirating blood into a special pipette until the 0.5 marks was reached. The blood was then mixed with Turk's solution to the 11 marks on the pipette. The pipette was shaken by making a figure of eight movements to ensure thorough mixing. The first two drops of the solution were discarded before transferring the liquid to a hemocytometer and covering it with a glass cover. Capillary action was used to transfer the liquid to the counting chamber. The number of leukocytes was counted in 5 out of the 16 small compartments in the hemocytometer and converted to obtain the number of cells per mm3. Leucocyte count equation:

Number of White Blood Cells = No. cells counted x dilution factor/area counted (mm^2) x depth (Blaxhall and Daisley 1973).

Blood smear preparations (Differential leukocyte observation)

Two glass slides were used to make blood smears (slide A and slide B). A drop of blood was placed on slide A, and slide B was placed over the drop of blood at an angle of 45°s to slide A. Slide B was then moved to the right and then to the left quickly and steadily to obtain a thin film of blood on each slide. The slides were then air-dried before being placed in methanol for 5 minutes, and then placed in Giemsa dye for 20 minutes. The slides were washed with running water for 5 minutes and dried. The dry preparations were observed using a microscope at 100x magnification. The leukocyte differential count was counted up to 100 cells and the percentage of each type (lymphocyte, monocyte, eosinophil, basophil, and neutrophil) was counted.

Proportion of a leukocyte cell type = number of the leukocyte cell type x 100% / Total number of leukocytes (Blaxhall and Daisley 1973).

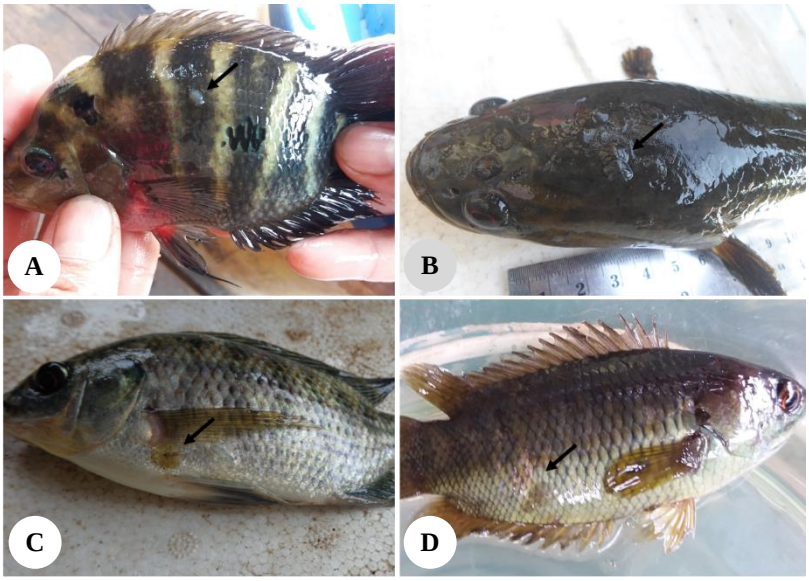

Figure 2. *Argulus indicus* infestation on freshwater fish in Lake Towuti District, South Sulawesi Province, Indonesia: A. *Cichlasoma trimaculatum*; B. *Channa striata*; C. *Oreochromis niloticus*; D. *Anabas testudineus.* Arrow showed location of *A. indicus* on host body

Data analysis

Prevalence, mean intensity and abundance were calculated according to the methods of Bush et al. (1997) tabulated. Histological observations were analyzed descriptively while the T-test at the 95% confidence level (P<0.05) was used to evaluate the significance of differences in hematological parameters between infested and non-infested fish.

RESULTS AND DISCUSSION

Results

The prevalence of *A. indicus* infestation in 373 fish from Lake Towuti was observed in four species of fish (Figure 2). These species were the three-spot cichlid *C. trimaculatum* (Figure 1A, n = 74)); the striped snakehead *C. striata* (Figure 1B, n = 113.); the Nile tilapia *Oreochromis niloticus* (Figure 1C, n = 84); and the climbing perch *A. testudineus* (Figure 1D, n = 102). The prevalence and mean intensity of infestation as well as the mean abundance of *A. indicus* varied between these species, being highest in *C. striata* with 81% prevalence, mean intensity of 5.71±1.02 and mean abundance of 4.06±0.81. Whereas, the lowest infestation was found on *C. trimaculatum* with 4.1% prevalence, mean intensity of 1.66±0.66 and mean abundance of 0.06±0.04 (Table 1)

The histopathological analysis revealed inflammatory responses in the skin of infested striped snakehead, climbing perch, tilapia specimens, and three spot cichlids (Figure 3). Histologically, there was increasing in melanomacrophage and hemorrhage, and sparse distribution of leukocytes as mild inflammatory reaction was observed in epidermal layer of *C. striata* (Figure 3.A). The skin of *A. testudineus* revealed leukocyte distribution and increasing hemorrhage in dermal layer (Figure 3B). Whereas, *O. niloticus* and *C. trimaculatum* show melanomacrophage dermal layer (Figure 3.C-D). The histologic change indicates the inflammation reaction to attachment of *A. indicus* in skin fish. Specifically, *C. striata*, as the high infestation of *A. indicus*, show mild inflammation to parasite attachment in the skin and low inflammation on *C. trimaculatum* as the low infestation.

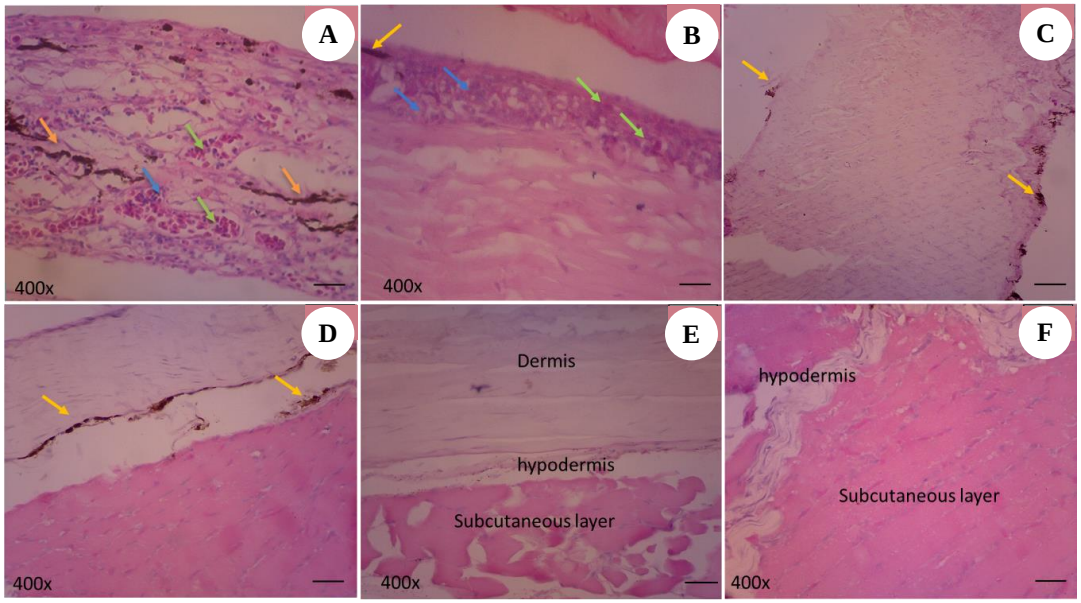

Figure 3. Histology of fish skin infested with *Argulus indicus* (A-D) and normal skin (E-F). Blue arrows: Inflammation; Green arrows: Hemorrhage; Orange arrows: Melanomacrophages. Bar: 100 µm. A. Increased number of melanomacrophages in epidermis and dermis, inflammatory and hemorrhagic cells in the epidermis of *C. striata*; B. Haemorrhage and spread of inflammatory cells (leukocytes) in the dermis of *Anabas testudineus*; C. Melanomacrophages in the dermis of *Oreochromis niloticus*; D. Melanomacrophages in dermal layer of *Cichlasoma trimaculatum*; E-F. The Normal skin of *O. niloticus* and *C. trimaculatum*

Table 1. Prevalence, mean intensity and abundance of *Argulus indicus* in freshwater fish from Lake Towuti, South Sulawesi, Indonesia

Host species	∑Fish examined	∑Fish infected	∑Parasites	Prevalence (%)	Mean intensity (X ± SD)	Mean abundance (X ± SD)
Anabas testudineus	102	49	87	48%	1.78±0.12	0.85±0.18
Cichlasoma trimaculatum	74	3	5	4.1%	1.66±0.66	0.06±0.04
Oreochromis niloticus	84	4	7	4.7%	1.75±0.40	0.08±0,04
Channa striata	113	91	520	81%	5.71±1.02	4.06±0.81

Table 2. Blood parameters of *A. indicus* infested and non-infested fish from Lake Towuti, South Sulawesi, Indonesia

Species	Health status	Erythrocyte (×109 cells/mL)	Leukocyte (×106 cells/mL)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	Neutrophil (%)
A. testudineus	Not infested	2.35 ± 0.08^{ns}	2,82 ± 0.28^{a}	67.00 ± 2.00^{a}	14.67 ± 1.53^{a}	5.67 ± 0.58^{ns}	6.00 ± 1.73^{ns}	6.67 ± 2.08^{ns}
	Infested	1.75 ± 0.38^{ns}	4.98 ± 0.44^{b}	55.00 ± 2.00^{b}	30.00 ± 2.00^{b}	4.00 ± 1.00^{ns}	6.33 ± 1.53^{ns}	4.67 ± 1.53^{ns}
O. niloticus	Not infested	2.20 ± 0.03^{a}	2.66 ± 0.13^{a}	68.70± 2.08^{a}	14.00 ± 2.00^{a}	6.33 ± 1.15^{ns}	5.67 ± 1.53^{ns}	5.33 ± 1.53^{ns}
	Infested	1.31 ± 0.33^{b}	5.53 ± 0.20^{b}	62.67 ± 2.53^{b}	22.00 ± 4.58^{b}	4.67 ± 2.52^{ns}	6.00 ± 3.61^{ns}	4.67 ± 2.58^{ns}
C. trimaculatum	Not infested	2.27 ± 0.11^{a}	2.92 ± 0.15^{a}	70.30 ± 1.53^{a}	13.70 ± 1.53^{a}	6.67 ± 1.15^{a}	4.67 ± 1.15^{ns}	4.67 ± 1.15^{ns}
	Infested	1.20 ± 0.11^{b}	5.83 ± 1.05^{b}	64.00 ± 1.00^{b}	23.67 ± 3.21^{b}	3.33 ± 0.58^{b}	6.00 ± 2.08^{ns}	3.00 ± 0.58^{ns}
C. striata	Not infested	3.01 ± 0.31^{ns}	3.33 ± 0.11^{a}	69.33 ± 2.08^{a}	18.00 ± 2.00^{a}	4.00 ± 1.00^{ns}	5.67 ± 0.58^{a}	3.00 ± 1.00^{a}
	Infested	5.96 ± 0.61^{ns}	7.48 ± 0.08^{b}	47.33 ± 2.08^{b}	31.33 ± 2.31^{b}	6.00 ± 1.00^{ns}	8.33 ± 1.53^{b}	7.00 ± 1.00^{b}

Note: ns: not significant (P>0.05); The mean values in columns with different superscripts indicate the significantly different (P<0.05)

The fish blood sample analysis (Table 2) showed a significant difference in hematological parameters (P<0.05) between *A. indicus* infested fish and non-infested fish of the same species. Parasitized *C. striata* showed a significant increase (P<0.05) in the total of leukocytes and percentage of monocytes, basophils, and neutrophils. In contrast, the percentage of lymphocyte cell decreased compared to the unparasitized fish of the same species. *O. niloticus* and *C. trimaculatum* revealed a significant increase (P<0.05) in the total of leukocyte and monocytes percentage and decrease (P<0.05) in the lymphocyte percentage between infested fish and uninfested fish (Table 2). Whereas parasitized *A. testudineus* showed significant hematological value in the total of Leukocyte and Monocyte percentage and decrease in the lymphocyte percentage (P <0.05).

Discussion

In this study, all four fish species obtained from Lake Towuti were infested with *A. indicus*. The highest infestation level was in striped snakeheads and the lowest in three spot cichlids. This is consonant with the general perception that the fish most likely to be infected with *A. indicus* are those often found in the lower region of the water column and are less active, such as striped snakeheads and climbing perch. This is related to the strategy of *A. indicus* in looking for the closest host they can find, so that striped snakeheads and climbing perch have a higher probability of encountering *A. indicus* than tilapias or cichlids which tend to remain closer to the water surface and swim more actively (Stewart et al. 2017).

Parasitic species often exhibit a heterogeneous infestation pattern and are highly dispersed in host populations. *Argulus* fish lice have developed ways to optimize transmission from one fish to another. Their ability to detect visual, physical and chemical cues connected to the host can lead to rapid host discovery and enhance the potential for host infestation (Mikheev et al. 2015). *Argulus* need a host to survive so they have developed various tactics to be able to infest hosts. In the metanauplius stage, *Argulus* with incompletely developed organs will only wait near the substrate until a fish moves close to them, and then they will immediately move quickly to attach to the host's body (Mikheev et al. 2007). This is what causes *Argulus metanauplii* to infest more heavily those fish that are often found on or close to the substrate. Adult *Argulus* can swim quite fast to find a host, but they cannot maintain speed over long distances; in order to conserve energy, they will tend to attach themselves to the first potential host they encounter, making less active fish the most likely and easiest hosts for them to infest (Mikheev et al. 2015). *Argulus* can also manipulate the behavior of host fish to increase transmission effectiveness, while the complex *Argulus* life cycle that exploits several consecutive host species during different life stages is another way to increase the success of transmission (Stewart et al. 2018).

Argulus parasite infestations cause direct damage to the skin of the host fish through attachment and feeding mechanisms (Walker et al. 2011). Skin damage results from the mechanical action of maxillary suction by adult lice and the hooks or spines of the larval and juvenile stages as well as their sharp mandibles (Stewart et al. 2017). In addition, damage can occur due to various toxins or digestive enzymes secreted through the pre-oral stylet and labial spines (Walker et al. 2011). Skin infections are characterized by an inflammatory response at the site of attachment and erosion due to feeding by the parasites as well as the infiltration of inflammatory cells into the affected tissue (Parida et al. 2018).

The skin is the main target organ during ectoparasitic infection and local or systemic inflammation is a factor that regulates susceptibility or resistance to infection, and a large number of host molecules involved in immunity modulate this inflammatory response or process either directly or indirectly (Kar et al. 2016). Previously reports on host preference in *Argulus siamensis*, indicate that *Labeo rohita* depends on the degree of inflammatory response induced by parasitic activity (Kar et al. 2013). This study investigated the histopathological changes in the skin of wild *C. striata*, *A. testudineus*, *C. trimaculatum*, and *O. niloticus* due to the attachment of the parasite *A. indicus*. Tissue damage was visible on the skin of *C. striata* and *O. niloticus*. Leukocyte cells were widespread on the skin of *C. striata* infested with *Argulus*, indicating an inflammatory response, with hemorrhaging also present and accompanied by the spread of melanomacrophage. In *C. trimaculatum* only melanomacrophage was observed on the skin at the site of *Argulus* attachment. This difference in symptom severity is in line with the level of parasite infestation which was highest in *C striata* and lowest in *C. trimaculatum*.

Parasitic infestation generally causes local feeding and adhesion damage, especially when infection rates in individual hosts are very high. These areas of damage can facilitate the entry and development of secondary bacterial, fungal and viral infections (Kumar et al. 2016). Hosts can generally tolerate low-grade skin infections and are able to repair damage caused by low numbers of parasites relatively quickly. In severe infestations, however, the host cannot create an effective defense against the parasite; thus, the direct and secondary damage tend to become more intense. Inflammation, bleeding, and ulceration associated with attachment and feeding by parasites occur (Walker et al. 2011). Histologically, the act of feeding by ectoparasites can be seen to disrupt the dermal and sub-dermal tissues including muscles (Parida et al. 2018). The histological observations made in this study are consonant with these general findings.

Fish parasites can cause blood disorders including anemia, leucocytosis, and thrombocytopenia, while *Argulus* parasite infestations can cause stress reactions with behavioral and physiological responses in host fish (Rocha et al. 2018). For example, Ali et al. (2010) demonstrated anemic conditions in infested fish, with a decrease in the number of erythrocytes and hematocrits in host blood due to parasite feeding activity as well as failure of host osmoregulation due to skin lesions. However, the hematocrit and erythrocyte counts can also be increased with the release of stored blood cells from the spleen, loss of plasma, or by swelling of red blood cells as a stress response in *Argulus*-infected fish (Jones and Grutter 2005). The analysis of blood from *A. indicus*-infested and non-infested fish showed significant differences between the two groups (Table 2). *Argulus* infestation had a significant effect on the number of leukocyte cells and the percentage of monocyte cells in all observed fish species ($P< 0.05$), while only *C. trimaculatum* and *O. niloticus* had significant differences in erythrocyte cell count. Increased percentages of basophil and neutrophil cells were also observed in *C. striata*, the fish species with the highest parasite attachment intensity. The study of Panjvini et al. (2016) also shows an increase in WBC in fish infected with the parasite. The increasing leukocyte count can indicate the defense mechanism and response of cellular immune mechanisms against parasites. *Argulus* infested fish have also shown an increase in monocyte cells (Tavares-Dias and De Moraes 2007). Monocytes are the main phagocytes in fish; during the infection-fighting process, monocytes will migrate from blood vessels to inflammatory foci (Esteban et al. 2015). In addition to monocytes, neutrophils are also involved in the defense of fish against parasites. Hematological changes may be related to physiological characteristics as well as disease or condition of the fish. In addition, in the host-parasite relationship, the severity of health changes may be related to the mechanism of attachment of the parasite, its life cycle, and in particular, the number of parasites (Fazio 2019).

Our results confirmed that At least four species of fish were found to be infested with *A. indicus*: the climbing perch (*A. testudineus*), three spot cichlid (*C. trimaculatum*), Nile tilapia (*O. niloticus*), and striped snakehead (*C. striata*). The most severely infested was the striped snakehead, as the behavior patterns of this fish and *A. indicus* infestation tactics result in a high probability of interaction between the host and parasite species. High levels of infestation increase the inflammatory response, with symptoms observed in fish skin, including melanomacrophage, the spread of leukocyte cells, and hemorrhaging. Hematological parameters also showed significant differences between infested and non-infested fish, in particular an increase in the number of leukocyte cells and the percentage of monocyte cells in infested fish, which indicates an inflammatory response to *A. indicus* infestation in fish.

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