CHAPTER 5

ASPECTS OF THE PATHOLOGY OF *LAMPROGLENA CLARIAE* (COPEPODA: LERNAEIDAE) ON THE GILLS OF CLARIAS GARIEPINUS FROM THE VAAL RIVER SYSTEM

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INTRODUCTION

The parasite *Lamproglena clariae* Fryer, 1956 is endemic to Africa; it was first described from material collected from Lake Malawi and was subsequently found in the East, West, Southern, and Central Africa (Fryer 1968; Marx & Avenant-Oldewage 1996). Adult female specimens of *L. clariae* attach to the gill filaments of *Clarias gariepinus* Burchell, 1822, a widely distributed and an extremely economically important fish species valued in subsistence fisheries and aquaculture in both Africa and Europe. Coinciding with the growing economic value of this fish is the increased interest in its parasite load and what effects they might hold for the aquaculture industry (Reed et al. 2003). In aquaculture, fish are often maintained at high densities, facilitating copepod transfer amongst hosts (Bowers et al. 2000) and parasites have the chance to multiply and increase in numbers achieving heavy burdens (Khalil 2003). Many species of parasitic copepods are pathogenic to freshwater fish and they are especially important in regions where there is intensive aquaculture (Woo & Shariff 1990).

The fish tissue as an environment of adult female copepods can be modified by means of their attachment and feeding strategies. Lernaeids feeding directly on blood can cause primary anaemia in fish (small or young) and those that produce hyperplasia in gill filaments can reduce respiratory capacity in fish (Thatcher 1998). Though impacts on the host have usually been reported in terms of pathological lesions caused by attachment and feeding of the adult stages, as well as localized mild epithelial responses of hosts to juvenile attachment, many studies report pathology associated with heavy infestations (Tully & Nolan 2002).

When parasitic females of species of the genus *Lamproglena* attach to gills, unlike other lernaeids, which develop new structures for adhesion and loose evidence

of external segmentation, they preserve a recognizable copepod habitus, with partially retained external segmentation. The segments are enlarged and elongated and the thoracic legs are reduced (Marx & Avenant-Oldewage 1996; Paperna 1996).

Lamproglena clariae attaches midway along the length of the gill filament and the genital segment is in line with the apex of the filament. This leaves the abdomen and egg sacs in the water current passing through the gills. They prefer the median part of the fourth gill arch and the parasite size correlates with filament length, which in turn correlates with fish size. The positive correlation between maxillipede length and the gill filament length and width further supports the statement that the size of the host determines the size of a parasite. Maxillipeds grow according to the size of the gill filament size after attachment has occurred and they also correlate positively with the parasite length (Tsotetsi et al. 2004).

The adult female grips the gill filament with the strong maxillae and use maxillipeds as both attachment and feeding appendages, penetrates the gill tissue with these appendages and consumes blood, the head then becomes embedded in the host tissue (Marx & Avenant-Oldewage 1996). Hence, this study aimed at determining mechanical damage caused by attachment and feeding on the gill filaments of the host.

MATERIALS & METHODS

Specimens of *C. gariepinus* were collected by means of gill nets in the Vaal Dam (S 26° 52.249', E 28° 10.249') and transported in aerated dam water to the laboratory (±120 km from the dam).

Blood was collected from the dorsal aorta of the fish using the 21G needle in sterile evacuated blood vacutainers containing Ethylenediamenetetra-aceticacid

(EDTA) as an anticoagulant. Blood was transferred into haematocrit tubes with EDTA and centrifuged in a microhaematocrit centrifuge at 600 rpm for five minutes. Packed cell volume was determined with a haematocrit reader.

Fish were killed by a single cut through the spinal cord; gills were dissected free from the head and studied with a dissection microscope for gross pathology. Gill filaments with adult female specimens of *L. clariae in situ* were photographed, fixed in a solution of alcohol, formaldehyde and acetic acid; and preserved in 70% ethanol.

Some fixed specimens were dehydrated in an ascending series of ethanol solutions. The specimens were then infiltrated with a low viscosity Aliphatic Epoxy Resin. They were sectioned (5μm) with a rotary microtome, stained with Heidenhein's azan solution (Humason 1979) and mounted. Sections were studied with a compound microscope to determine the pathology associated with the adult females and their mode of attachment. Uninfected gill filaments were also examined after similar histological procedures.

Intensity of infestation was determined by counting the number of *L. clariae* specimens collected from each fish (n=108) and correlation between the infestation intensity and fish haematocrit was determined.

RESULTS

Histological examination of the normal gill filament through cross sectioning (Fig. 1A) showed mucous cells scattered among the one cell thick layer of squamous epithelium, a supportive connective tissue layer, the median cartilage rod **c.r** and blood capillaries. Magnified portions of this sectioning showed the mucous cells **m** (Fig. 1B), a blood capillary running along the medial margin of the filament containing erythrocytes **e** (Fig. 1C), and an epithelial layer (Fig. 1D) made up of

squamous epithelial cells **s** (114µm thick), mucous cells and supportive connective cells **c**. The size of a single mucous cell was determined and found to be approximate 21x 25µm.

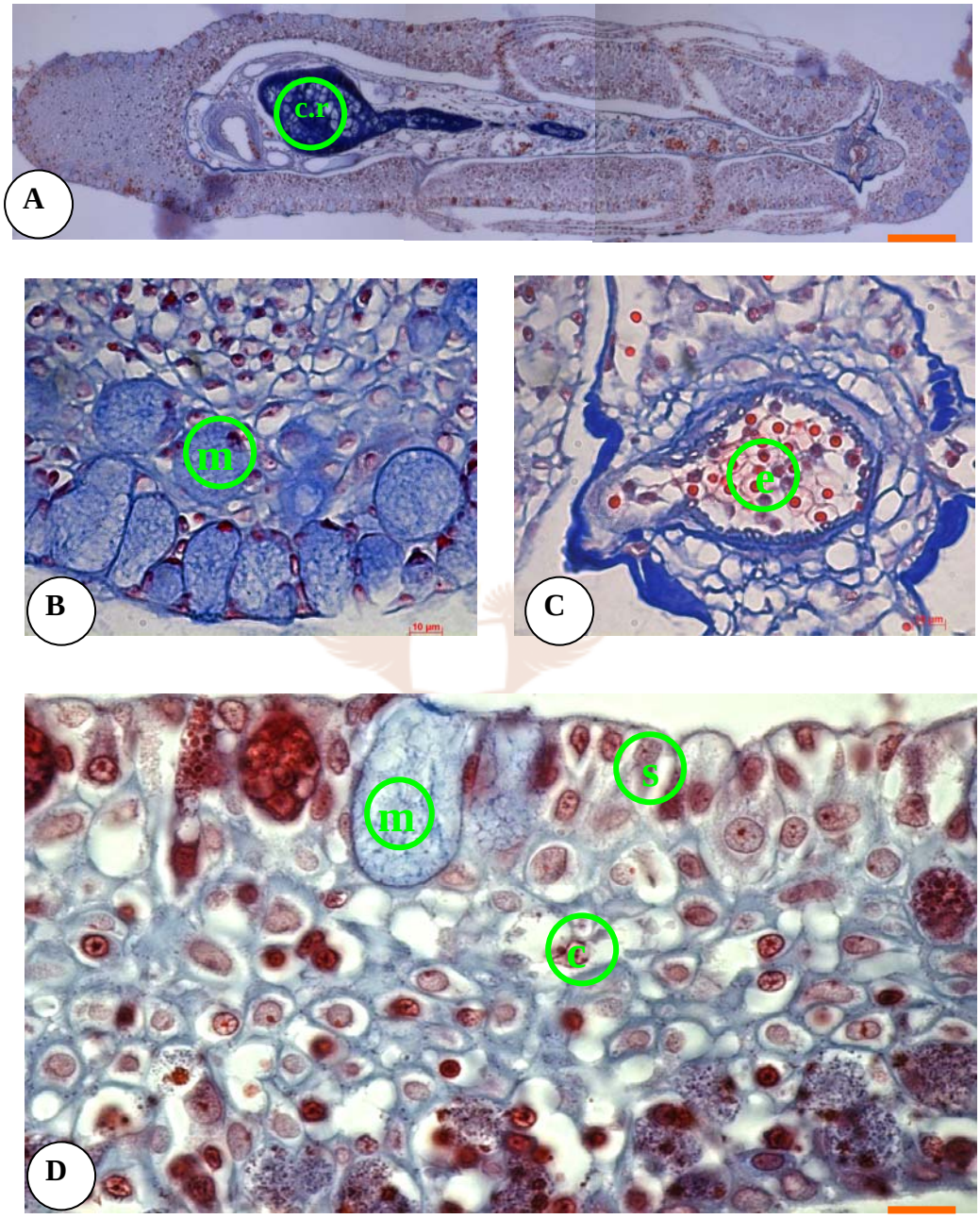
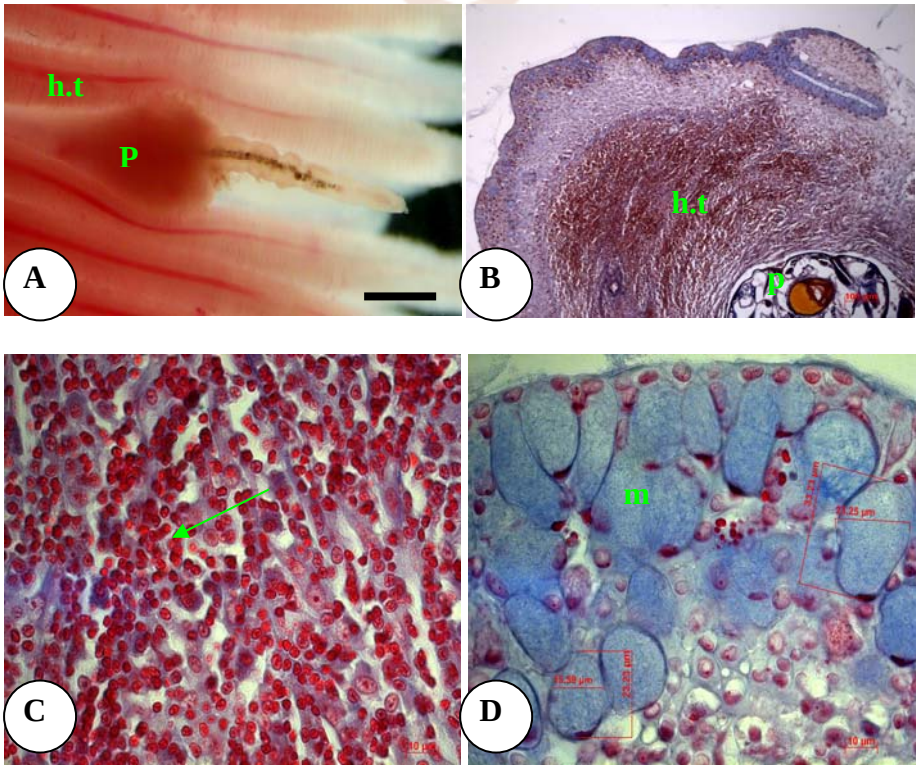


Fig. 1. Normal gill tissue, cross section (A). Mucous cells (B), blood capillary (C) and an epithelial layer (D). Scale bars: (A) =200µm, (D) = 20µm.

Pathological changes in the gills were divided into three phases, which were coupled with the development of the female adult *L. clariae*. The first phase is marked

by the presence of a newly attached early adult female stage of the parasite. This stage has functional swimming legs and segmentation is very pronounced, in contrast to the gravid adult in which segments are enlarged and elongated and the thoracic legs are reduced. Gross morphology through dissection microscopy showed localised inflammation, characterised by redness and excessive swelling of the host tissue over the head region of the parasite **p** embedded in this region whilst the remainder of the filament was unaffected (Fig. 2A). Histological examination also showed proliferation of the host tissue **h. t** around the head region of the parasite **p**, embedding it into the host tissue (Fig. 2B). An acute inflammation characterised by haemorrhage, oedema and neutrophilia (Fig. 2C) was observed. Hypertrophy and hyperplasia of epithelial cells, particularly mucous cells (33 x 23µm) (Fig. 2D). Supportive connective tissue resulting in the thickening of the epithelial layer to approximate 150µm (Fig. 2E); and necrotic tissue (Fig. 2F) adjacent to the attached parasites were also observed.



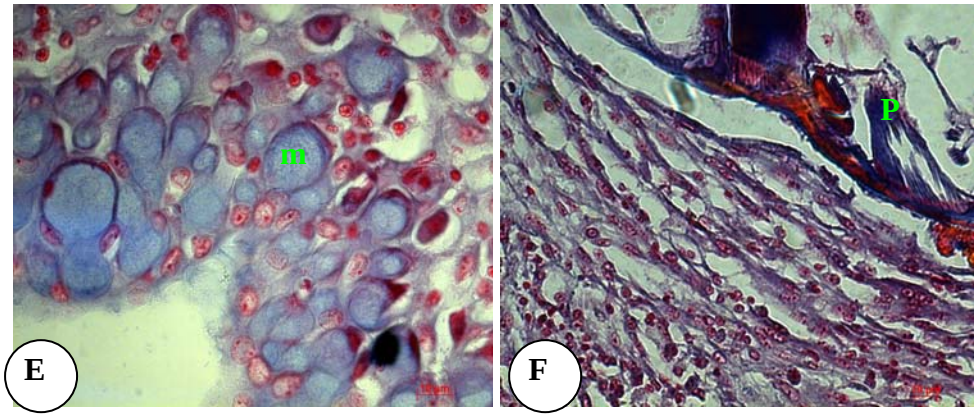


Fig. 2. Proliferation of the host tissue around the head region of the parasite, gross morphology (A), histological view (B). Neutrophilia, oedema (represented by an arrow) and haemorrhage (C), Hypertrophy (D) Hyperplasia (E), Necrotic tissue near the attached parasite (F). Scale bar (A) = 1mm.

The second phase was associated with the young adult stage and characterised by reduction of swimming legs, partially retained external segmentation, elongated and enlarged segments and visible ovaries. Gross morphology characterised by more severe host reaction as almost the total gill filament was inflamed and a red fluid, presumably blood, was observed in the intestine of the parasite (Fig. 3A). Histological study revealed proliferation of the host tissue surrounding the head region of the parasite. The host tissue in the vicinity of the parasite was eroded presumably by the scraping and rolling movement applied by maxillae and maxillipeds of the parasite, indicated as arrows in the figure (Fig. 3B). This action would bring the gill tissue towards the buccal opening (Fig. 3C). The ingested host tissue was observed in the buccal cavity, oesophagus and midgut of the parasite (Fig. 3D). Prominent muscle strands orientated in longitudinal, transverse and oblique planes present in both maxillae and maxillipeds facilitate stretching and rolling of both these appendages (Fig. 3E). Hyperplasia and hypertrophy of epithelial cells resulting in the thickening of the epithelial layer was also revealed (Fig. 3F). No haemorrhage, neutrophilia nor oedema was observed during this phase.

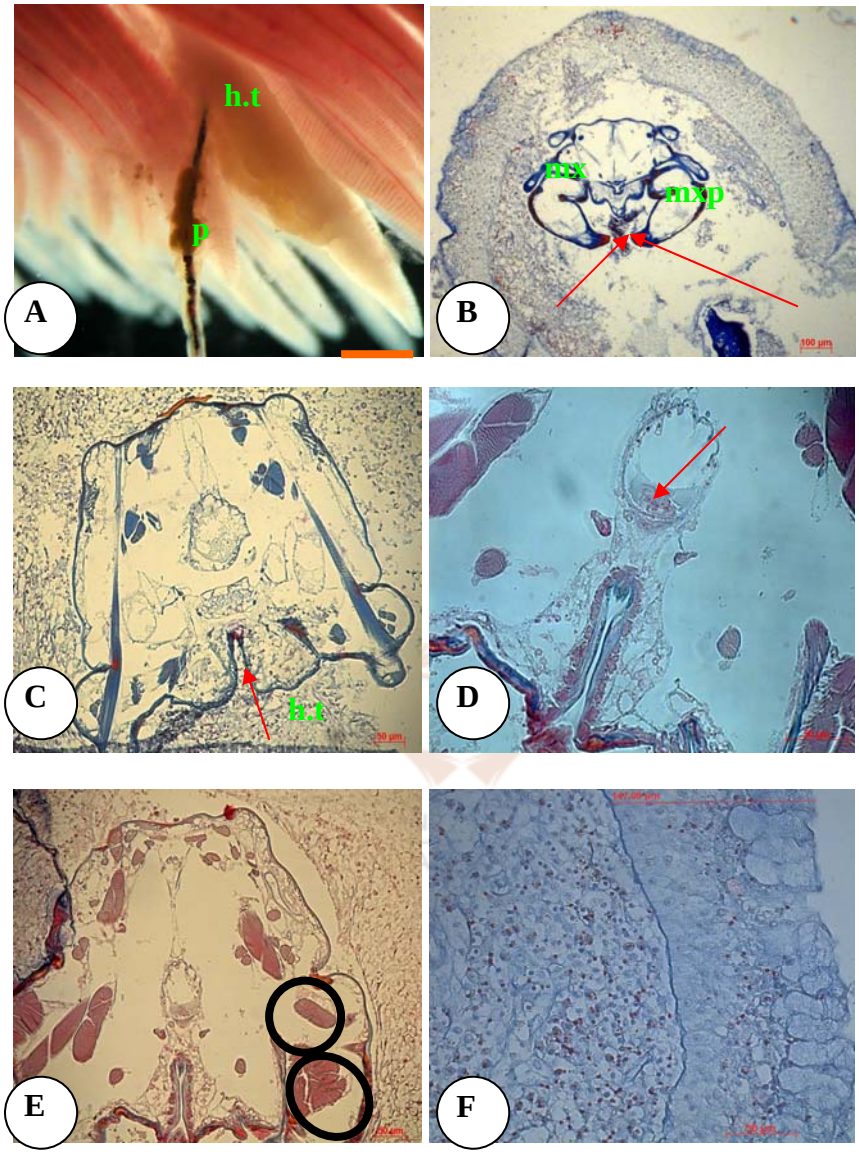


Fig. 3. Infected host tissue, gross morphology (A), histological view (B), ingestion, host tissue (C), ingested host tissue (D), muscles, maxilla, maxillipedes, (E), thickened epithelial layer (F).

The third phase is associated with the occurrence of the gravid adult stage marked by partially retained external segmentation in parasite, enlarged and elongated segments, reduction of the thoracic legs and presence of egg sacs. Gross morphology reveals a more localized reaction, as only the attachment zone was affected. Reduced

level of inflammation compared to the previous phase is observed; the digestive tract is still filled by red fluid (Fig. 4A). Histological examination was performed through longitudinal sectioning and revealed hyperplasia of epithelial cells within lamellae (Fig. 4B) resulting in their fusion (Fig. 4C). Necrosis was present in the vicinity of the parasite as evident from disrupted cell structure, loss of cytoplasm, highly reactive nuclei and reduction in the size of the nuclei (Fig. 4D). Neither haemorrhage nor neutrophilia was observed.

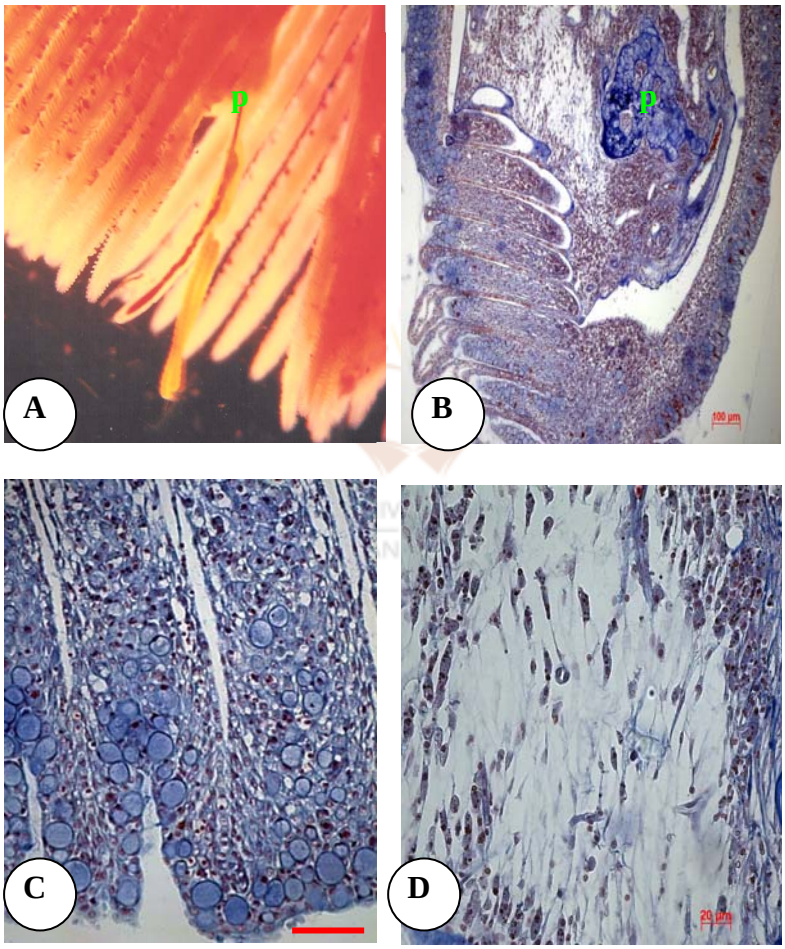


Fig. 4. Infected gill tissue, gross morphology (A), histological view, longitudinal sectioning, (B), Fusion of gill lamellae (C), Necrotic tissue (D) near the attached parasite. Scale bar: (C) = 18µm

Pathology was only observed on the gill filaments with attached parasites and the rest did not show any pathological lesions.

Infestation intensity ranges between 0 and 32, haematocrit values of the host fish range between 6% and 52% and no correlation (R^2= 0.0025) was observed between the infestation intensity and the host fish haematocrit (Fig. 5).

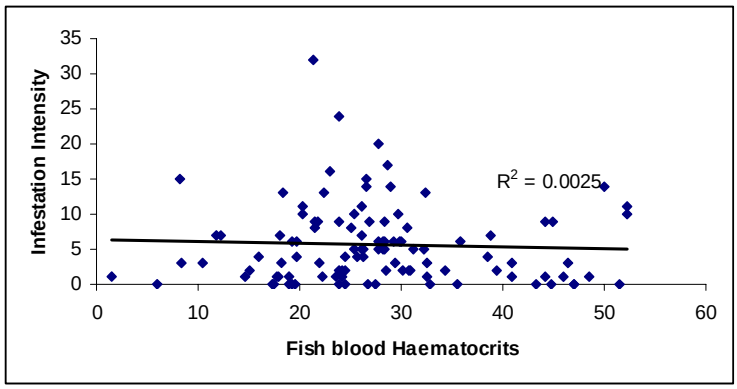


Fig. 5. Correlation, haematocrit values and *Lamproglena clariae* infestation intensity, *Clarias gariepinus*.

DISCUSSION

During the first phase of attachment, signs of the acute inflammation similar to common inflammatory response elicited by metazoans characterised by neutrophilia and monocytosis in blood and accumulation of neutrophils and macrophages at the site of injury or infection (Shariff & Roberts 1989; Roberts 1989; Suzuki & Iida 1992) was observed in the current study. Although neutrophils were present during the early acute inflammatory response, no phagocytic activities were observed. Neutrophilia occurs within an hour of an inflammatory stimulus and commonly reaches a peak after 48 hours (Secombes 1996). The acute inflammation resulted from increased capillary permeability which allows escape of erythrocytes and neutrophils into the surrounding tissue to easily reach the invader and disrupts the vascular integrity of the gill.

According to Ellis (1986), cellular response is typically biphasic, especially in response to potentially pathogenic organisms, and is marked by an increase in blood neutrophils, preceding the appearance of monocytes and macrophages. The role of eosinophils in fish inflammatory response is not clear. Although they are known to be involved in antiparasitic responses in a few fish species (Cone & Wiles 1985; Reimschuessel et al. 1987), they are absent in most other species.

Lepeophtheirus salmonis causes a similar effect on the gills of *Oncorhynchus* sp. The inflammatory infiltrate is predominated by neutrophils and it was suggested that cell mediated immunity does not play a major role in the elimination of *L. salmonis* from the hosts (Johnson & Albright 1992). Neutrophils and macrophages are the predominant cell types reported at sites of inflammation of a wide variety of both naïve and previously exposed fish hosts infected with parasitic copepods (Joy & Jones 1973; Boxshall 1977; Shields & Goode 1978; Paperna & Zwerner 1982; Shariff & Roberts 1989). *Ergasilus sieboldi* also causes extensive gill damage and severe haemorrhage with inflammation, and blockage of blood vessels of the gill filaments which leads to atrophy of gill tips (Bauer 1970).

The host reaction observed in the second phase was probably caused by both attachment and feeding. The results indicate that the parasite was still undergoing development as ovaries were already visible, but egg sacs were not yet developed. This observation implies that the parasite requires energy from the host tissue to be able to complete the development, hence feeds on the host tissue. The observed erosion of host tissue within the reach of the maxillipedes was possibly caused by the rolling movement of maxilla and maxillipeds which are believed to gradually destroy the epithelium. Avenant-Oldewage (1994) suggested that the rolling movement of mandibles in *Dolops ranarum* gradually eroded the epithelium. Furthermore, the

presence of the compressed host tissue in the vicinity of the claws of maxillipeds, shows that this tissue is being pushed into the buccal cavity of the parasite. The adequate musculature of the maxillae and maxillipedes allows for stretching, contraction and rolling of these appendages whilst feeding. This was indicated by the presence of eroded host tissue not only near the maxillipeds' claws, but also even further from the parasite. The host tissue observed in the digestive tract of the parasite indicates that *L. clariae* also feeds on host tissue.

Bowers et al. (2000) suggest that stress caused by *L. salmonis* rise with increased size or development of the parasite; however in this study the gravid adult stage caused less pathology when compared to the earlier stages. This is possibly due to varying parasite demands for food, as the parasite undergoes development. Early adult stages need more energy from the host than the gravid ones, therefore causes more harm through both feeding and attachment. The absence of tissue erosion during the third phase suggests that the parasite stopped feeding. Demands placed on the host are greatest during the phases of vigorous metabolic activity (e.g reproduction) of the parasite as was previously suggested by Kabata (1958).

In *Opiocephalus* sp., *Lamproglena* sp. induces a distorted growth of the tip of the gill filament in such a way that there is hypertrophy of connective tissue and a local degeneration of capillaries. The head of the copepod becomes deeply embedded and it was suggested that this was not the result of active burrowing of the parasite, but of tissue growth around it, stimulated by irritation set up by the head-appendage, perhaps after more than a season sojourn (Sproston et al. 1950). Similar gill damage was caused by *Salmincola californiensis* on the gills of *Oncorhynchus mykiss* (Kabata & Cousens 1977). In the current study distorted growth of the tip of the gill filament was not observed. This suggests that distorted growth of the tip of the gill filament

depends on the attachment site of the parasite on the gill filament. The former two species attach to the tips of the gill filament, whilst *L. clariae* rather attaches midway along the gill filament (Marx & Avenant-Oldewage 1996; Tsotetsi et al. 2004).

Extensive epithelial hyperplasia observed in the current study is similar to host reactions caused by other gill parasitic copepods such as *Sinergasilus lieni* on silver carp and bighead (Molnar & Scekely 2004), *Myracetyma* sp. on *Plagioscion squamosissimus* (Thatcher 1998), *Dissonus manteri* on *Plectropomus leopardus* (Bennett & Bennett 1994) and *Ergasilus labracis* on *Morone saxatilis* (Paperna & Zwerner 1982). Extensive epithelial hyperplasia actually produces more epithelial tissue on which a copepod can feed. However, the thickening of the epithelial tissue can be disadvantageous to the host by interfering with its ability to allow gaseous exchange to occur (Thatcher 1998). Hyperplasia may also be seen as an attempt by the host to seal off the parasite from the surrounding tissue (Fustish & Millemann 1978; Kabata 1984).

Hyperplasia and hypertrophy of epithelial cells resulting in fusion of gill lamellae was also recorded in other copepods on gill filaments. The study on *Nemesis robusta* revealed that the tissue proliferation partially or completely blocked interlamellar water channels, which prohibited water passage between secondary lamellae (Benz & Adamson 1990). Juvenile *Lernaeocera branchialis* caused the ends of gill filaments to thicken and lamellae to fuse as a result of tissue proliferation (Kabata 1958). However, gill epithelial hyperplasia and hypertrophy of *O. nerku* caused by *S. californiensis* resulted in the fusion of filaments (Kabata & Cousens 1977).

The absence of haemorrhage and neutrophilia during the second and third phases of host reaction indicate that these are chronic phases of inflammation and the

gradual loss of the degree of intercellular oedema suggests that improvement of the gill structure takes place as Roberts et al. (2004) previously suggested for fishes infested with *S. californiensis*.

Necrosis of the host tissue in the vicinity of the parasite agrees with findings of Paperna (1996) that the area around attachment site of the parasite (*Lernaea cyprinacea*) may ulcerate with resulting focal necrosis. The rest of the gill filament remained normal.

Haematophagous feeding mode could lead to anaemia and weakening of the fish (Thatcher 1998). However, the results from the current study showed no correlation between the intensity of infestation and haematocrit values. This suggests that *L. clariae* does not cause anaemia to its host fish. The low haematocrit values associated with anaemia observed were neither directly nor indirectly proportional to the intensity of *L. clariae* infestation. This suggests that they were caused by other factors than feeding of *L. clariae*.

Furthermore, the absence of a correlation between the fish haematocrit values and infestation intensity could be due to the parasite size in relation to its host size, as previously suggested (Kabata & Cousens 1977; Kabata 1981; Bennett & Bennett 1994) for other copepods. The length of *L. clariae* ranges from 6mm-7.2mm with an average of 6.1mm and correlated positively with the size of the studied host fish which ranged between 40.6 and 121cm. This suggested that the parasite size correlates positively with the host size (Tsotetsi et al. 2004). This is in contrast to the findings of Woo & Shariff (1990) who showed that adult specimens of *L. cyprinacea* were particularly harmful to young fish because of their relatively large size. The low mean intensity of 6.6 observed under natural condition (Tsotetsi et al. 2004) suggests

that it was too low to have any impact on the haematocrit values and that *L. clariae* causes limited pathological effect to its host fish.

However, high intensities may be detrimental to the fish as many studies report increased pathological effects associated with severe infestation (Dogiel et al. 1961; Bennett & Bennett 1994). Paperna (1996) suggested that high infestations in *L. clariae, L. intercedens* and *L. monodi* may interfere seriously with respiration of their host fishes. Furthermore heavy infestations and the possibility of repeated infestations may result in irreversible and cumulative branchial tissue modification with serious hydrodynamic consequences (Benz 1980).

Proliferation of mucous cells in the current study is similar to observations of Dezfuli et al. (2003) caused by *Ergasilus sieboldi* infestation. Hyperplasia of epithelial cells, particularly mucous cells associated with an increase in the production of mucous can cause respiratory distress and suffocation of the fish (Bennett & Bennett 1994; Martins et al. 1999) and fusion of lamellae would result in restriction of air passages and thus hinder the process of respiration of the host. Thus there is no doubt about the potential detrimental effect that this copepod can have on its host either directly because of mode of feeding and attachment or indirectly because of secondary effects. Changes in gill tissue could also adversely affect the excretion of the fish since the gills are also involved in other physiological functions (Barassa et al. 2003). Infestations in high density fish stocking may reach high intensities and no evidence of antiparasitic response was observed.

The current study showed that *L. clariae* causes three phases of host reaction associated with its adult stage development; the gravid adult stage caused less pathology when compared to the earlier stages. It causes hyperplasia and hypertrophy

leading to fusion of gill lamellae. Although these host reactions were not harmful to the fish, high infestations may be detrimental.