CHAPTER 4

ASPECTS OF THE LIFE CYCLE OF *LAMPROGLENA CLARIAE* (COPEPODA: LERNAEIDAE) FROM THE VAAL RIVER SYSTEM

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Lamproglena clariae Fryer, 1956 is endemic to Africa and attaches to gill filaments of freshwater fish of the family Clariidae (Marx and Avenant-Oldewage, 1996). The adult stage of this parasite has been extensively studied and described (Fryer 1956, 1961, 1964; Marx and Avenant-Oldewage, 1996). A brief description of the nauplius stages of this parasite was recorded by Fryer in 1956 however, attempts to rear these nauplii did not succeed. The only species in this genus whose life stages has been described is *L. chinensis* Yü, 1937. Sproston *et al*. (1950) described only male and female adults of *L. chinensis* and a metanauplius. Fortunately in 1962 Kuang managed to study and record the whole life cycle of this parasite through laboratory observations. Five nauplius, five copepodites, a pre-adult (cyclopoid) and an adult stage were recorded. This life cycle differs from that of *Lernaea cyprinacea* Linnaeus, 1758, another member of the family Lernaeidae, which has only three nauplius, five copepodite, a cyclopoid and adult stages (Grabda, 1963). The current study aimed at determining the number of life stages of *L. clariae* in comparison with those of *L. chinensis* and *Lernaea cyprinacea* and further studying and recording their morphology.

MATERIALS AND METHODS

Collection of specimens

Specimens of *Clarias gariepinus* (Burchell, 1822) were collected over a period of four years (2001-- 2004) from the Vaal Dam. They were collected by means of gill nets and transported to the laboratory.

Laboratory Procedures

Fish were killed by a single cut through the spinal cord and gills were dissected out. In 2001 and 2002 both the larval and adult stages of *L. clariae*

specimens were collected from the gill filaments of the fish. Larval stages were preserved in 70% ethanol and adult stages were preserved after the egg sacs were carefully removed from them. In 2003 and 2004 only adult specimens of *L. clariae* were observed and collected from the gill filaments of the fish. Eggs were carefully removed from the adults, placed in the Syracauses and glass Petri dishes approximately 5cm in diameter with dam water and another set placed in algal dam water. Both sets were incubated in the laboratory at standard laboratory temperature artificially set at 18^{o}C in summer and 22^{o}C in winter. A third set of eggs was incubated in dam water in a controlled experimental room at daylight cycle (12 hours of darkness and 12 hours of light, resembling the summer light cycle) at 27 ^{o} C. To observe the number of naupliar stages, ten nauplii were each placed in a glass Petri dish immediately after hatching and observed every two hours with the aid of a dissecting microscope until they reached the first copepodite stage. The number of exsheathed cuticles from each Petri dish was counted and some of the larvae collected immediately after hatching and moulting were preserved in 70% ethanol. The first infective larvae (first copepodite) were introduced into a fish tank (8.3x 10^4 cm^3) containing *C. gariepinus* bred and reared in the laboratory and never exposed to *L. clariae* infestation. However, ten of the first copepodites were left in algal water and observation continued.

A day after infestation the fish was transferred into a bigger tank (1.1x10^6 cm^3). Fish tanks with infested fish were kept in a controlled room at daylight cycle. Fish were later killed at various intervals (Table 1), various larval stages of the parasite recovered and preserved in 70% ethanol. Preserved specimens were cleared in 90% lactic acid, mounted and drawn with the aid of a Zeiss light microscope equipped with a drawing tube.

Table1: The incubation intervals of the first copepodite stage of *Lamproglena clariae* on fish

Experimental Period	Infestation period (Days)
January 2001	7, 14, 21
January 2003	14
April 2003	7, 14, 22
January 2004	7
March 2004	1, 1, 1, 2

In the two final experiments fish were physically infested with parasites that is, the copepodites were physically put on the fourth gill arch of the fish using a brush. Fish were anaesthisized with Two-Phenoxy ethanol after one and two days respectively, then gills were dissected out and parasites were searched from both the gills and water. These experiments only aimed at finding the second copepodite stage, hence the fish were killed after one/two days of infestation.

Grabda (1963) stated that copepodite stages of *Lernaea cyprinacea* are not as host specific as adult specimens. Hence in the current study, small (about 6 cm long) *Tilapia* specimens were also infested with the first copepodite specimens, although (Tsotetsi *et al*., 2004) showed that adult female specimens of *L. clariae* are host specific to members of the Clariidae family.

Overall, twenty-two repetitions were done with 1043 parasites on forty seven *C. gariepinus* specimens. Prevalence and Mean Intensity were calculated according to definitions by Margolis et al. (1982).

Only experiments done in January 2001, January and April 2003, January, February, March, April and May 2004 reached the first copepodite stage. They were regarded as successful and are the ones that will be mentioned and discussed.

RESULTS

High parasite prevalence was observed during late summer month (January) and autumn months (February--May). High percentages of gravid females were also observed during these seasons. Although no fish and consequently no parasites were collected during the spring and early summer seasons, non gravid female parasites were collected in August and September. Eggs removed from adult females in each experiment were of colours varying between white, bright and dark yellow, with a number of eggs ranging between eighteen and forty four. Although only dark yellow eggs hatched into nauplius stage and the rest did not hatch, a high hatching success was observed (Table 2).

Table 2: Hatching success of eggs collected from adult females throughout the study period

Collection Period	Prevalence (%)	Gravid Females (%)	Hatching success (%)
January 2001	75	14	91
April 2002	82	7	0
August 2002	0	0	-
October 2002	0	0	-
November 2002	0	0	-
December 2002	0	0	-
January 2003	50	50	100
April 2003	100	50	100
August 2003	67	0	-
October 2003	0	0	-
November 2003	0	0	-
January 2004	88	35	100
February 2004	100	50	31
March 2004	33	50	100
April 2004	47	17	100
May 2004	100	11	100
September 2004	75	0	-

Key:

Gravid females %: Gravid females/ Gravid + Non gravid females

Hatching success: Eggs that hatched (dark yellow)/Total number of eggs

A difference in developmental rate was observed in the set incubated in the laboratory. A faster developmental rate was observed during the warmer months with longer photoperiod than during colder months with shorter photoperiod (Fig. 1).

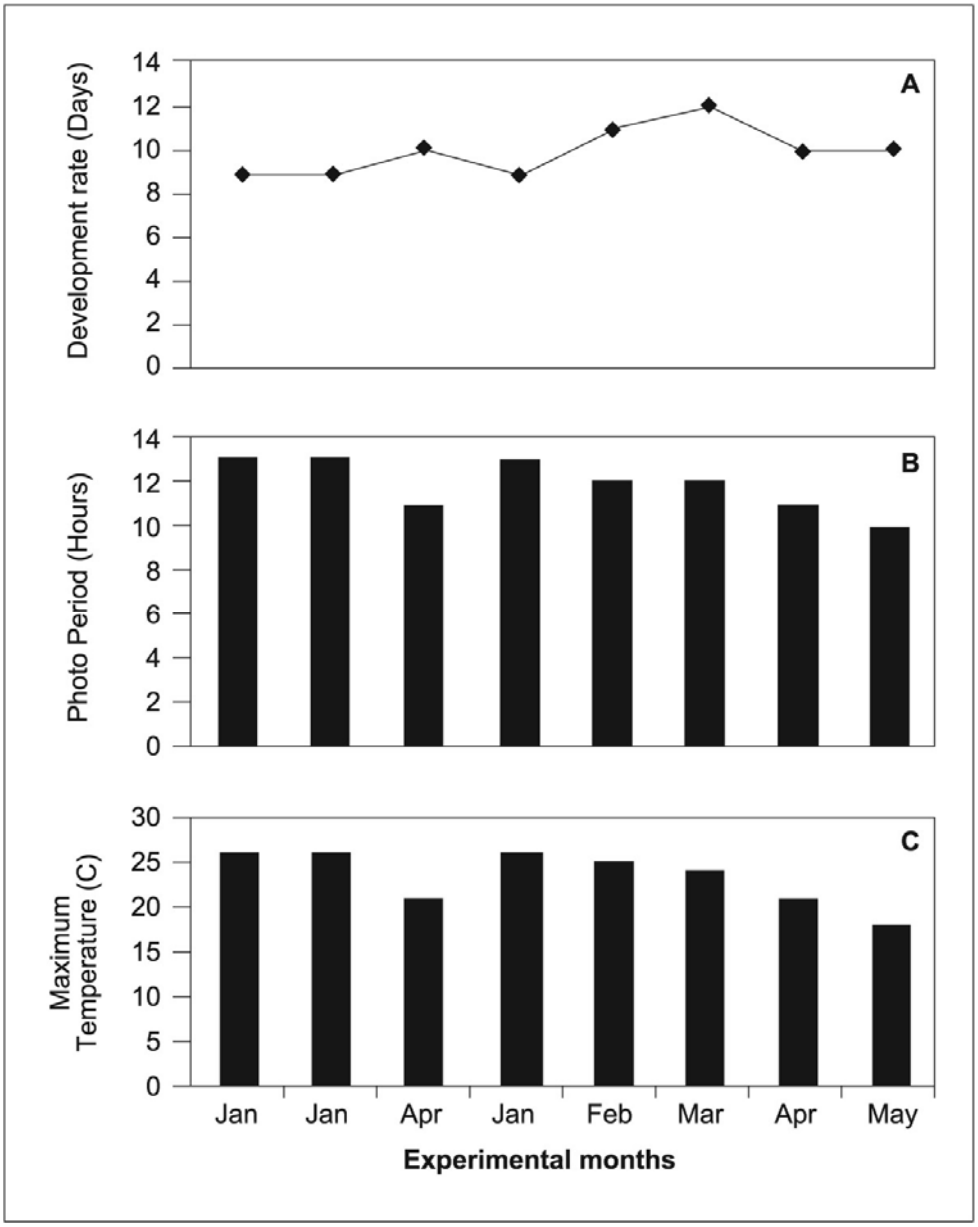

Figure 1: Comparison between the development rate (A) of larval stages of *Lamproglena clariae* reared in the laboratory under different photoperiods (B) and different temperatures (C).

However, there was no difference in the development rate of the parasite from the egg to the first copepodite stage between sets incubated in the controlled experimental room and those incubated in the laboratory. They both took nine days to develop during warmer months.

The incubated eggs each hatched into a nauplius, which molt into a second nauplius after twenty four hours, then molt for the second time after about forty hours into a third nauplius stage. This stage lasted for approximately seven days, then molt into a first copepodite stage. Three cuticles were observed in culture medium for each nauplius that developed from the first nauplius to the first copepodite stage.

Copepodites incubated in algal water did not develop further, but died after six days. Copepodites used to infest the *Tilapia* fish were collected from the gills of the fish after three days and were still at the first copepodite stage.

The size of experimental *C. gariepinus* specimens infested ranged between 33-45cm in length and 250-500g in weight.

Various stages were recovered from infested fish at various incubation periods (Table 3).

Table 3: Various stages of *Lamproglena clariae* larvae recovered from experimentally infested fish

Experimental Period	Infestation period (Days)	Recovered parasites (n)	Stages recovered
January 2001	7, 14, 21	4	3 Copepodite + Adult
January 2003	14	3	Copepodite
April 2003	7, 14, 22	3	2 Copepodite + Adult
January 2004	7	2	Copepodite + Immature Adult
March 2004	1, 1, 1, 2	4	Copepodite1

There was a difference in the mean intensities of parasites used to infest the fish and those recovered after infestation and a very low infestation success ranging between 0 and 33% was observed (Table 4).

Table 4: The number of parasites at the first copepodite stage used to infest fish throughout the study period

Study Period		Mean intensity	Recovered Mean intensity	Infestation success (%)
January	2001	20	4	20
January	2003	41	3	7
April	2003	19	3	16
January	2004	38	2	4
February	2004	7	0	0
March	2004	12	4	33
April	2004	19	0	0
May	2004	28	0	0

Both the recovered specimens and larval stages collected in the field were used to describe the life cycle. Specimens of each developmental stage were studied and described as follows:

NAUPLIUS STAGES

All the nauplius stages are oval shaped with translucent bodies filled with a mass of yolk, which is reduced as the nauplius develops. The first stage has three pairs of appendages; the uniramous antennules, biramous antennae and biramous mandibles. All these appendages bear smooth setae. The second and third stages have an extra pair of appendages presumably, the maxillule, appearing as papillae with a pair of smooth setae. They also have a naupliar eye positioned mid anteriorly and are indicated as ‘X’ on the attached drawings. There is an increase in the number of setae on appendages as they molt from one stage to the next. At rest, antennules and

antennae are directed forward and cover each other; mandibles are situated obliquely forward and bent to sides. Attached drawings are made from preserved specimens; therefore the arrangement of appendages fails to correspond to their proper natural arrangement. At the posterior end they have various number of setae used for balancing.

Nauplius 1 (Fig. 2a)

Elliptical body, three pairs of appendages, a pair of posterior setae. Its size (n=20) ranges between 0.510--0.516 mm length, and 0.268--0.310 mm width.

Antennules (Fig.2b) uniramous, three podomeres. The distal podomere with one lateral and two apical setae; the median podomere with one lateral seta. The proximal podomere without setae.

Antennae (Fig.2c) biramous, protopodite (coxapodite + basipodite) without setae. The exopodite with four podomeres; two setae on the distal, one each on the third and second podomeres. The endopodite with two podomeres, two distal setae.

Mandibles (Fig.2d) biramous, protopodite without setae. Exopodite with four podomeres, two setae on the fourth podomere and one seta on the third and second podomeres respectively. Endopodite, one segmented with two distal setae.

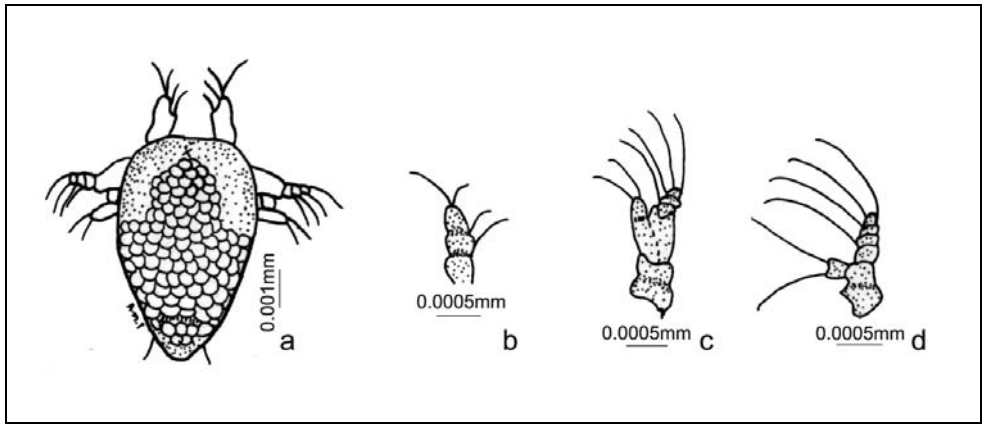

Figure 2: *Lamproglena clariae*, dorsal view, Nauplius stage 1, (a), Antennule (b), Antenna (c) and Mandible (d).

Nauplius 2 (Fig.3a)

Elliptical body, slightly reduced amount of yolk, four pairs of appendages and two pairs of posterior setae. It ranges (n=20) from 0.517--0.518mm length, 0.31--0.33mm width.

Antennules (Fig.3b) uniramous, three podomeres. Distal podomere with one lateral and three apical setae, median podomere with one lateral seta. Proximal podomere without setae.

Antennae (Fig.3c) biramous, protopodite without setae. Exopodite with four podomeres, three setae on the distal, one seta on the second podomeres respectively. Endopodite with two podomeres, two setae on the distal podomere.

Mandibles (Fig.3d) biramous, exopodite with four podomeres, two setae on the fourth podomere, one seta on the third and second podomeres respectively. Endopodite with two podomeres, two setae on the distal podomere. The protopodite has a small protrusion on the side. The fourth appendage, presumably the first maxilla (Fig.3e) appears ventrally in a rudimentary form, about two thirds of the body length from the posterior end. They are cylindrical lobes bearing one long outer and shorter inner setae.

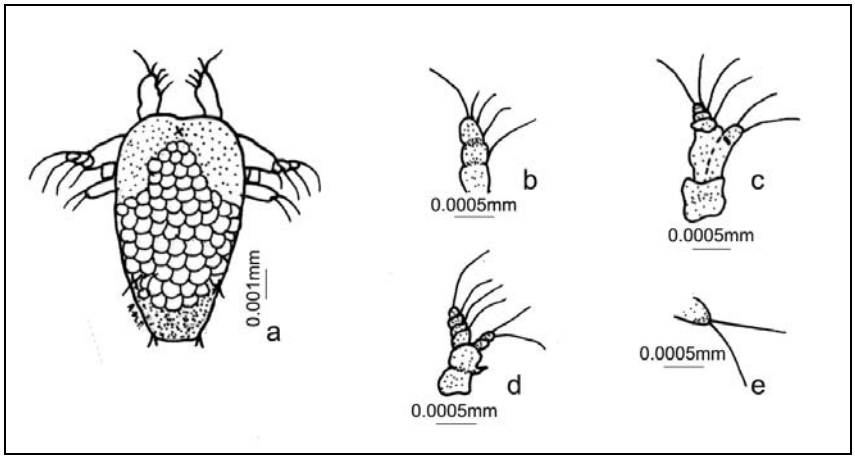

Figure 3: Nauplius stage 2, *Lamproglena clariae*, dorsal view (a). Antennule (b), Antenna (c), Mandible (d) and Maxillule (e).

The difference between Nauplius 1 and Nauplius 2 is the addition of a podomere in the endopodite of the antennae and mandible. Addition in the number of setae particularly of the antennules and an additional appendage (maxillule).

Nauplius 3 (Fig. 4a)

Elliptical body, narrowed towards the posterior end, a decrease in the yolk amount, four pairs of appendages, bears three pairs of posterior setae. In its late stage, the yolk amount is much reduced and the furcal rami are present, bearing three pairs of unequal setae. Its size ranges from 0.52mm--0.56mm long and 0.23mm--0.26mm wide.

Antennules (Fig.4b) uniramous, indistinctly segmented, seven apical and two lateral setae.

Antennae (Fig.4c) biramous, the exopodite with four podomeres, two setae on the distal, one seta on the third and second podomeres respectively. The endopodite with two podomeres, two setae on the distal podomere.

Mandibles (Fig.4d) biramous, the exopodite and endopodite attached to the protopodite. The exopodite with four podomeres, two setae on the fourth podomere, one seta on the third and second podomeres respectively. The endopodite with two podomeres, two setae on the distal podomere. The protopodite has a small protrusion on the side. The first maxilla (Fig.4e) still appears as a papilla with a pair of setae, but positioned more anteriorly to the midventral side of the nauplius body.

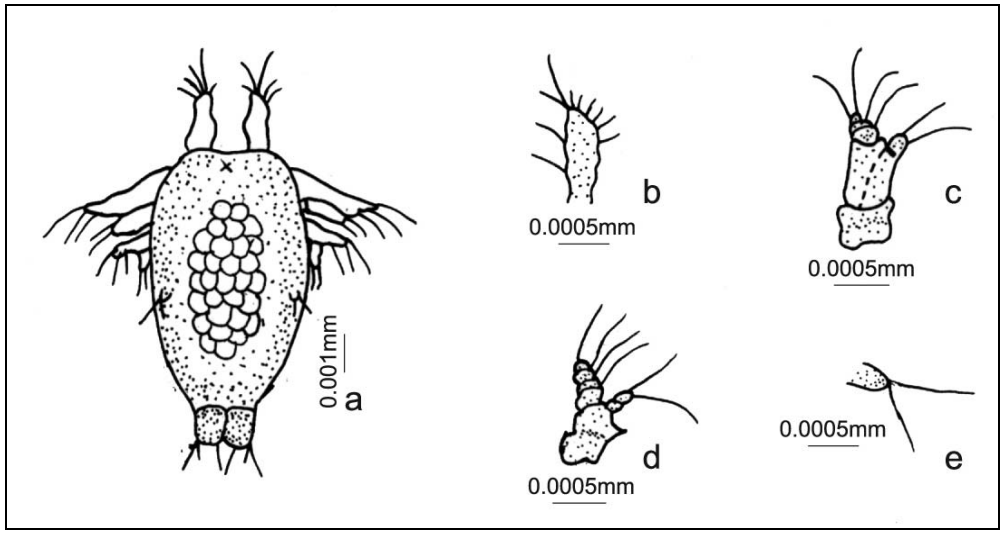

Figure 4: Nauplius stage 3, *Lamproglena clariae* dorsal view (a). Antennule (b), Antenna (c), Mandible (d) and Maxillule (e).

The number of podomeres in body appendages remains the same as in the previous stage, but the number of setae on antennules increases.

COPEPODITE STAGES

All the copepodite stages have three body somites; the cephalothorax, free thorax and abdomen. The somite cephalothorax resulted from incorporation of the thoracic appendage, maxillipede to the cephalic region. Hence, four appendages were observed on the cephalothorax, and the number of segments and appendages on the free thorax and abdomen differed from one stage to the next.

The body is transparent with dark coloured, almost black intestine. The naupliar eye is visible throughout the copepodite stages and is positioned in central line at the anterior body edge.

Copepodite 1(Fig.5a)

The total body length (n=10) is 0.74 mm. Four pairs of appendages observed on the cephalothorax were antennules, antennae, maxillae and maxillipeds.

The cephalothoracic region

Antennules, uniramous with three podomeres, eleven setae on the second and third podomere (Fig.5b). Antennae, biramous, exopodite with three podomeres, a pair of apical spines. The endopodite, much reduced, appears as a bud, one segmented, a pair of apical setae (Fig.5c). The maxilla, one segment appearing as a claw arising from the copepod body surface (Fig.5d). The maxillipede, unisegmented with five terminal claws of unequal sizes (Fig.5e).

No mandibles nor maxillule were observed at this stage.

The thoracic region

The thorax, three free segments, the first two segments with a pair of swimming legs each. The third segment, a pair of marginal setae, a pair of setae medially. The first pair of swimming legs, biramous, one segmented, both the exopodites and endopodites are attached to a basipodite bearing setules. The exopodite with setules on the surface and both exopodites and endopodites with long setae (Fig.5f). The second pair of swimming legs, biramous. exopodites and endopodites with a number of setules, but the exopodites have fewer, and bears one lateral spine (Fig.5g).

The abdominal region

The abdomen, two segments. No sexual/genital structures are visible at this stage. Each furcal ramous with five setae varying in size, the innermost being the longest (Fig.5h).

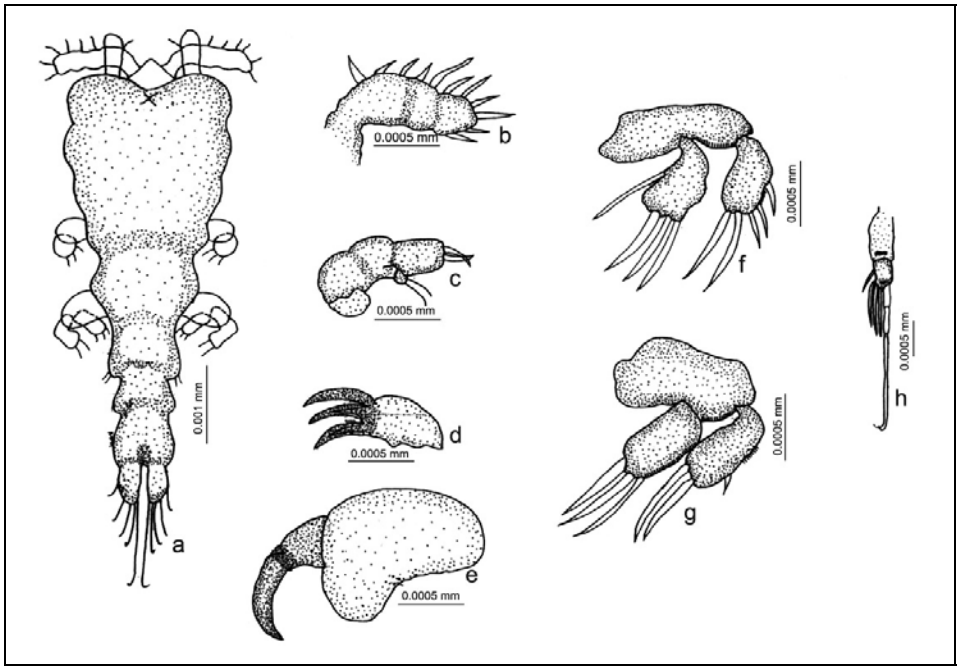

Figure 5: Copepodite stage 1, *Lamproglena clariae*, dorsal view (a), Antennule (b), Antenna (c), Maxilla (d) and Maxilliped (e), first swimming leg (f), second swimming leg (g) and furcal ramous (h).

This stage marks the final stage of the free living phase in the life cycle of *L. clariae*. The following stages are parasitic, thus were collected from fish.

Copepodite 2 (Fig.6 a)

The total body length (n=3) is 1.204 mm.

The cephalothoracic region

The cephalothorax, four pairs of appendages; antennules, antennae, maxillae and maxillipeds. Antennules with three podomeres, a total of fourteen marginal setae (Fig.6b). Antennae, uniramous, three podomeres with a pair of apical spines (Fig.6c). The maxilla, one segment with a claw, arises from the copepod body surface (Fig.6d). The maxillipede, unisegmented with five terminal claws (Fig.6e).

Although the number of body appendages of the cephalothoracic region is the same as in the previous stage, few differences were observed; an addition in the

number of setae on the antennules and reduction of biramous antennae into uniramous antennae were observed.

The thoracic region

The thorax, five free segments, the first four segments with a pair of swimming legs each. The fifth segment bears a pair of setae. Sexual structures are still not visible at this stage. The first pair of swimming legs, biramous, exopodite and endopodite, consist of two segments. The first segment of the exopodite without setules, but with a lateral spine. The second one, spare setules, setae, three lateral spines. The first segment of the endopodite, with setules, the second one with setules and setae (Fig.6f). The second pair of the swimming legs is similar to the first except that the first segment of the exopodite bears a lateral spine (Fig.6g). The third pair of legs only differs from the first two in that, the first segment of the endopodite is the only one that bears setules (Fig.6h). The fourth pair is similar to the second pair (Fig.6i) and the fifth pair is less developed and appears as a lobe with setae (Fig.6j).

Addition of two segments on the thoracic region, addition of a segment on each swimming leg and the difference in the number of spines and setae on the swimming legs were observed.

The abdominal region

The abdomen, two segments, a pair of furcal rami (Fig.6k) with five smooth setae of various sizes. The second and third setae of the furcal ramous are the longest contrary to their arrangement in the first copepodite. The arrangement of setae on the furcal rami stays the same throughout the following stages.

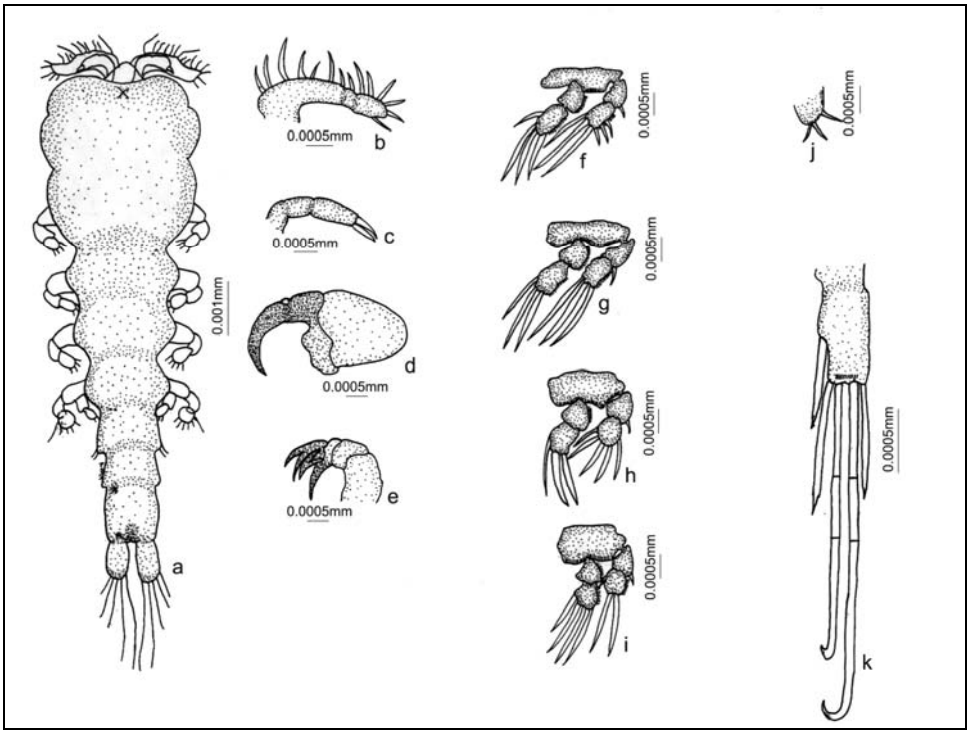

Figure 6: Copepodite stage 2, *Lamproglena clariae*, dorsal view (a). Antennule (b), Antenna (c), Maxilla (d) and Maxilliped (e), first swimming leg (f), second swimming leg (g), third swimming leg (h), fourth swimming leg (i), fifth leg (j) and furcal ramous (k).

Copepodite 3 (Fig. 7a)

The total body length (n=5) is 1.488 mm.

The cephalothoracic region

The cephalothorax appendages (Fig.7b-Fig.7e) are similar to the previous stage, except for an addition of one apical seta on the second antennae.

The number of body appendages of the cephalothoracic region is the same as in the previous stage, except for the addition in the number of setae on the antennae.

The thoracic region

The thorax has five free segments with a pair of swimming legs each. The first four pairs of legs (Fig.7f-7i) biramous, two segments (both the endopodite and exopodite). The fifth pair (Fig. 7j), less developed, uniramous, three setae, appears on

the genital segment. The genital segment, two internal oval structures. There is a vestigial pair of sixth swimming legs appearing as setae on the distal part of the genital segment.

The segments on the thoracic region remain unchanged, but changes are observed on the fifth segment where genital structures are observed and the fifth pair of swimming legs being more developed than in the previous stage.

The abdominal region

The abdomen, three segments, furcal rami, each ramous with five setae of various sizes.

An additional segment on the abdomen is observed and the arrangement of setae on the furcal rami remains unchanged.

Copepodite 4 (Fig.8a)

The body length (n=8) is approximately 2.5625 mm long. The body somites and the cephalothoracic appendages are similar to those of previous stages.

The number of body appendages of the cephalothoracic region is the same as in the previous stage, except for the addition in the number of setae on the antennules.

The thoracic region

The free thorax, still five segmented. The first four segments, a pair of biramous, two segmented swimming legs each (Fig. 8f--8i); the fifth pair of legs appearing as a papilla, the sixth pair of legs appearing as setae on the marginal edges

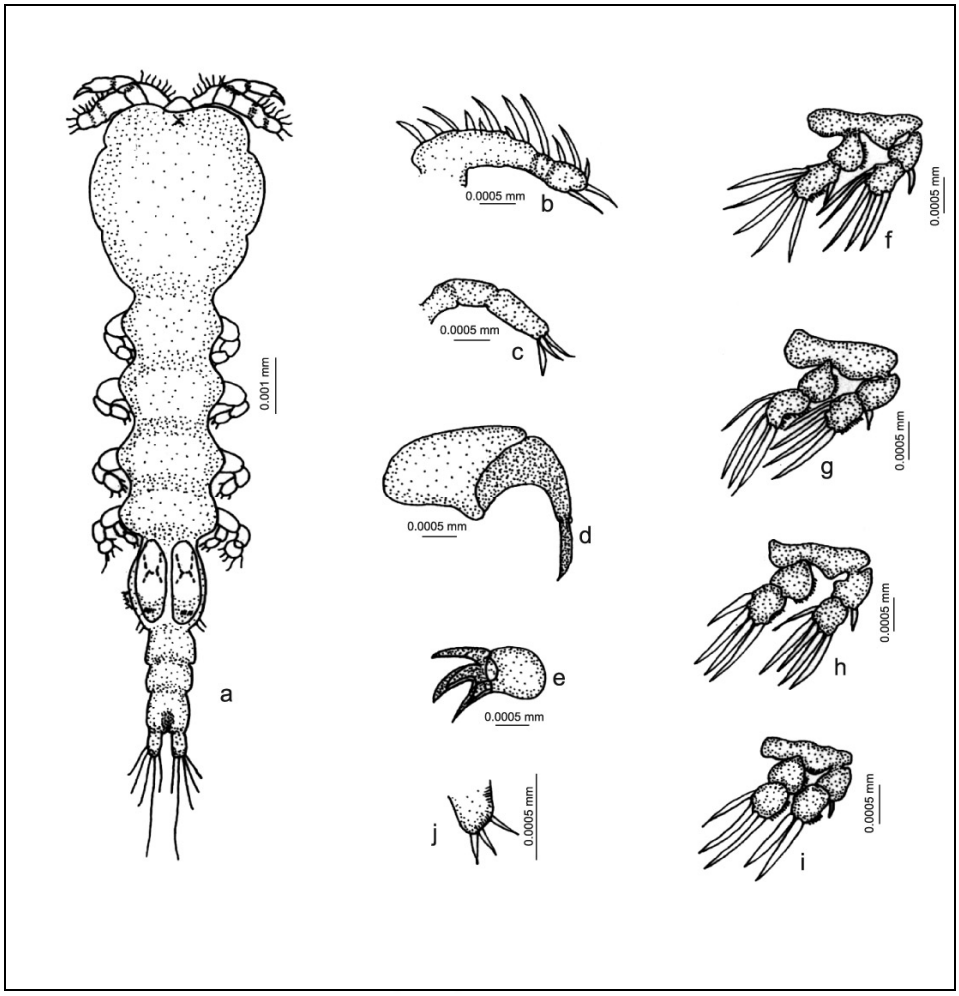

Figure 7: Copepodite stage 3, *Lamproglena clariae*, dorsal view (a). Antennule (b), Antenna (c), Maxilla (d) and Maxilliped (e), first swimming leg (f), second swimming leg (g), third swimming leg (h), fourth swimming leg (i) and fifth swimming leg (j).

of the genital segment. This segment has increased in size compared to the previous stage such that the fifth pair of legs is situated above the genital structures.

The abdominal region

The abdomen, three segments, a pair of furcal rami with five setae of various sizes.

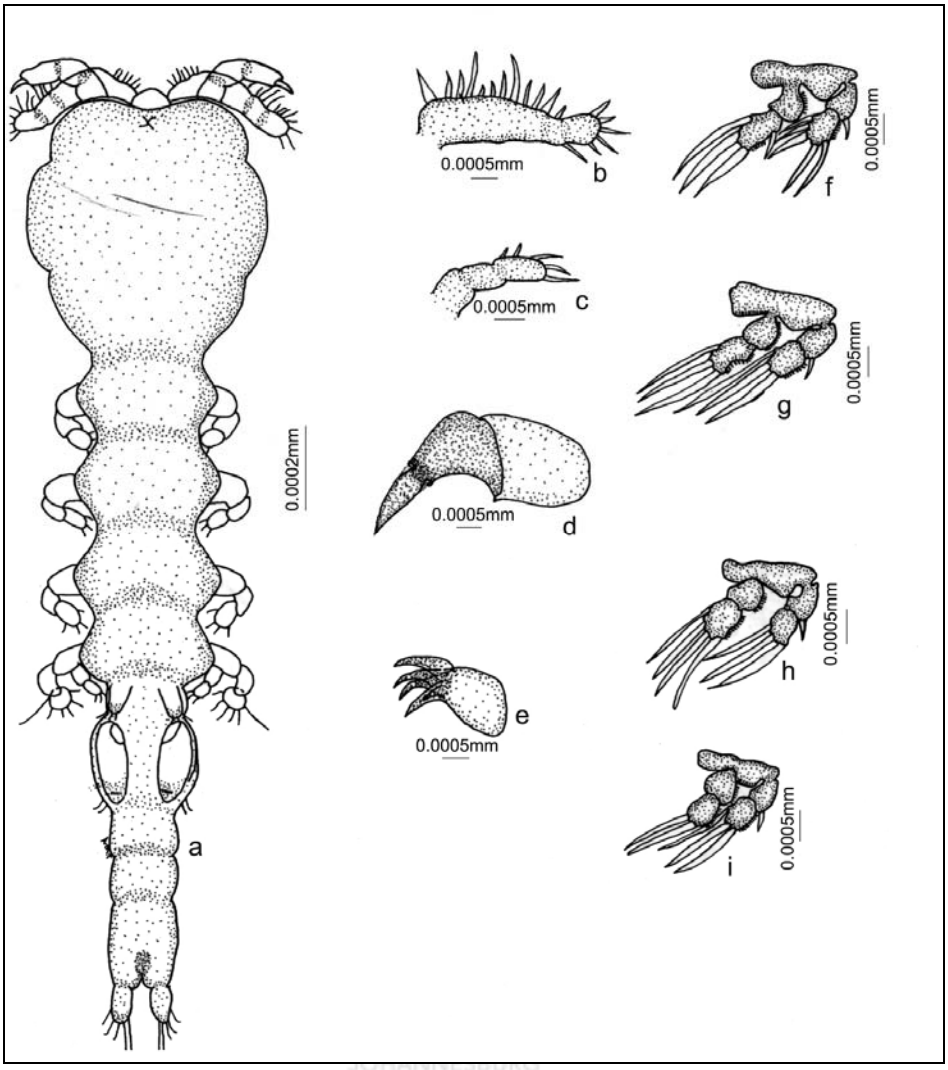

Figure 8: Copepodite stage 4, *Lamproglena clariae*, dorsal view (a). Antennule (b), Antenna (c), Maxilla (d) and Maxilliped (e), first swimming leg (f), second swimming leg (g), third swimming leg (h) and fourth swimming leg (i).

Cyclopoid (Fig. 9a).

The total body length (n=3) is 2.6525 mm. The body somites and the cephalothoracic appendages are similar to those of previous stages.

The thoracic region

The thorax, five segments. The first four segments, a pair of biramous, two segmented swimming legs each. The fifth segment, a pair of the fifth swimming legs,

oval genital structures, a pair of setae at the marginal edges (Fig. 9f--9i). The fifth and sixth pairs are similar in structure to those in the previous stage.

The abdominal region

The abdomen, four segments, a pair of furcal rami, each ramous with five setae of various sizes.

An addition of an abdominal segment was observed at this stage.

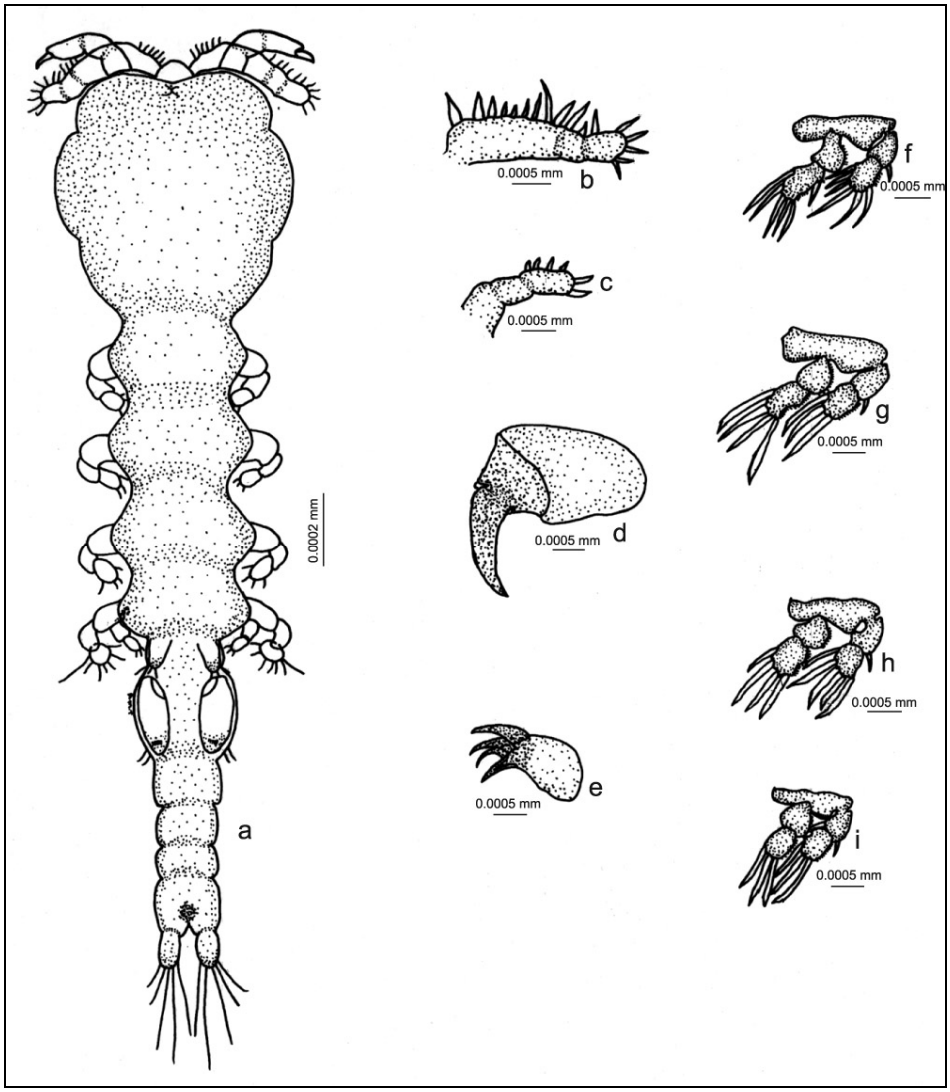

Figure 9: Cyclopoid stage, *Lamproglena clariae*, dorsal view (a). Antennule (b), Antenna (c), Maxilla (d) and Maxilliped (e), first swimming leg (f), second swimming leg (g), third swimming leg (h) and fourth swimming leg (i).

Adult (Female)

The body has three somites; cephalothorax, thorax and the abdomen. Antennules, antennae, maxillae and maxillipedes are the only appendages observed on the cephalothorax region at this stage. The thorax has four pairs of reduced (became smaller than in the preceeding stages) legs and a genital segment, no appendages were observed on the three-segmented abdomen. Segmentation is blurred at this stage, not as clear as in previous stages. The furcal rami have five reduced setae of various sizes.

Results of the current study revealed that the adult stage of this parasite undergoes different phases during its development. The newly attached female posses the functional swimming legs and segmentation is still pronounced. This is followed by the early adult phase with reduced swimming legs, partially retained external segmentation, elongated and enlarged segments and visible ovaries without egg sacs. The matured adult females posses egg sacs and is described in details by Marx and Avenant-Oldewage (1996).

The observed life stages of *Lamproglena clariae* can be diagrammatically presented as follows:

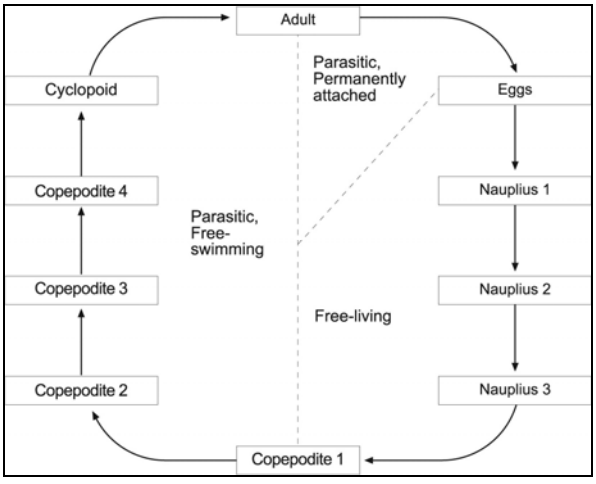

Figure11: Diagrammatic presentation of the life cycle of *Lamproglena clariae*, Fryer 1956.

DISCUSSION AND CONCLUSION

The high prevalence and percentages of gravid females observed in autumn, just before the winter season in the current study is similar to the results of Tsotetsi *et al*. (2004) whereby parasite abundance and prevalence decreased during the summer period and an increase in parasite prevalence, intensity and abundance occurred before winter. These observations support the observation made by Marx and Avenant-Oldewage (1996) that reduced water levels in winter concentrate hosts and parasites, which increases the probability of infection. Bruton (1979) further states that spawning in *C. gariepinus* usually takes place in summer and it is during this period when hatched parasite larvae are abundant and can infect their hosts. This suggests that the hatched nauplius stages of *L. clariae* would have been abundant, molt through to the infective copepodite stage, and might have infected their hosts during this period, developed and matured in their host towards winter; hence higher percentages of gravid females were collected during this period (January--April) in the current study.

In some of the eggs removed from the adult females, nauplii with red nauplian eyes were already visible, but they took more than two days to hatch. This can be explained in terms of the change in environment (from natural to incubation environment). In contrast to the natural environment whereby the abdomen of *L. clariae* is extended into the open gill cavity, allowing for egg sacs to be aerated, in the incubation environment there was no aeration supplied to the eggs. Bennett (1999) suggested that aeration and water movement are important for successful hatching in copepods such as *Dissonus manteri*. Under these conditions hatching success is 94.8%, but decreases to 48.4% in static water with no aeration.

Observations of the faster development rate of larval stages during warmer months with longer photoperiods in the current study suggest that both temperature and photoperiod determined the rate of larval development. However, the similarity in larval development rate between sets of larvae incubated in the controlled experimental room and laboratory with different temperatures suggests that photoperiod plays more of a role on development rate than temperature, since parasites incubated in the laboratory experienced constant temperatures ranging between 18^{o}C and 22^{o}C. Previous studies have shown that larval development depends on temperature (Paperna, 1996; Escribano and McLaren, 1992; Tully, 1992; Gonzalez and Carvajal, 2003; Tully and Nolan, 2002).

No difference was observed between the development of nauplii reared in dam water and green water (with algae), though Kumazawa (2000) states that some freshwater copepods require green algae and larger organisms to complete their life cycle. The gradual decrease in the amount of yolk in the current study as nauplii developed further indicates that development proceeded at the expense of body yolk, without additional external food and that nauplius stages of *L. clariae* are free living.

Dying of the first copepodites incubated in algal water without moulting to the second copepodid stage showed that this stage depends on the host for development. This observation differs from observations made on the life cycle of *Lernaea spp.*, whereby in addition to nauplius stages, the first and second copepodid stages were obtained from egg culture (Nakai, 1927). However, that observation does not prove that the second Copepodite stage is either parasitic or free living, since it is possible that it would not survive and moult to the third stage without a host.

The three nauplius stages observed currently corresponds to the number of nauplius stages reported for *L. cyprinacea* (Grabda, 1963), but differs from that

reported for *L. chinensis* (Kuang, 1962). This is surprising because it would be expected that the congeneric *L. clariae* and *L. chinensis* would follow the same pattern. However, it is most probable that Kuang mistook the late second and third nauplius stages for the fourth and fifth stages, since these stages (early and late second stages; and the early and late third stages) look somewhat different from each other.

The low infestation success observed can be explained in terms of variation in parasite attachment success and the immune system of the host fish.

Contact with the host of *Lepeoptheirus salmonis* is facilitated by a burst swimming response to linear water accelerations with a frequency of 3-12 HZ, which lasts for 1-3 seconds (Heuch and Karlsen, 1997). A fish moving within centimetres of the copepodite generates this type of acceleration. However, the response to physical stimuli such as small scale water disturbances does not explain the host specificity of parasites as many fish species may generate similar disturbances. There is no chemotactic response prior to attachment to the host, *Salmo spp.* (Bron *et al*., 1993). Chemosensory mechanisms may be important in determining if a copepodite attaches successfully to the host. Settlement and moulting will not occur if the host is not the specified one for the parasite. This also explains why the *L. clariae* specimens did not moult to the next stage when attached to *Tilapia* specimens.

The piscine immune system is very responsive to antigenic stimulation (Woo and Shariff, 1990) and since gills are highly vascularised they have a more pronounced immune response (Johnsons and Albright, 1992). Active host rejection has been reported on cyclopoid copepods *L. cyprinacea* and *L. polymorpha* in both naïve and previously exposed fish (Shields and Goode, 1978; Shariff and Roberts, 1989; Woo and Shariff, 1990) and reduction of these parasites was believed to be due to cellular response.

The number of copepodite stages observed in the current study differs from number of copepodite stages of both *L. chinensis* and *L. cyprinacea*. In the current study four copepodite stages were observed, whilst five copepodite stages were observed in the life cycles of both *L. chinensis* and *L. cyprinacea*. Morphology of observed copepodite and cyclopoid stages of *L. clariae* corresponds to those of these copepods in terms of number of segments, except for the copepodite stage with three swimming legs. It is, hence suspected that there is a missing stage in the current study between the second copepodite which has two pairs of swimming legs, and the third copepodite stages which has four pairs of swimming legs. According to Kabata (1979) morphological development from one stage to the next is mainly marked by the addition of segments and the appearance of additional swimming appendage. This suggests there should have been a stage with three pairs of swimming legs. If that stage were observed, then it would correspond to the third copepodite stage in the *L. cyprinacea* and *L. chinensis* life cycles. On the other hand considering the number of attempts made to experimentally determine the life cycle of this parasite, the collection of other larval stages from the field; it may be assumed that this stage does not exist in *L. clariae* and that this species has four copepodite stages. Anyway this still calls for further investigations.

A gradual increase in size was observed as the life stages of this copepod progressed. Even though no male specimens at the third copepodite stage was observed or collected in the current study, it is assumed that it would be similar to the female one, since there are little morphological differences between sexes in copepods, the male being distinguished from the female mainly by the structure and proportions of its genital complex (Kabata, 1979).

Observation of only four pairs of appendages on the cephalothoracic region of copepodite stages in the current study is similar to observations made by Marx and Avenant-Oldewage (1996) on the adult stage of this parasite. According to Yamaguti (1939) and Sproston *et al*. (1950) the first maxilla of *Lamproglena* are reduced to mere lobes with knobs at their anterior corners, mostly obscured by maxillae. However, in adult *L. chinensis* they are more highly developed than they appear to be in any other *Lamproglena* species (Sproston *et al*., 1950). According to Kabata (1979) it is very difficult to isolate mandibles and maxillulae because they are covered by well developed maxilla. However, these appendages were observed in larval stages of *L. cyprinacea* (Grabda, 1963).

Reduction of the swimming legs of the copepodite stages observed after attachment and moulting on the host is an adaptation to parasitic mode of life of the adult female. An immobile copepod has no need for a stream lined, well-articulated body or for locomotory appendages. As expected, *L. clariae*, like many parasitic copepod species, partly lost its segmentation and reduced the locomotory appendages as it became attached to the host. When the females become permanently attached to the host, the onus lies on the male to establish contact between the two sexes, hence it must remain mobile at least until it has located the female. The male is often less modified than the female, particularly in its locomotory ability (Kabata, 1992). The cyclopoid stage marks the final stage of the male *L. clariae*, since it does not attach to the host but remains free-living. Male specimens were found moving about females attached to the host gills, and this showed that male *L. clariae* are associated with females after attachment.

Various recovery intervals of copepodites from the infested fish made it difficult to determine exactly the period a copepodite takes to moult from one stage to

the next. However, in the life cycle of *Lernaea cyprinacea* it was observed that each stage lasted one to two days (Grabda, 1963).

The observed life stages indicates that *L. clariae* can be regarded as an apormophous parasitic species which shows secondary reductions in the loss of segmentation, development of neck and reduction of the swimming appendages, antennules, antennae and furcal rami.