Egypt. J. Agric. Res., 80 (4), 2002.

A CONTRIBUTION ON BIOLOGY OF *LERNAEA CYPRINACEA* (COPEPODA) PARASITIZING CULTURED COMMON CARP *(CYPRINUS CARPIO)*

SALEH, O. A. AND R. A. M. RAMADAN

Central Laboratory of Aquaculture Research, Abbassa, Agricultural Research Centre, Ministry of Agriculture, Dokki-Giza – Egypt.

(Manuscript received 28 May, 2002)

Abstract

The biology of *Lernaea cyprinacea* (*L. cyprinacea*) parasitizing carp (*Cyprinus carpio*) was studied at Abbassa Fish Hatchery (AFH) and Central Laboratory of Aquaculture Research, Abbassa (CLAR) during 2000 –2001. The life cycle, morphometric characteristics and host parasite relationship of this species were described in this study. All developmental stages of *L. cyprinacea* life cycle in vitro under different temperatures were distinguished by the reflexed claws on its antenna. The development of the antennually segmentation and setation pattern are traced from third copepodids to adults of both sexes. Filling the fish ponds with water five days before nursing the fry or fingerlings of carp (*Cyprinus carpio*) is sufficient to break the life-cycle and prevent the infection.

INTRODUCTION

Lernaea species are the most harmful ecto-parasites of cultured fresh water fishes. Its destructive activity is attributed to its large size, mode of attachment, loss of fish blood and fish exposure to secondary infection by fungi, bacteria and possibly virus, (Paperna, 1980 & Post, 1983). The parasitic copepod, *L. cyprinacea* reduced about 30 % of the fry production of *Cyprinus carpio* and *Puntius goniontus* and killed about 1.8 billion fry. The frequency and severity of such outbreaks have been increased with the increasing Aquaculture activities (Kabata, 1985). *L. cyprinacea* requires only one host to complete its life cycle. At 27°C, the larval stages take 12-17 days to develop to adult males or premetamorphosed females, adult males die within 24 hours. Females are fertilized and either attack the same host or swim to another host, then chew and bore their way into tissues as they metamorphosed into adults. Within one day, they produced their first batch of eggs, these hatch within 24-36 hours, later, the egg sacs shed. A new pair of egg sacs are produced within 1-3 days, the largest egg sacs are produced 5 to 10 days postmetamorphosis and the parasite die within 30 days at 28- 32 °C, (Shariff and Sommerville, 1986). Lernaeids or anchor worms are common pests in fresh water aquaculure of cyprinds and to lesser extent, of salmonids and other fish. Epizootic in cultured fish is often associated with high mortality (Berry *et al.*, 1991). In order to develop methods for the prevention and cure of Lernaeosis in fish, its pathomorphic and epidemic characteristic must be studied, (Shuxinhua, *et al.* 1998 and Ramadan, 2000).

MATERIALS AND METHODS

Fifty cultured *Cyprinus carpio* fish specimens were collected from CLAR ponds and AFH and transported alive to the laboratory. They were infected with macroscopic adult ovigerous females *L. cyprinacea* (Fig. 21, a & b). Fish were classified into four experimental groups held at different water temperatures 23 °C, 25 °C, 27 °C and 30 °C, (10 fishes / each) for infection procedure. The dead fish were removed from aquarium and fixed in 10% formalin.Water was filtered through a fine mesh sieve (Plankton net 20 um and 80 um). The mesh retained numerous, copepodid stages which were examined. All drawings were made with the aid of camera lucida. Aquaria contained continuously well aerated water (pH of 7.4 - 7.5) nitrate content less than 10 mg / L, no nitrite and ammonia, and a total hardness of 24) was maintained in all experimental groups. All experiments were carried out in a controlled-environment room temperature 27 -28 °C and thermostat regulated water temperatures 23 °C, 25°C, 27 °C and 30 °C for infection procedure and parasite counting. Fish subjected to12:12 h light:darkness cycle (Paperna,1996). Two earthen ponds were dried and used to study the effect of filling the ponds with water without fish host. The fish (300 sample) were examined through three examinations after introducing the fish (7 days intervals), in addition to 100 fish samples from another unprepared nursing pond as a control were examined after 7 days.

RESULTS

Parasitic adult females (Fig.21a&b) produced egg sacs containing 100-250 eggs (Fig.1). At 23 °C, hatching occurred after 84 h. Hatched nauplii, (Fig. 2) molt into second stage nauplius within 25 h (Fig. 3), and further molt to the third stage naupluis (Metanauplius) within 48 h after hatching. Free copepodite stage appeared 169 h (7days) after hatching. Copepodites attached to the gills and occasionally also on the walls of buccal cavity and on the skin. The copepodites underwent four successive molts, the size of the copepodites increased from molt to molt from 0.33 - 0.80 mm (Fig. 5, 6, 7 & 8). At fifth stage, the copepodites underwent sexual dimorphism, where females being larger 0.82 - 0.95 mm than males 0.70 - 0.85 mm (Fig. 9 & 10). This period lasted 16 days after hatching. Copepodites abandoned their attachment on their host and either left their host in search of a new host fish of the same or different species , or relocated themselves from the gills to the skin on the same host.The stages underwent further molt to the last copepodite stages (sixth stage) cyclopoid stage (Fig. 11 & 12). At this stage, size difference and sexual dimorphism became even more distinct female (0.92 - 1.20 mm) and male (0.85 - 1.0 mm). After 18 days from hatching, larval development was terminated. Male gradually disappeared, females continued their transformation into adults through elongation of the body and loss of external segmentation and degeneration of legs. The first egg sac in the mature female was ob-

served 23 days after hatching, (Table 2), and the newly hatched nauplii were elleptical in shape, about 0.145 mm long and 0.80 mm wide. The naupli molt within 50 h, depending to some extent on water temperature, to become metanauplius. Metanauplius was slightly larger, about 0.170 mm long by 0.85 mm wide, and spines appeared at the base of each mandible. the first indication of body segmentation appeared in metanauplius. Twenty to 40 h after metanauplius was formed, the exoskeleton was Shed again to form the first copepodid with a body about 0.310 mm long by 0.110 mm wide .The first copepodid had two pairs of swimming legs and antennulae became more segmented. The first copepodid must find a host within three days or it would perish. The first copepodid on a host's skin or gills continued to metamorphose. The second, third, fourth and fifth copepodid were formed, each with more body segmentation and with swimming legs (Table 1). An additional abdominal segment was added at the fifth copepodid stage. The male measured about 0.780 mm in length and 0.170 mm in width at this time, had developed a pair of testes in the thorax with the sperm ducts leading to the genital segment. The female measured about 1.040 mm in length and 0.200 mm in width, developed a long and heavy genital segment, (Plat A). Copulation occurred at the sixth copepodid stage of development. The male placed two sac-shaped spermatophores, (Fig. 12) on the genital segment of female, left the female and died. The fertilized females continued to metamorphose, increased rapidly in length and burrowed into the integument or gills of the host (Fig. 13, 14, 15, 16 & 17). Anchor processes developed on the head, and the ovaries began to move posteriorly from the anterior thorax to a point near the junction of the thorax with the abdomen. The body continued to elongate, and the cephalic horns branched from the head. The female wass now in position to produce eggs (Fig. 18, 19 & 20). Studying the effect of filling the dried earthen ponds by water five days before introducing fry or fingerlings to them was sufficient to break the life cycle and prevent infection. The infection in the controlled pond was 6% due to the presence of available host (fish).

DISCUSSION

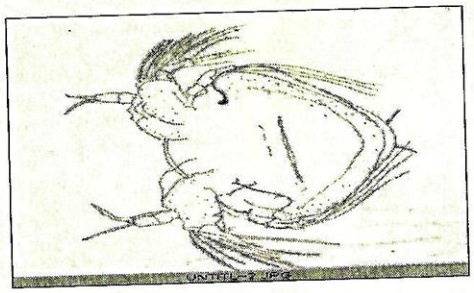
The present study threw light on the degree of temperature at which developmental stages of *L. cyprinacea* grow and emphasis had been placed particularly on swimming legs, body and antennae segmentation as the main features of development in *L. cyprinacea* which in these essential details agreed with the forms given by Kashara (1962), Grabada (1963), Shariff and sommerville (1986), Paperna (1991) and Boxshall *et al.* (1997). Filling the dried earthen ponds by water five days before introducing fry or fingerlings was sufficient to break the life-cycle and prevent infection. This is attributed to death of the copepodids during this period due to absence of available host. This result supported those reported by, Shariff and sommerville (1986), Woo and Shariff (1990) and Ramadan (2000).

Table 1. In vitro developmental stages of Leanaea cyprinacae.

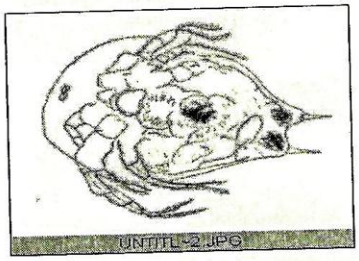
Larval Developments		Body length mm	Temperatures °C			
			23	25	27	30
Naupulis	First stage	0.140-0.150 (0.145)	3.5 day	3 day	38 h	24 h
	second stage	0.145-0.165 (0.155)	Within 25 h	Within 21h	within 18h	witin 12h
Metanaupulis stage		0.166-0.180 (0.173)	48 h 2 days	42 h 1.75 days	24h 1 day	18 h
Copepodid Stages	First stage	0.27-0.35 (0.310)	169 h 7 days	169 h 7 days	132h 5.5 days	45 h 2 days
	Second stage	0.36-0.40 (0.380)	207 h 8.5 days	146 h 6 days	129 h 5.5 days	98h 4 days
	Third stage	0.42-0.50 (0.440)	285h 12 days	195 h 8 days	157 h 6.5 days	135 h 5.8 days
	Fourth stage	0.56- 0.64 (0.60)	360h 15 days	247h 10 days	205h 8.5 days	144h 6 day
	Fifth stage	0.88-0.89 (0.92)	389h 16 days	285h 12 days	236h 10 days	194 h 8 days
	Sixth stage	1.02-1.07 (1.04)	424h 18 days	350 h 15 days	285h 12 days	237 h 10 days
	First egg sac appear	1.08-1.5 (1.20)	553 h 23 days	524 h 21 days	428h 18 days	356h 15 days

Table 2. Length of each stage of learnea development.

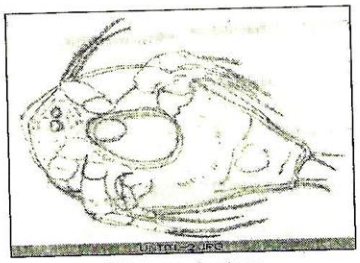
Days	Temperature			
	23 °C	25 °C	27 °C	30 °C
Napaulis stage	5	3	2.5	2
Copepodid stage	17	13	11	8
Adult stage	25	21	19	17



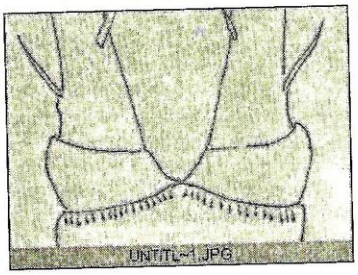
First nupulis stage



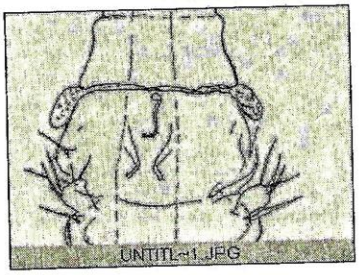
Second nupuls stage



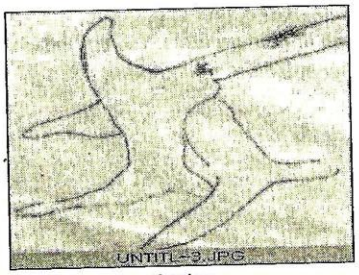
Third nupuls stage



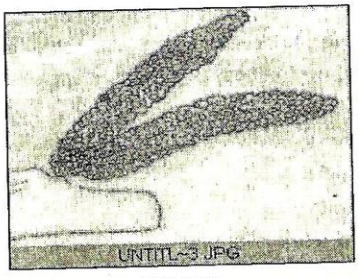
Anal segment



Genital segment



Anchor



Egg sacs

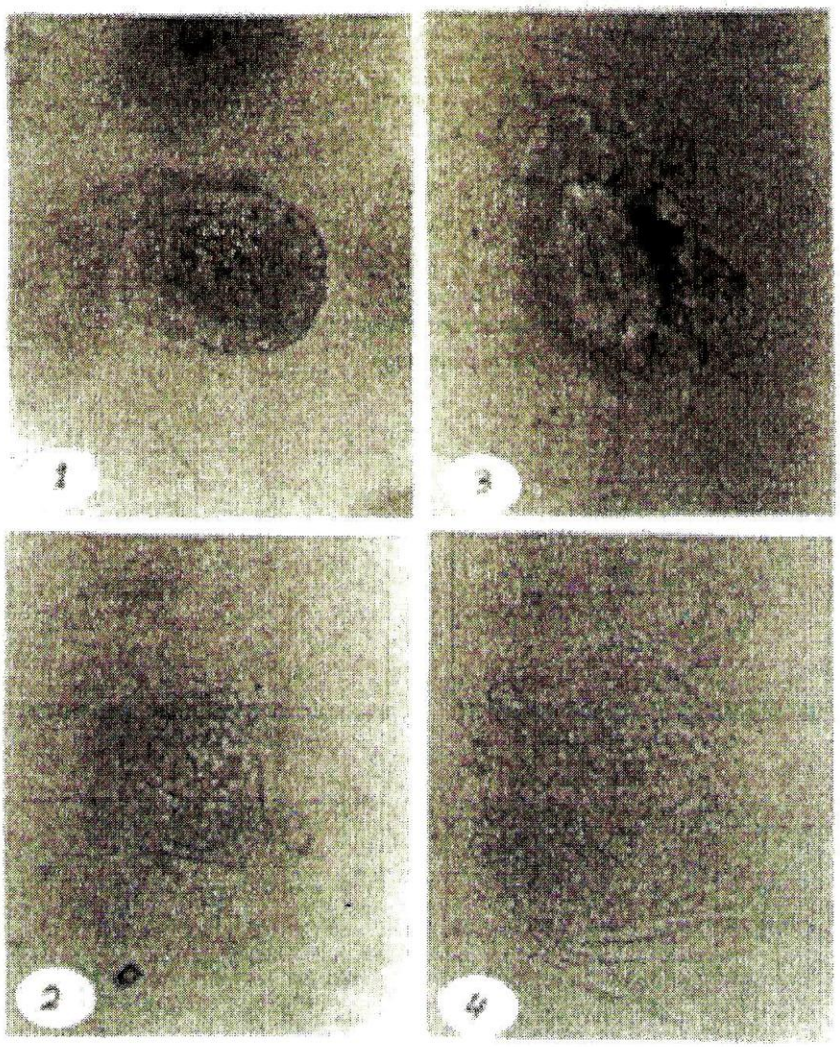


Fig. 1. Egg contain fully developed naupulis x 50

Fig. 2. Naupulis I: Newly hatched nauplii are unsegmented, elliptical, six legs X 250.

Fig. 3. Naupulis II: Unsegmented, elliptical, six legs, one pair of primitive eyes began to appear, aquatic free swimming, planktonic stage X 250.

Fig. 4. Metanaupulis: Spines appear at the base of each mandible. The first indication of body segmentation appears, and after 20-40 h X 250.

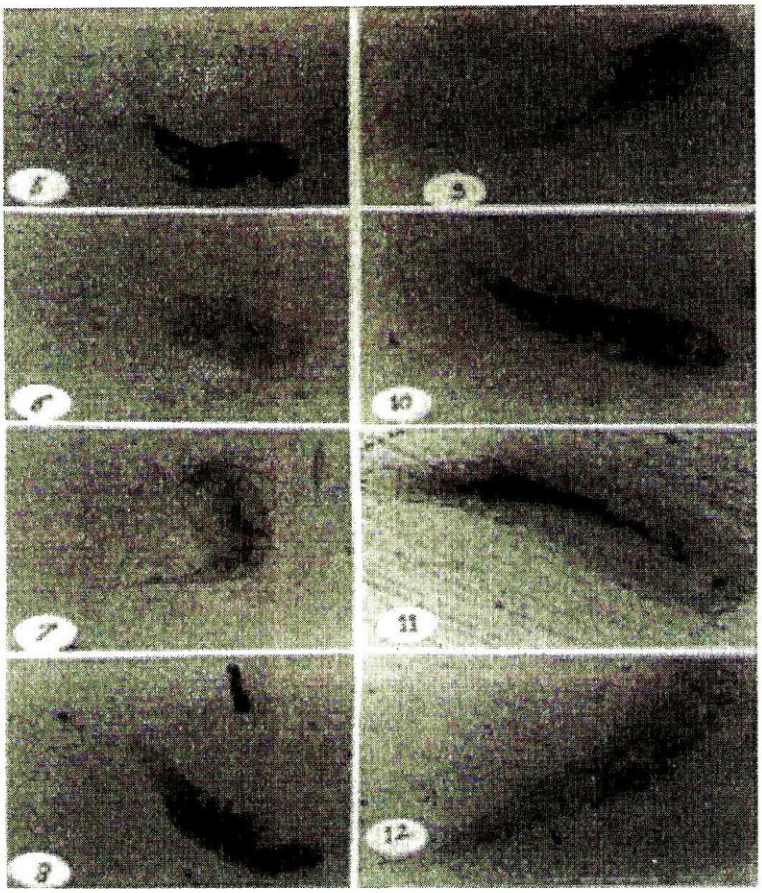


Fig. 5. Copepodid I: The first copepodid stage has two pairs of swimming legs, and the antennulae be come more segmented. This stage must find a host within three days or it will perish X 250.

Fig. 6. Copepodid II: the second Copepodid stage more elongated with a body about 275 mm long by 142 mm wide. This stage has three pairs of swimming legs, and the antennulae become more segmented X 250.

Fig. 7. Copepodid III: Cephalothorax, incorporating first pedigerous somite and second to fifth pedigerous somites, genital somite and single free abdomine somite. Antennule uniramous, 4-segmented X 250.

Fig. 8. Copepodid IV: Genital somite and two free abdominal somite X 250.

Fig. 9. Copepodid V (Male): Genital somite and three free abdominal somite X 250.

Fig.10. Copepodid V (Female): Genital somite and three free abdominal somite without spermatophores X 250.

Fig.11. Copepodid V (Male): Genital somite and four free abdominal somite without spermatophores X 250.

Fig.12. Copepodid V (Female): Genital somite and four free abdominal somite with spermatophores following copulation perior to insertion in host X 250.

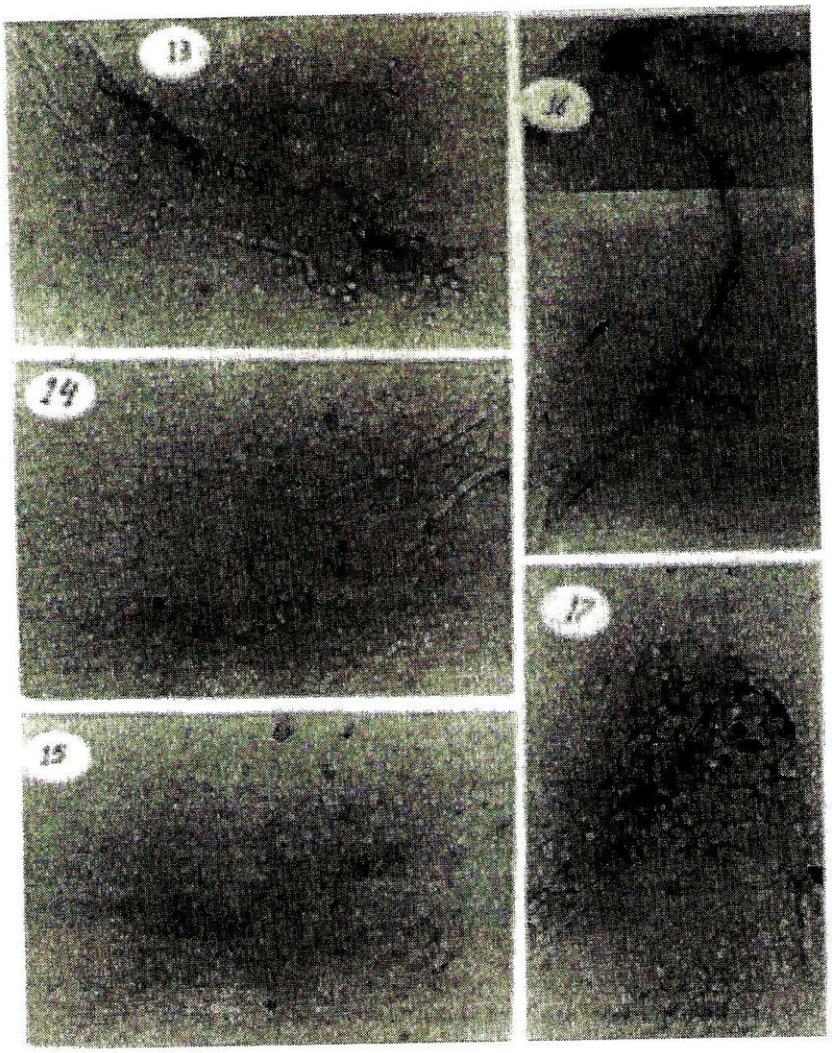


Fig. 13. Female initial period of entry (third pair of sweeming legs) x 500.

Fig. 14. Female initial period of entry (fourth pair of swimming legs) X 500.

Fig. 15. Female initial period of entry (fifth of swimming legs) and Caudal rami X 500.

Fig. 16. Female initial period of entry (whole) X 250.

Fig. 17. Female initial period of entry (Anchor) one pair of eye, mouth part and first pair of swimming legs X 500.

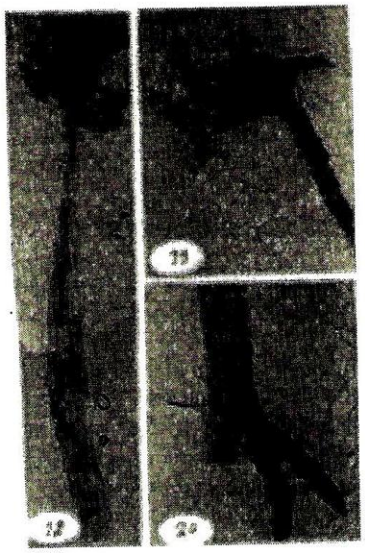
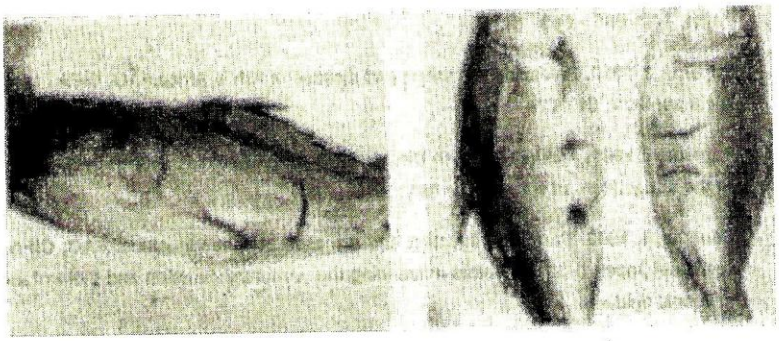


Fig. 18. Female after penetration and initial period of spawning (whole) X 500.

Fig. 19. Postmetamorphosis female adult stage (anterior end) X 100.

Fig. 20. Postmetamorphosis female adult stage (posterior en d) egg sacs X 100.



a b

Fig 21a. and b. Adult female of L. cyprinacea on Cyprinus carpio.

ACKNOWLEDGEMENTS

The researchers wish to thank Dr.Hamdy, M.A. El Gawady, Prof. of Parasitology, Suez Canal University for advices during conduct of this research. We also thank Dr.A. S.M.I. Diab head of fish health (ICLARM) for advices and encouragements.

REFERENCES

1. Berry Cr. Jr,. Gj. Babey, T. and Shroder. 1991. Effect of Lernaea cyprinacea (crustacea : copepoda) on stocked rainbow trout (Oncorhynchus mykis). J. wildlife dis. 27 (2) : 206-213.

2. Boxshall,G.A., M.A. Mont, and A. Schwarzbold. 1997. Anew species of Lernaea L. from Brazil, with notes on its ontogeny.Systematic parasitology 37:195 - 205. Immunohistological localization of tyrosine in mucus-secreting cell layers of Atlantic salmon Salamo slar L. J. Fish Dis., 13: 233 – 238.

3. Grabada, J. 1963. Life cycle and Morphogenesis of Lernaea cyprinaecae L. Acta Parasitologica Polonica, 11: 169 -198.

4. Kabata, Z. 1985. Parasites and Diseases of fish cultured in Tropics. Tylor and Francis, London & Philadelphia

5. Kashara, S. 1962. Studies on the biology of the parasitic copepoded Lernaea cyprinacea and the methods for controlling the parasites in fish culture ponds Contributions of fisheries laboratories Faculty of Agr. Univ. of Tokyo No. 3: 103 - 196 Naturalist, 102: 685 - 688.

6. Paperna, I. 1980. Parasitic infestations and disease of fish in Africa.FAO, CIFA., technical paper, 7: 51 - 62

7. Paperna, I. 1991. Annual Rev. Fish Dis., 1: 115 – 194. Diseases caused by parasites in the aquaculture of warm water fish.

8. Paperna, I. 1996. Parasites, infection and diseases of fishes in Africa. FAO, CIFA., technical paper, 3 , 31. Factors influencing the structure ,function and biota of the salmonid epidermis.

9. Post, G. 1983. Textbook of fish health. TFH. Publication Neptune City, N. J. 256 pp.

10. Ramadan, A. M. 2000. Morphological and Immunological studies on ectoparasites of some freshwater fishes. Thesis Ph. D., Suez Canal Unversity.

11. Shariff, M. and C. Sommerville. 1986. Effects of Lernaea polymorpha on the growth of big head carp , Aristichthys nobilis. ICOPAVI handbook. Abesract no. 598 and 227.

12. Shu-xinHua, Jin-Xiel, Dia-Zhenyan, Liu-hongoi, Xiao-Keyu, Shu-Xh, Jin-XL, Dia-Zy, Liu-Ho and Xiao-Ky. 1998. A study on the Lernaeosis of large-mouth bass. J. hunan-Agricultural-University, 24:5, 401-403, 5ref.

13. Woo-PTK, and M. Shariff. 1990. Lernaea cyprinacea L. (Copepoda: Calididea) in Helostoma temmincki cuvier& Valenciennes: the dynamics of resistance in recovered and native fish. J. fish dis. , 13:6, 485-493.

دراسة على دورة حياة اللرنيا سبرنسيا Lernaea cyprinacea التي تصيب المبروك العادي بالمزارع السمكية

اسامة عبد الرحمن صالح ، رمضان انور محمد

المعمل المركزي لبحوث الثروة السمكية بالعباسة- مركز البحوث الزراعية - وزارة الزراعة - الدقى- الجيزة - مصر

تعتبر اللرنيا سبرنسيا *Lernaea cyprinacea* من اخطر الطفيليات الخارجية التي تصيب اسماك المياه العذبة وترجع خطورتها إلى حجمها الكبير وطريقة التصاقها بالأسماك التي تودي إلى فقد بعض من دم هذه الأسماك وتهتك بالأنسجة وبالإضافة إلى تعرض الأسماك للعدوى بالمسببات المرضية الأخرى مثل البكتيريا و الأوليات والفطريات.

وقد تمت دراسة على دورة حياة اللرنيا سبرنسيا *Lernaea cyprinacea* التي تصيب المبروك العادي في كل من المعمل المركزي لبحوث الثروة السمكية والمفرخ السمكي بالعباسة وذلك خلال الفترة من ٢٠٠٠-٢٠٠١م .

وقد تمت هذه الدراسة بالمعمل تحت درجات حرارة مختلفة (٢٣× م - ٢٥× م - ٢٧ ×م - ٣٠× م) وذلك لتحديد طول فترة الحياة من بداية فقس بيض اللرنيا حتى ظهور أكياس البيض بالإناث الناضجة. تم وصفت جميع مراحل النمو المختلفة وصفا تفصيليا مدعما بالرسومات بالاستعانة بالكاميرا لويسيدا والصور الفوتوغرافية. ولكسر دورة حياة اللرنيا سبرنسيا-*Lernaea cyprina* تم دراسة تأثير ملء الأحواض الترابية الجافة بالماء وتركها بدون أسماك لمدة خمسة أيام وقد *cea* أظهرت النتائج أن ذلك كاف لكسر دورة الحياة ومنع الإصابة حيث وصلت نسبة الإصابة ٦٪ بالأحواض التى تم وضع الأسماك بها مباشرة.