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EFFECT OF GONADAL INFECTION BY NEMATODE PARASITE ON THE FECUNDITY OF THE SEA BREAM *LETHRINUS NEBULOSUS* IN RAS MOHAMED MARINE PARK (NORTHERN RED SEA)

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ABSTRACT

The sea bream *Lethrinus nebulosus* was sampled during the spawning season of 1996 (May-July) in Ras Mohamed Marine Park. Out of 119 specimens collected (46 females and 73 males), 11 females (23.9 % of examined females) were found to be parasitized by unidentified nematode. This parasite is found to be specific for fish ovary, causing a reduction in the absolute fecundity of the infected females compared with normal ones (averaged 4.97 x 10^5 and 7.54 x 10^5 eggs/female, respectively).In all infected females, 2-4 nematode worms of 30-40cm in length / each ovarian lobe, were coiled together in a mass occupying the ovarian lumen and seemed to block the oviduct preventing egg releasing. Histological examination showed that ovaries of heavily infected females did not contain mature ova with a depression of the vitellogensis process. All infected ovaries were characterized by certain degrees of atresia depending upon the level of infection. The synchronization between reproduction of the fish and the parasite, and the parthenogenetic nature of the parasite are thought to increase the biological potential of the parasite to colonize and infect other host individuals and possibly other populations.

INTRODUCTION

Members of the family Lethrinidae are economically important group of fish which are commonly appreciated in many countries. The sea bream *Lethrinus nebulosus* which has a wide distribution from western

Pacific to Indian Ocean, is the most frequent economic species among lethrinid fishes. It represents 26 % of the total landing in Qatar, 25 % in New Caledonia, 25 % in Japan and 20% of the total lethrinid catch from the Gulf of Suez which represents about 35 % to 40 % of the total catch (Sanders, *et. al.*, 1984a,b; Sabry, 1997).

Several biological studies have been carried out on lethrinid fishes, in South Pacific (Kuo, 1988; Kuo & Lee, 1990; Dalzell, 1992; Moran *et al.,* 1993), Indian Ocean (Bertrand, 1986, Bautill & Samboo, 1988; Hamza & Kasim, 1994), Arabian Sea (Baddar, 1987; Al-Sayes *et al.,* 1988; Mohamed *et al.*, 1988a,b; Ezzat *et al.*, 1992), and Red Sea (Hashim & Shakour, 1981; Kedidi, 1984; Kedidi & Bouhlel, 1985; El-Gammal, 1988; Bawazeer, 1990). On the Egyptian Red Sea, some biological studies were performed on *L. nebulosus* (Salem, 1971, 1976; Sander *et al.*, 1984a,b; Sabry, 1997).

Although, the previous studies have reported on the biology of this fish along the Egyptian coasts, the effect of parasitism on its gonads was not a target of any of them. However, Saoud *et al.* (1986) identified some helminth parasites in the digestive system of *L. nebulosus* from the Arabian Gulf.

The present work deals with a nematode parasite which was found to infest the ovaries of *L. nebulosus* in its spawning ground at Ras Mohamed Marine Park and its effect on the reproductive capacity of this important fish species.

MATERIAL AND METHODS

A random sample of 119 specimens of *L. nebulosus* was obtained during the spawning season (May - July) from the spawning ground of Ras Mohamed Marine Park. Forked length and body weight were measured to the nearest millimeter and gram, respectively. The fish were then dissected to expose its gonads. After gonads were sexed, the ovaries were weighed in 0.01 grams and the incidence of infection was determined. Ovaries were fixed in 10 % formalin for estimation of the fecundity and histological investigation.

Since no differentiation in development of ovaries of *L. nebulosus* has been assured (Ebisawa, 1990), so a piece of ovaries were dissected out randomly from one lobe for histological observations.

Dissected ovaries of normal and infested specimens were transferred into 70 % alcohol after one or two days of fixation. They were processed for usual sectioning. Sections were cut at 6 - 8 um, stained by Ehrlich's acid haematoxylin and counterstained by eosin (Drury & Wallington, 1980).

The female *L. nebulosus* is a multiple, spawner (Ebisawa, 1990). Therefore, all stages of oogenesis were found in mature ovaries. The patch fecundity as a total number of maturing and mature ova were counted for each ovary. For this purpose, five pieces were taken from each ovary. Each piece of 0.1 - 0.7g was placed in Petri dish and slightly pressed by fingers with some water to detach the developing ova from ovarian tissues. The number of maturing and mature ova was counted in each piece using a hand counter under a binocular microscope. The counts of the five replicates were averaged and the patch fecundity was calculated for each female.

RESULTS

A total of 119 specimens of sea bream *L. nebulosus* were examined. Out of them 46 were sexed as females and 73 as males (i.e. sex ratio 1.0 :1.59)All examined males were non-parasitized, while 11 of the 46 females were found to be parasitized by unidentified adult nematode i.e. 23.9% of examined females. Therefore, the parasite infection seems to be specific, infecting only the ovaries, and non of testes or other organs of examined fishes were found infected by this nematode.

Table (1) summarizes the different parameters in both parasitized and non parasitized females. It is clear that gonad weight, gonad index and fecundity were greatly affected by parasitism. Higher fecundity was recorded in non parasitized compared with parasitized females. For instance, the patch fecundity measured absolutely in parasitized females represented two-third of non-parasitized (averaged 4.97×10^5 and 7.54×10^5 eggs, respectively).

The level of infection of ovaries by the nematodes ranged from moderate to heavy. Out of the eleven infected females, 4 were heavily infected (i.e 8.6 % of all examined females). In both cases the number of worms per each lobe of ovary ranged between 2 to 4 worms, even in the case of heavy infected ovaries. The length of the worm ranged between 30 to 40 cm. Therefore, the ovarian lumen in both cases seems to be blocked by coiled parasites (plate 1 A&B).

Ovarian histological structures of normal and parasitized females are shown in plate (1C- E). In mature normal females (plate 1-C), the ovary was dominated by mature oocytes and all other stages of oogenesis were represented (i.e. oogonia, Chromatin nucleolus stage, peri-nucleolus stage oocyte, primary yolk globule stage, secondary yolk globule stage and tertiary yolk globule stage oocytes, Ebisawa, 1990). In moderate infection, the parasite was located inside the central ovarian lumen causing severe effects on follicles of the central portion of the ovary while, the remaining peripheral regions were representing the last ovarian areas to be affected by the parasite.

In heavy infected ovaries (Plate 1 D&E) the parasitic worms were densely occupying the central ovarian lumen, and extending to the peripheries representing more than 70 % of the cross section. Although all moderate infected ovaries were found in mature conditions and dominated by mature ova, the oogenesis in heavy infected ovaries was almost stopped at pre-vitellogenesis stages (Plate 1E). The main features of these ovaries were:- all oocytes were in pre-vitellogenesis stages (nucleolus stages) the atretic oocytes (mainly of late stage) nearly lost their normal shapes and internal structures and dominated the central portion of the ovary (Plate 1D&E).

Two main phenomena for this parasite could be noticed from the histological sections. Firstly, the parasite was found to feed upon the ovarian materials where some unidentified ovarian tissues and granulosa cells were found inside the parasite intestine (plate 2A). Secondly, the reproduction of the nematode seemed to be a parthenogenetic type where: no males were recorded and all worms have ovaries and uteri. These uteri appeared filled with different embryonic stages of the parasite (Plate 2B). This suggested that the spawning season of *L. nebulosus* is synchronized with the peak of reproduction of this parasite. In other words, there is a synchronization between reproduction of the parasite and its host.

DISCUSSION

Some marine parasites are known to inhibit the reproductive activity of their hosts and lead to castrate them. This is common in certain molluscan hosts (Obreski, 1975; Huxham *et al.*, 1993; and Hanafy *et al.*, 1996). Meanwhile, very little is so far known about the infection of fish gonads by parasitic nematodes in general, and the Red Sea fishes in particular.

Rhode (1993) summarized the effect of parasitism on fish population by reducing population abundance through causing mortality, affecting the reproductive organs and reducing the number of offspring.

Although the prevalence of the nematodes in the females of *L. nebulosus* population in the spawning ground of Ras Mohamed Marine Park was 23.9 %, only 8.7 % of all the examined females were heavily infected and the fish maturation process was depressed. It is thought that even in moderately infected females, the worms were coiled together forming a mass causing mechanical occlusion of the oviduct and inducing sterility. Therefore, heavily infected females were likely to be sterile due to mechanical blockage of the oviduct and/or suppression of maturation process. On the other hand, moderately infected females contained mature ova and the fecundity generally reached two thirds of the non-parasitized ones. Rhode (1993) found that the microsporan *Glugea hertwigi* prevents the reproduction of the infected fish by mechanical occlusion of the fish vents and also destroy gonad tissue in smelt *Osmerus operlanus*.

This study showed that all heavy infected females had ovaries in primary growth phase, i.e. in early and late peri-nucleolus stages (pre-vitellogenesis stages) and with oocytes in late atretic stages. The sterility of fishes due to parasitism is known in few fish species. For instance, Several coccidians are known to infect the testes of clupeoids inducing sterility (Sindermann, 1966). Similary, the coccidian *Eimeria sardinae* parasitizes the testes of clupeoid fish like sardines, sprat and Atlantic herring, and often leads to sterility (Rohde, 1993).

Although the relationship between the parasite and the female *L. nebulosus* is still not clear, some factors are believed to increase the biological potential for the parasite to infect other host individuals and even other populations. These include synchronization between the spawning season of the fish and the parasite and parthenogenetic nature of parasite. These factors are supposed to increase the success of the parasite infection where *L. nebulosus* (from different locations) is known to aggregate in a restricted spawning ground. In addition, the new infected individuals in the spawning ground are thought to enable the parasite to transport and build up a new population in new areas after re-migration of fish spawners to their original areas. Price (1980) claimed that parthenogenesis, besides enabling a single parasite individual to colonize a new host, has another advantage: which is permitting maturation at an early larval stage (progenesis), since adult characters required for mating are unnecessary. Rapid maturation may lead to an increased rate of population growth and a small size of reproducing animals.

The depression of oogenesis is mainly related to the consumption of fish ovarian material by the parasite. Many of granulosa cells and some unidentified ovarian tissue were found in the intestine of the worms. It is known that the main functions of granulosa cells is the deposition of yolk in the developing ova and acts also in its degeneration in case of unovulation. In addition to its nutritive and phagocytic functions, the granulosa may also be concerned with the elaboration of ovarian hormones which are synthesized by the ovum or the granulosa or the corpus luteum which develops from the granulosa (Hoar & Randall, 1969). This may explain the previtellogenesis condition of the heavy infected ovaries.

A fine balance exists between parasites, their hosts and the environment. Fish parasites are consequently extremely sensitive to changes in their aquatic habitat (Siddall *et al.*, 1993). There is a growing body of literature demonstrating the parasitic fauna of fish in relation to aquatic ecosystem, and their potential role as biological indicators of water quality (Khan & Thulin, 1991; Poulin, 1992). Rhode (1993) showed that all parasites have restricted niches, characterized by certain hosts, microhabitats and macrohabitats. The life cycle of the present parasite, macrohabitats, its geographical range and seasonality of both the parasite and its host especially during the resting stage of the host ovaries. All that could be ascertained that there is a specificity of this parasite to the ovaries of sea bream *L. nebulous.* According to Rogers (1975) the infective stages in some parasitic species especially nematodes, settle in definite microhabitats and further development occurs there due to specific stimuli. In some cases the parasite has a preference for one sex of the host. Paling (1965) found that a particular population of monogenian *Discocotyle sagittata* on *Salmo trutta* affects 5-7 year old males more heavily than females, and, according to Williams (1965), *Calicotyle kroyeri* is never present in gravid female ray, *Raja radiata*, although non-gravid females may be infected. The preference of nematode parasite for the female *L. nebulosus* may be related to the proper food for parasite provided by fish ovary and / or to the female sex hormone or both together.

This is the first study about the effect of gonadal infection on the reproductive capacity of a fish population in general and on the commercial fish species *L. nebulous* in the Red Sea in particular. In conclusion, further investigation on the seasonality and the effect of this parasite on the reproductive capacity as well as the rate of recruitment of *L. nebulosus* from different localities of Red Sea seems necessary.

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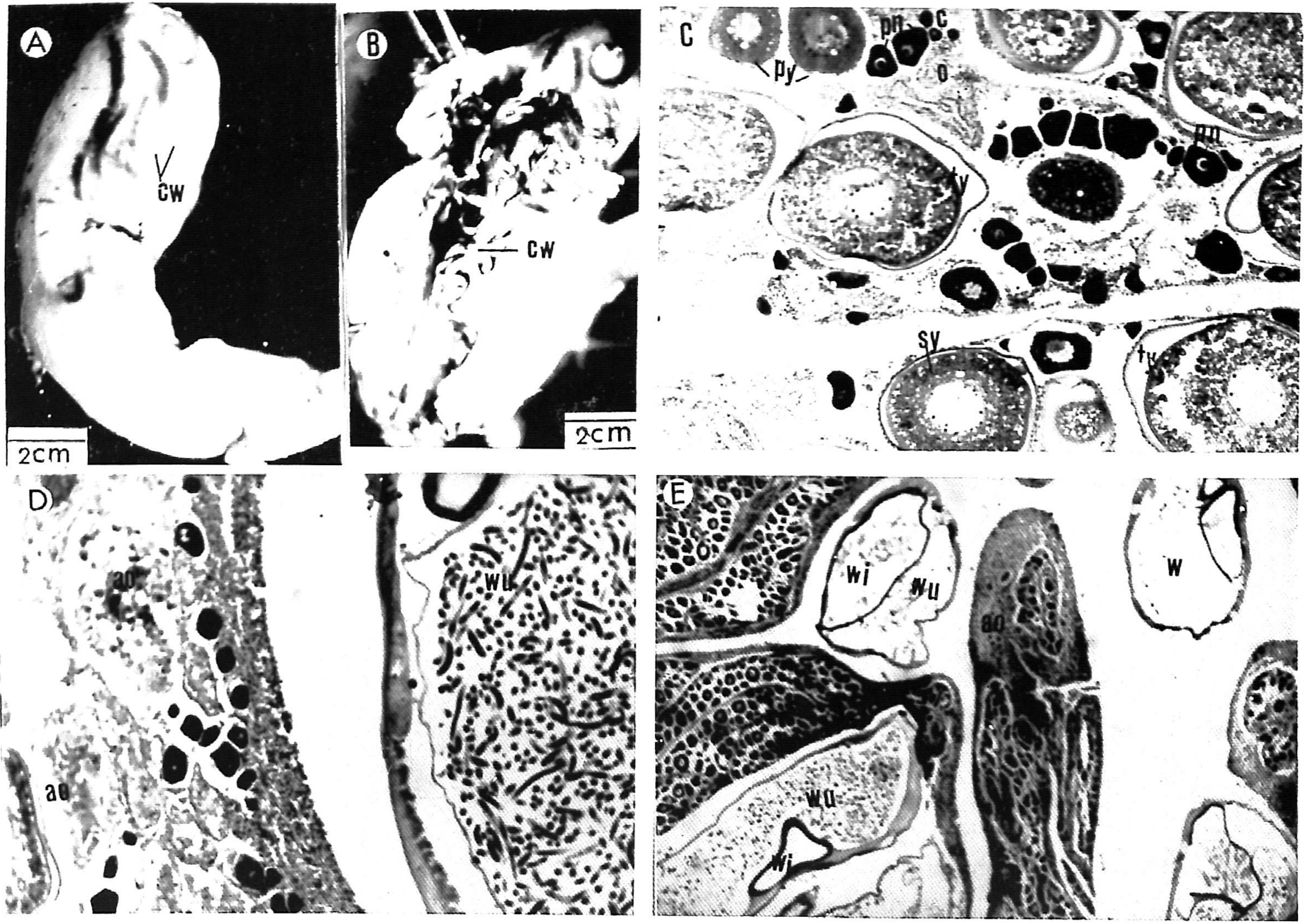
Legend of Figures

Plate 1. Ovary of *L. nebulosus*: A. external appearance of infected ovary, note the coiled worms (CW); B. dissected ovary showing the worms coiled together to form a mass occuping the central ovasian lumen; C. non-parasitized ovary (100x) showing different stages of oogenesis, o- nest of oogonia, c- chromatin nucleolus stage oocyte, sy-secondary yolk globule oocyte, ty-tertiary yolk globule oocyte;; D. infected ovary (200x) showing late stage of atretic oocytes (ao) and worm uterus packed with differnt stages of embryonic development; E. general view of heavily infected ovary (25x), note the whole ovary in early stage of maturation (pre-vitellogenesis stage) and the worms occupying a large area of the section.

Plate 2. A. infected ovary (400x) showing unidentified ovarian materials (ot) and granulosa cells (gc) inside the worm intestine (wi) B. a cross section in the worm uterus (400x) showing different stages of embryonic development, cd-different stages of cell division, j-juveniles.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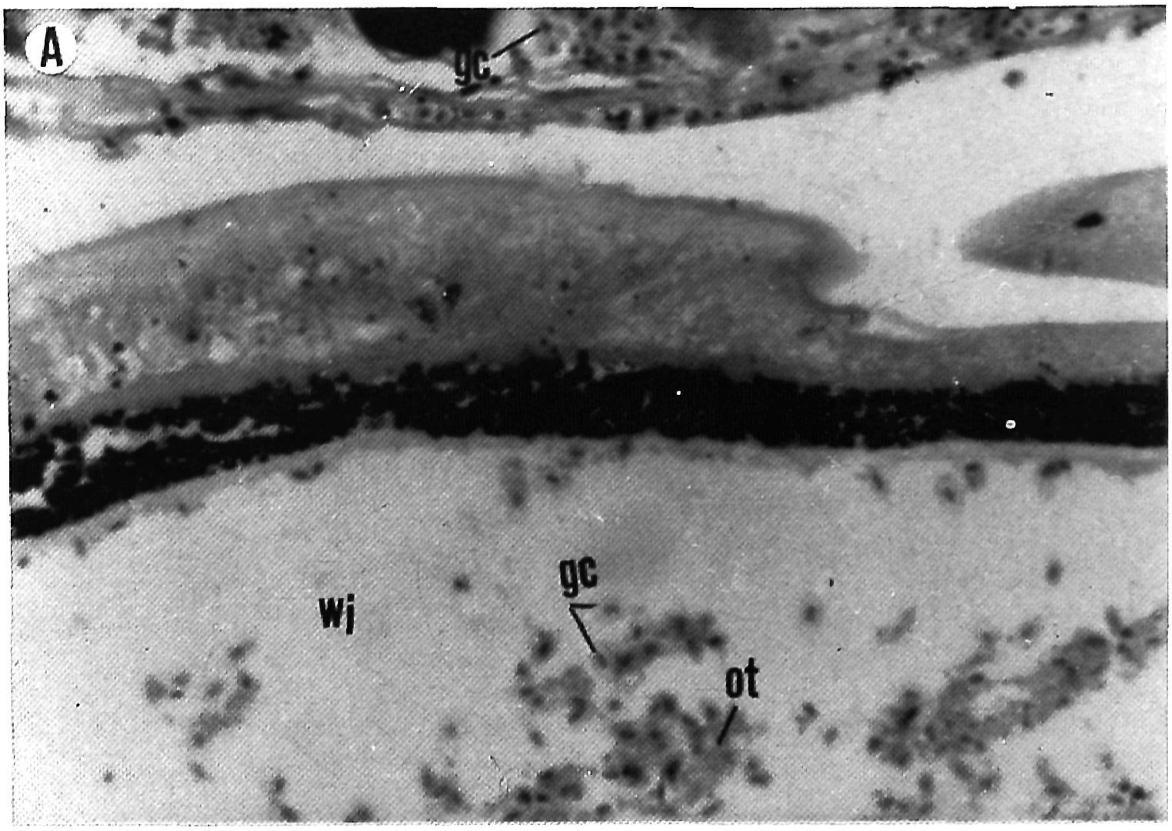
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Table 1: Mean, standard deviation (SD) and range of forked length (cm), wet weight (g), gonad weight (g) gonad index and absolute fecundity of non- parasitized and parasitized females of seabream *L. nebulosus* collected from Ras Mohammed spawning ground.

Parameter	Infected females		Non-infected females	
	Mean ± SD	range	Mean ± SD	range
Forked length	46 ± 5.17	37-52.5	45.5 ± 4.8	36-57.5
Wet weight	1740 ± 514	904-2476	1770 ± 557	829-3360
Gonad weight	32 ± 14.1	54-59.3	50 ± 34.4	13.2-133
Gonad index	1.90 ± 0.86	0.45-3.83	2.69 ± 1.42	0.99-6.82
Absolute fecundity (10^5)	4.97 ± 2.4	0-8.5	7.54 ± 5.58	1.47-1.99
Incidence of infection	23.90%			

تأثير إصابة المناسل بأحدى الديدان الخيطية على خصوبة سمكة الشعور (ليسرنيس نيبيولوسيس) من محمية رأس محمد (شمال البحر الأحمر)

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تمت دراسة اصابة وتأثر مناسل اسماك الشعور ليسرنيس نيبيولوسيس باحدى الديدان الخيطية التى لم يتم تعريفها بعد-فى موقع تكاثر هذه الاسماك (الفرشة) بمحمية رأس محمد خلال موسم التكاثر (مايو-يوليو ١٩٩٧) وبفحص مناسل ١١٩ سمكة (٤٦ أنثى، ٧٣ ذكر). وجد أن مناسل ١١ أنثى منها مصابه بهذا النوع من الديدان (٢٣.٪ من إجمالى عدد الإناث) فى حين انه لم يتم تسجيل ايه إصابة لمناسل ذكور هذا النوع من الأسماك.

وقد وجد أن لهذه الديدان تأثير سلبى كبير على خصوبة إناث هذا النوع من الاسماك، ففى المتوسط كانت الخصوبة المطلقة للاناث غير المصابه ٧,٥٤ × ١٠° بيضه للأنثى فى حين كانت ٤,٩٧× ١٠°بيضه للأنثى فى الاناث المصابه.

وقد اوضحت الدراسة أن هذه الديدان تودى إلى عقم الإناث فى حالة الاصابة الشديدة وذلك بوقف عملية نمو البيض عند المراحل الأولى والسابقة لمرحلة تكوين المح وبالرغم من أنه فى حالة الإصابات المتوسطة فإن المبايض قد احتوت على بيض ناضج إلا انه يعتقد أن الديدان (والتى توجد بمتوسط ٢-٤ لكل فص من المبيض وبطول يتراوح بين ٣٠-٤٠ سم للدودة الواحدة (والتى تلتف على بعضها مكونه كتلة تملأ التجويف الاوسط للمبيض) قد تودى الى منع تحرر البيض والذى يودى بدوره الى العقم.

كذلك وجد توافقاً بين تكاثر كل من الاسماك والديدان ويعتقد ان هذه الديدان عذرية التكاثر وتتحرر منها الاطوار البالغه فى موسم تكاثر الاسماك (التى تتجمع من اماكن مختلفة فى منطقة محدودة للتكاثر) مما يعطى فرصه كبيرة للطفيلى لاصابة افراد اخرى من الاسماك والانتشار فى اماكن أخرى عند عودة هذه الاسماك لمواطنها الاصلية . وترجع اهمية هذا البحث للأهمية الاقتصادية لاسماك الشعور وقدرة هذا الطفيلى على الاصابة والانتشار وتأثيره الملحوظ على معدلات التكاثر.