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Isopoda infestation and its effects on the reproductive maturation of *Nemipterus japonicus* in Red Sea

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Abstract

This study had been carried out at the National Institute of Oceanography and Fisheries in cooperation with Animal Health Research Institute. A total of 250 marine fish (*Nemipterus japonicus*) of different body weights were randomly collected in different seasons from the Red Sea at Suez Gulf. The Isopoda was found unilaterally in most cases in the opercular cavity as a single parasite per fish. It was identified as Cymothoid isopoda (*Irona nanodies*). The total prevalence of infestation was 41.6%, the highest recorded infestation was in summer (53.3%) followed by spring, autumn and winter, where the prevalence rates were 39.7%, 37.5% and 31.1% respectively. The prevalence of infestation was slightly higher in females than in males (43% and 40.4% respectively). Clinical signs, postmortem lesions and parasitological findings as well as the isopod-fish relationship were discussed. The potential effect of isopods on the reproductive investment of the host was measured by gonad maturation and gonado-somatic index (GSI). Isopoda infestation was studied during periods of spawning where the fishes were more susceptible to infection. Parasitized females had fewer, smaller and less mature ova compared to un-parasitized females, and lower gonado-somatic index (GSI) for male and female.

Keywords: Isopods, *Nemipterus japonicus*, *Irona nanodies*, Reproduction.

1. Introduction

Isopods are associated with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting, or damaging infested fishes. Isopods are primarily found in warm water. They infect fish as well as other crustaceans. Larval isopod parasites of the family Gnathiidae are abundant on the gills of tropical marine fish and represent a primary source of food for cleaner fish (Ravichandran *et al*., 2009).

Among marine fish parasites, nearly 25% are crustaceans, mainly represented by Copepoda, Brachiura and Isopod (Pavanelli *et al*., 1998; Eiras *et al*., 2000). Isopod crustaceans are part of the greatest fish ectoparasite group and are easy to identify due to their macroscopic size, morphological features as well as they are easily found on the outer part of Fish bodies, namely; gill chambers and the mouth cavity (Eiras *et al*., 2000; Thatcher, 2000 and Gustavo *et al*., 2009). The presence of these blood sucking ectoparasites may affect these body parts, causing gill filament atrophy, removal of branchial arcs, anemia and obstruction of the mouth cavity and thus destruction of the tongue, compromising the whole of the fish behaviour and leading, sometimes, to the death of the animal (Chavez-Lopez *et al*., 2005; Rhode, 2005). The effects and infections deriving from these ectoparasites vary according to status and balance on the host–parasite relationships, where they can cause lesions ranging from low impact up to irreversible difficult situations resulting sometimes in the host's death, as above (Leonardos & Trilles, 2003). Primary reproductive investment can be represented by a simple measure of gonadic mass and the gonadosomatic index (GSI), the latter being considered an indicator of somatic and reproductive investment in mature fish (Chen *et al*., 2004; Malavasi *et al*., 2004).

This paper aimed to discuss the isopod ectoparasites of marine fish *Nemipterus japonicus* and clarify their effect on the reproduction of host fish.

2. Materials and methods

Fish:

The present investigation was done using trawl in different seasons from Red Sea. Freshly caught marine fish (*Nemipterus japonicus*) were randomly collected. The collected fish samples were transported to the lab in plastic bags partially filled with its natural water within a short time according to Langdon and Jones (2002).

Clinical and Post-mortem examination of fish:

The fish samples under investigation were grossly examined for determination of clinical signs and any isopod parasite. The samples were examined carefully, externally and internally for detection of any abnormalities. Gills, fins, skin, branchial cavity, musculatures and internal organs were thoroughly examined according to Stoskopf (1988).

Parasitological examination:

The opercula were opened for removing the macroscopic parasite specimens from the infested fish. The crustacean parasites were collected and washed several times in warm normal saline solution, transferred into test tubes, fixed in 3% formalin and preserved in 70% alcohol. There was no need for microscopical examination and staining, as these parasites were large enough to identify according to Lucky (1977) and Schmidt (1992).

Reproductive studies

Total length (cm), body weight, gonad weight (g) and sex were recorded (Table 1 & 2 for male and female). GSI was calculated for each individual as gonad weight/body weight X 100. The means and standard deviations (SD) are shown in Tables 1 and 2. To determine the effects of isopods on host fish, data on ova numbers and developmental stages were collected and analyzed in different seasons from 2010 to 2011. However, the developmental stage of fish was determined macroscopically while the ovum diameter from parasitized and un-parasitized females were measured using an ocular micrometer. Ova were staged according to the method of Kume *et al*. (2000), being classed into four categories based on oocyte maturation:

- Stage I (Peri-nucleolus stage or immature ova);
- Stage II (Yolk–vesicle stage or secondary staged ova);
- Stage III (Primary, secondary, tertiary or middle staged ova that were grouped together);
- Stage IV (Maturation stage or ripe ova).

Size of ovum was classed as follow:

1. Small (< 0.1 µm),
2. medium (0.1–0.25 µm),
3. Large (0.26–0.49 µm),
4. Extra-large (0.5–0.7 µm), to compare size differences within the developmental stages of parasitized and un-parasitized fish.

Table 1. Seasonal isopod infestation and reproductive parameters of males *Nemipterus japonicus* (Average body weight and Average weight of gonads: mean±SD are shown).

Season	No. of samples	Average body weight (g)	Average Gonad weight (g)
autumn	59/22	131.4 ± 23.68	0.23 ± 0.064
winter	16/4	71.79 ± 1.92	0.06 ± 0.014
spring	27/11	31.27 ± 1.11	0.18 ± 0.22
summer	34/18	51.28 ± 21.67	0.23 ± 0.09

Table 2. Seasonal isopod infestation and reproductive parameters of females *Nemipterus japonicus* (Average body weight and Average weight of gonads: mean±SD are shown).

Season	No. of samples	Body weight (g)	Gonad weight (g)
autumn	13	112± 22.14	0.96 ± 0.28
winter	29	68.50± 11.62	0.49 ± 0.27
spring	31	34.30±9.65	0.73 ± 0.44
summer	41	41.28± 21.67	0.38 ± 0.15

3. Results and discussion

3.1. Clinical and Postmortem examination

The clinical signs of infested fish showed a slight unilateral protrusion of the operculum revealing small slit and partial gills atrophy. These results are nearly similar with the findings recorded by Woo (1995), Noga (1996), Eissa (2002) and Mohamed &Abo-Esa (2007). On the other hand, post-mortem examination of the infested marine fish revealed that the parasite occupies the entire area of the gill filaments leaving no space for any parasite (plate1). The pressure of large-sized parasite often leads to mechanical damage and atrophy of lamellar structures with a consequent impairing the opercular respiratory movements. These results supported those of Kabata (1985), Mahi Ghobashy (2000), Eissa (2002), Mohamed &Abo-Esa (2007) and Gustavo *et al*. (2009).

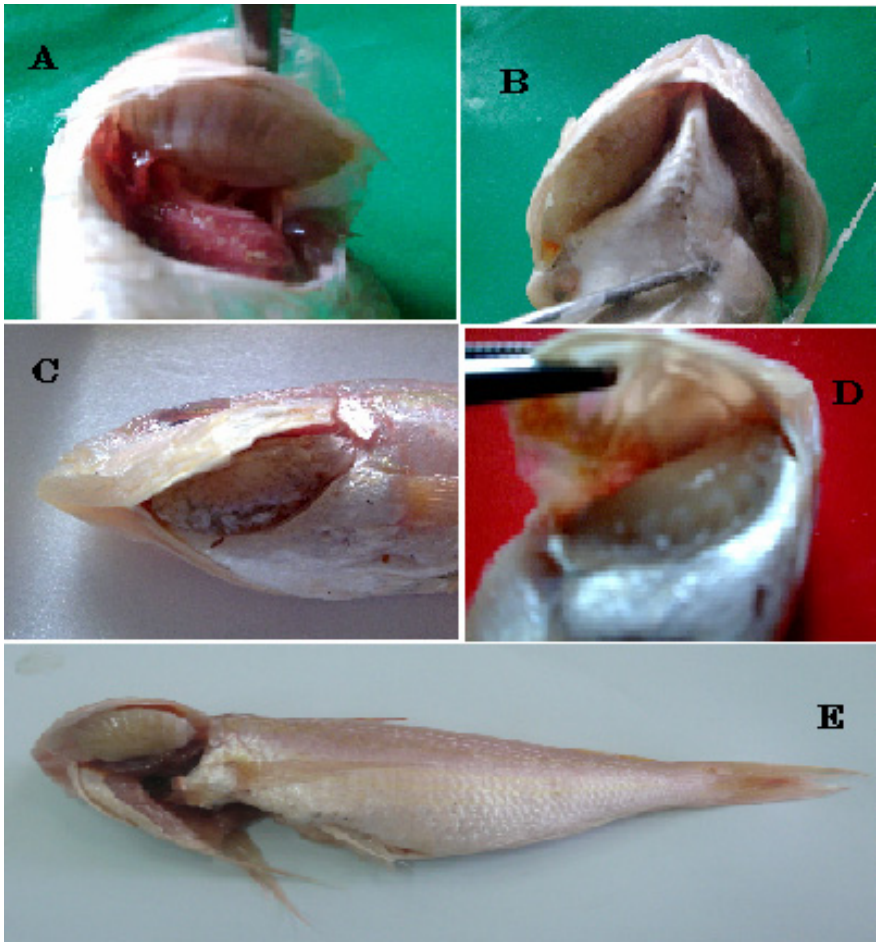

Plate 1. A: Isopoda attached to the opercular cavity of *Nemipterus japonicus.* B: Infested fish showing slight protrusion of operculum. C: Gill's chambers showing attached Isopoda. D: Isopoda cover all the gills. E: Infested fish showing the site of attachment and the size of Isopod to the size of the fish.

3.1.1 Effects of isopods on the host

In fact, Cymothoids are harmful to fish in several ways. Menace feed voraciously and easily kill fry and fingerlings through the tissue damage they cause. Permanently attached adults stunt the growth of fish and retard or inhibit reproduction, probably because of the nutritional drain though more subtle mechanisms such as through hormonal changes have not been ruled out. Those in the gill chamber are usually associated with stunted gills, partly from pressure atrophy and partly from damage associated with feeding activity and attachment which impair opercular respiratory movements. They have also been frequently associated with anemia which caused by blood sucking isopods.

The pathogenic microbes and parasite presence could damage the physiological and reproductive activities of the host fish, (Kabata, 1985; Ravichandran *et al*., 2009). Thereby a regional difference for the proliferation of microbes was observed. Also the contamination of this area more frequently by the respiratory water current that carries bacteria along with food particles could have enhanced secondary bacterial invasion (Mohamed &Abo-Esa, 2007). The bacterial invasion in the branchial region reduces the respiratory area by clubbing and fusion of gill lamellae and affects respiration as well as nitrogenous wastes excretion (Vismanis and Kondratovics, 1997; Ravichandran, 2007). The infestation outcomes such as hyperemia, hemorrhagic lesions and penetration of dactyl us usually cause anemia, pressure atrophy, which often accompanies the presence of larger blood sucking parasites. This may lead to severe economic loss in the commercial species of fishes.

3.2. Parasitological examination

The detected parasites are belonging to order: Isopoda, suborder: Flabellifera Sars, Family: Cymothoidae, subfamily: Anilocrinae, Genus: *Irona nanodies*. (plate2). The parasite was segmented, creamy white in colour and the small head was deeply embedded in the first segment of peraeon with small black eyes. The body is large ranged from 1.5 up to 2.8 cm long relative to the size of the fish (plate3). The preopods are similar in appearance and structure, the

hinder pairs having some superiority of size. The 1st peropod is long; 2-7 progressively decreases in length. The parasite is characterized by its weakly valted body shape, cephalon posterior margin not trilobed, a usually wide pleon, antennule shorter than antenna. This morphological description agrees with that given by Kabata (1985) and Mahi Ghobashy (2000).

Plate 2. A: Parasitic Isopoda, *Irona nanodies*. B: Dorsal view C: Ventral view.

Plate 3. A: Different length of *Irona nanodies*. B: Different size.

3.3. Prevalence and seasonal variation

The total prevalence of infestation was (41.6%) This result nearly agrees with Mahi Ghobashy (2000) and Eissa (2002) who recorded 42.8% and 47% respectively. Results in Table (3) showed that the prevalence of infestations in male and female fish were 40.4% and 43% respectively. The highest infestation in male and female fish was recorded in summer 52.9% and 53.6% respectively while the lowest one was recorded in winter 25.0% and 34.5%. This result nearly agrees with Mahi Ghobashy (2000) and Eissa (2002) who recorded the highest infestation in summer, but disagrees with them in the prevalence of highest infestation 70% and 100% respectively. Mahi Ghobashy (2000) recorded that the highly infestations after summer was winter (38 %) followed by autumn (36.5%) and (0%) in spring. Meanwhile Eissa (2002) reported that the highly infestations after summer was autumn (52 %) followed by spring (36%) and (0%) in winter. This variation in prevalence may be attributed to the unequal samples and different sites from which samples are collected as well. The highest record of infestation in summer may due to high water temperature and spawning season as it extends from May to October i.e. from summer until the autumn, when the fish are more susceptible to any infection because of lowered immunity (Breikaa, 1992, El-Halafawy, 1995 and Mohamed & Abo-Esa 2007).

3.4. Effects of isopods on fish reproduction

The considerable reduced reproduction seen in parasitized female fish was manifested in several ways. The proportion of parasitized female fish with ova (16.7%) was significantly lower compared to unparasitized females (71.7%) ($P = 0.0001$). Parasitized females also had a different proportional mix of ova sizes compared to un-parasitized females ($P < 0.0001$) (Figure 1). This difference was largely due to parasitized females having 21.2% fewer extra-large ova than did the un-parasitized females. The proportions of various ovum developmental stages were also significantly different amongst parasitized and un-parasitized females (Figure 2). This data coincides with Fogelman *et al*. (2009).

Table 3: Prevalence of infested female and male *Nemipterus japonicus* by *Irona nanodies* in different seasons.

Season	Female			Male		
	No. of sample	No. of infested	%	No. of sample	No. of infested	%
autumn	13	5	38.5	59	22	37.3
winter	29	10	34.5	16	4	25.0
spring	31	12	38.7	27	11	40.7
summer	41	22	53.6	34	18	52.9
total	114	49	43.0	136	55	40.4

The several effects of *I. nanodies* on female host reproduction indicate that this isopoda greatly decreases the reproductive output of *N. japonicus* in spawning season. Parasitized females had fewer, smaller and less mature ova compared to un-parasitized females, and lower gonadosomatic index (GSI) for male and female (Figure 3&4). These data suggest that *I. nanodies* impair host female reproductive output, potentially by hindering ovum development, which in turn impeded or prevented ovum production. Adlard and Lester (1995) also reported that fewer viable ova were produced by parasitized female *Chromis nitida* than by un-parasitized females; however, they found insignificant differences in the sizes of ova produced by parasitized and un-parasitized females.

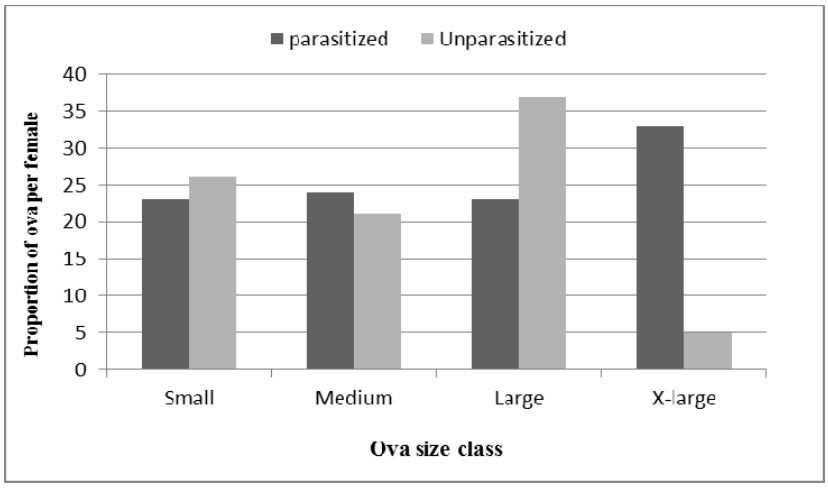

Figure 1. Frequency distributions of ova diameter size classes in the ovaries of parasitized and unparasitized (j) females from 2010 to 2011.

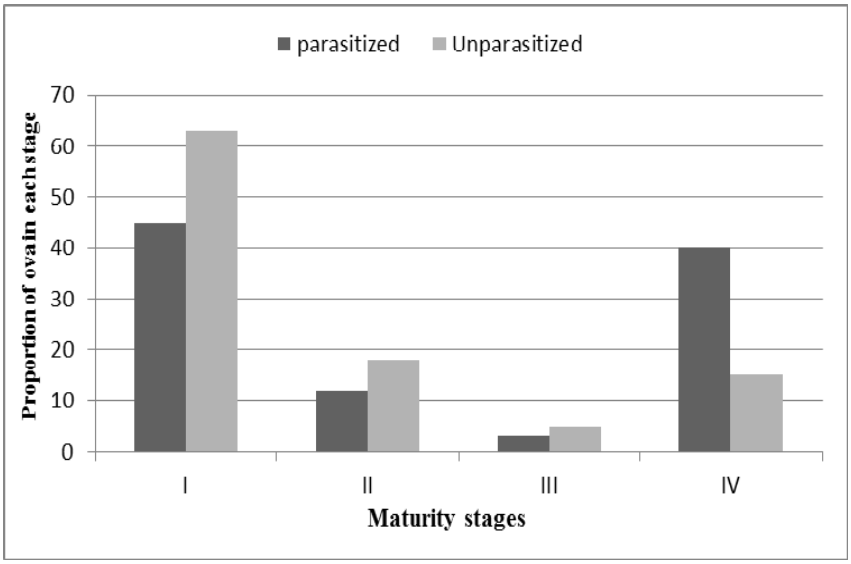

Figure 2. Frequency distributions of ovum developmental stages in the ovaries of parasitized and unparasitized by *Irona nanodies* in (2010-2011). (1) Peri-nucleolus stage (Peri-nuc); (2) yolkvesicle stage (Yolk-vess); (3) primary yolk, secondary yolk, and tertiary yolk stage combined (Pri-Ter); (4) maturation stage (Mat).

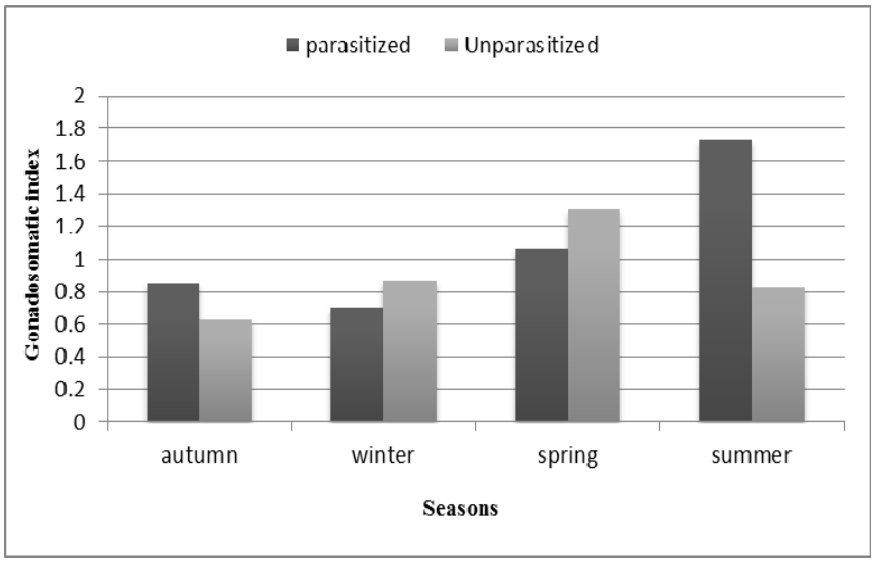

Figure 3. Frequency distributions of gonadosomatic index in the ovaries of female *Nemipterus japonicus* parasitized and unparasitized by *Irona nanodies* in (2010-2011) at different seasons.

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الإصابة بالقشريات الطفيلية متماثلات الأرجل وتأثيرها على النضج الجنسى لأسماك الصرع فى البحر الاحمر

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***المعهد القومى لعلوم البحار والمصايد ـ معمل تناسل وتفريخ الأسماك - السويس**
****معهد بحوث صحه الحيوان– قسم أمراض الأسماك ـ فرع الإسماعيلية***

تم إجراء هذه الدراسة في المعهد القومي لعلوم البحار والمصايد بالتعاون مع معهد بحوث صحة الحيوان. اجريت الدراسة على عدد 250سمكه من الأسماك البحرية (الصرع) ، والتي تم تجمعها بشكل عشوائي في مواسم مختلفة في خليج السويس من البحر الأحمر.

وجد طفيل متساوية الأرجل (الأيزوبودا) على جانب واحد فى معظم الحالات فى التجويف الخيشومى كطفيل وحيد لكل سمكة. وقد تم تصنيفه ووصفه. كان معدل انتشار الإصابة الكلي بالطفيل 41.6 ٪. وسجلت أعلى نسبة إصابة في فصل الصيف 53.3 ٪ تليها الخريف والربيع والشتاء مع معدل انتشار 39.7 ٪، 37.5 ٪ و 31.1 ٪ على التوالي.

تمت مناقشة مدى انتشار الإصابة في الذكور والإناث كما سجلت العلامات المرضية والصفة التشريحية ، وكذلك علاقة الأيزوبودا بالأسماك.

كما تم دراسة تأثير الأيزوبودا على القدرة التكاثرية للعائل (سمكة الصرع) من خلال تطور المناسل وقياس معامل النضج الجنسى (GSI) . وأثبتت الدراسة أن مناسل إناث الأسماك الأكثر عرضة للإصابة بالطفيل أصغر حجما وبها عدد أقل من البويضات. كما أن معامل النضج الجنسى للذكور والاناث أقل فى الاسماك المصابه عنها بالغير مصابة مما يؤثر بالتالى على معدل التكاثر للأسماك المصابة.