First occurrence of *Nerocila bivittata*: parasitic Isopods (skin shedders) on *Lithognathus mormyrus* (Osteichthyes, Sparidae) from Abu Qir Bay, Alexandria, Egypt

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Abstract: The present study deals with the infestation of *Lithognathus mormyrus* by *Nerocila bivittata* from Abu Qir Bay, Alexandria, Egypt. The market-sized of hosted fish were infested in approximately 10.26% of *N. bivittata* during summer season. The lateral side of the fish towards the end of trunk and begging of tail was the site most intensively infested by this parasite. At the site of attachment, the skin host was recognized by batches of clear brownish in colour with have external abnormalities such as skin ulcers, scale less and discoloration. The cymothoid isopod, *N. bivittata* damaged the epidermis, dermis and muscular tissue of *L. mormyrus* at the area of attachment by dactyls. These dactyls of the pereopods penetrated into the skin and anchored the isopod to the fish host. The parasite tore the epidermis layer of the fish host using their mouth parts and fed on the blood stream beneath. The infested fish were similar in length and lighter in weight and fecundity compared to the uninfested fish. The correlation coefficient "r" in relation between standard length and fecundity was significant for uninfested and infested fish.

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Key words: *Nerocila bivittata*; parasitic Isopods; *Lithognathus mormyrus.*

1. Introduction

Parasite diseases pose great problem in the culture and captive maintenance of marine fishes **(Al-Zubaidy and Mhaisen, 2013&2014).** Among marine fish parasites, nearly 25% are crustaceans, mainly represented by copepod, brachiura and isopod **(Eiras *et al*., 2000)**. Marine isopods play an important role in the food web, in particular in removing decaying material from natural or altered environments and they also represent an important factor of economic unbalance **(Espinosa and Hendrickx, 2001)**. They occur on fish host on the outer body surface or fins, in the mouth, gill chambers, nostrils or occasionally in self-made pockets in the flesh of their hosts **(Hoffman, 1998).** Isopods cause significant economic losses to fisheries by killing, stunting or damaging these fishes. They can also kill fish so that they do not survive **(Bunkley-Williams, *et al*., 2006).**

Cymothoidae represent one of the five families of Isopoda, are exclusively parasitic on fish. They predominantly attach or settle in the buccal cavity of fish, others live in the gill chamber or on the body surface including the fins. Their life cycle involves only one host (Holoxenic cycle) **(Lester &Hayward, 2006; Ramdane *et al*., 2007 and Alas *et al*., 2008).** They occur in marine, estuarine and freshwater habitats, especially in the near-shore coastal environment **(Sullivan & Stimmelmayr, 2008).** They feed on host blood or host haemolymph and can cause significant economic losses to fisheries **(Printrakoon and Purivirojkul, 2011).** *Nerocila* is a large genus of the family: Cymothoidae include at least 65 species living attached on the skin or on the fins of fish. Their appendages are highly modified to hold the body surface and tearing the strong body muscles of host fish **(Rameshkumar, *et al*., 2013).**

In the Mediterranean Sea, ectoparasitic Isopods, *Nerocila bivittata* is chiefly parasitic on fish belonging to the family Labridae (**Trilles, 1994 and Charfi-Cheikhrouha *et al*., 2000)**, but has sometimes been collected from hosts from other families: Scorpaenidae, *S. scrofa, S. porcus* (**Trilles, 1975**); Sciaenidae, *Sciaena umbra*; Mullidae, *Mullus surmuletus*; Gobiidae, *Gobius geniporus*; Serranidae, *Serranus scriba* (**Charfi-Cheikhrouha *et al*., 2000**); Sparidae, Gobiidae and Sciaenidae (**Öktener & Trilles, 2004**), Triglidae and Sparidae (**Bariche & Trilles, 2005**). Abu-Qir Bay receives different pollutants contributing to various waste sources. The shore line configuration and coastal sedimentation have been modified by artificial structures such as jetties at the inlet of Edku lagoon and seawalls emplaced at the outer margin of Rosetta promontory (**Firhy *et al*., 1994 and Khalaf-Allah, 2009**). This of course affects the wild fish community as a result of reproductive disorder and leads to serious of

histopathological alternation of vital organs in fish **(Khalaf Allah and Shehata, 2011**).

Sparidae is a very large family in the order perciformes. It is very widely distributed in Atlantic, Indian and Pacific Oceans. They are tropical and temperate littoral or inshore waters, sometimes brackish waters; young and small species gregarious in shallow waters; adults in deeper waters **(Bauchot and Hurau, 1986).** Most species are mainly carnivorous. Hermaphroditism (protogynous or protandrous) and gonad sex reversal is widespread in this family (**Whitehead *et al*., 1984; Turkmen & Akyurt, 2003 and Argyris, 2005**). In Abu Qir Bay, however, this family was represented by eleven genera with twenty one species (**Ibrahim and Soliman, 1996**).

The striped sea bream, *Lithognathus mormyrus* is highly economic importance in the Mediterranean Sea. Crustaceans, worms, mollusks, sea urchins and small fishes were the main food items consumed by this species. Reproduction occurred during spring and summer. Protandrous hermaphrodite fish and gonad sex reversal (**Jardas, 1985; Kraljevic *et al*., 1995 and Argyris, 2005**). The infected specimens by ectoparasitic Isopods causes lowest in fecundity **(Khalaf-Allah, 2009**).

Many works have been done on isopods parasites infection potential of different marine fishes, whereas no attempt has made of in the case of fish. Hence, to determine the harmful effect of parasites, the present study investigate the abundance, site of infection and histopathology of ectoparasitic Isopods, *Nerocila bivittata* (skin shedders) on *Lithognathus mormyrus* from Abu Qir Bay, Alexandria, Egypt

2. Materials and methods

1- Site of collection:

Fish samples were obtained from Abu Qir Bay (Fig. 1). The bay has a shore line of about 50 kms long in East of Alexandria City. The bay is located between Abu Qir head land south west to Rosetta mouth northeast. It lies between longitudes 30° 4` & 30° 21` East and latitudes 31° 16` & 31° 30` North. Three openings are connected to the bay, the first through Rosetta Nile Branch, the second through a channel to Lake Edku and the third to El-Tabia Pumping Station. In the western part of the bay, however, some rocky patches exist of which Nelson Island is obvious above the sea waters. The bay is generally shallow; water depth of the bay fluctuated between 1 meter near the coast to 16 meters at the open sea (**Younis, 2005 and Mohamed, 2006**).

2- Specimens collection:

A total of 384 specimens of *Lithognathus mormyrus* (Plate, 1A) were collected seasonally from the different localities of Abu Qir Bay, Alexandria during the period from December, 2012 to November, 2013, formed the materials for the present study. Bottom trawls, purse seines and beach seines were the main fishing methods used to collect the fish. Fishes were freshly examined for ectoparasitic Isopods. Infested and uninfested fish were preserved in 10% formalin solution for latter examination. In the laboratory, fishes were identified; standard and total lengths were measured to the nearest millimeters and recorded. Fishes were also weight-weighted in grams. The infected fishes were carefully dissected beginning at the site of infection, following the body of Isopods to reach its target organ in the host and the abundance and sites of infections were recorded. Isopods were separated and immediately preserved in 70% ethyl alcohol solution for latter examination. They were examined and photographed using 35mm Camera. Isopods were identified by protocols described by **Bowman and Tareen (1983).**

3- Fecundity:

To study fecundity, 9 ripe infested and uninfested females were selected during the spawning season (May - October). The gonads were weighted to the nearest 0.01 gm and preserved immediately in saline solution. Samples from the anterior, middle and posterior parts of each ovary were taken, weighed and placed in a Petri-dish. Ova were separated from the ovarian tissues with the aid of a dissecting needle and counted under a binocular microscope. Fecundity was calculated according to the following equation suggested by **Nikolosky (1963):**

$$\text{Fecundity} = \frac{\text{Average number of ripe eggs}}{\text{wt of sample (gm)}} \times \text{wt of ovary (gm)}$$

4- Histopathology:

For histopathological examination, normal and infested fish tissues were taken from the parasite attachment area by mouthparts and appendages and immediately fixed in alcoholic Bouin's solution for 24 hours. These specimens were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Vertical sections were cut at 5 to 7 microns, and stained with Harri's haematoxylin and subsequently counter stained with eosin. Finally, the slides were microscopically examined and photographed using camera mounted on light microscope and described.

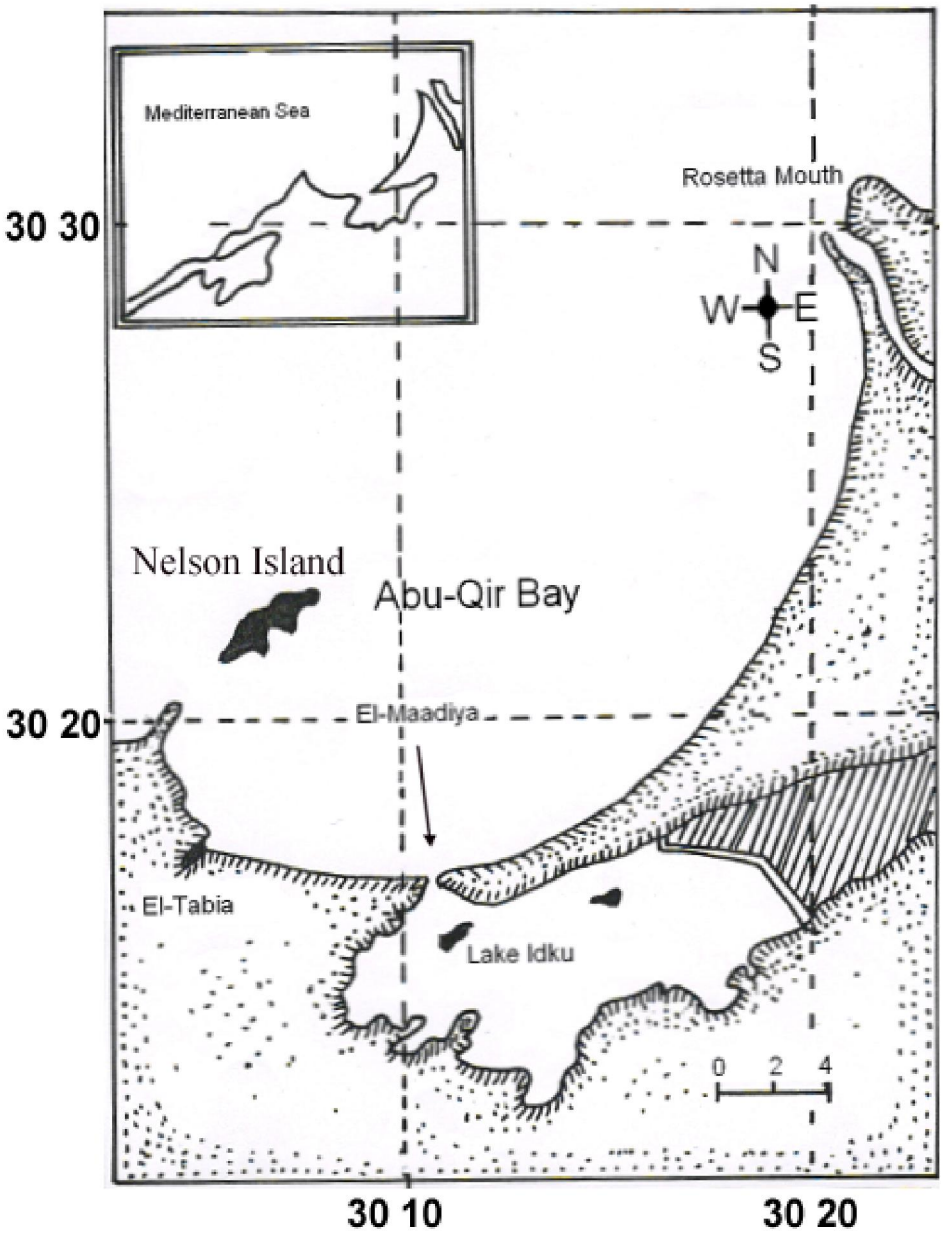

Fig. (1): Map of Abu-Qir Bay showing the study area

3. Results

1- Abundance of infection:

Data in Table (1) showed that, the annual percentage of infestation by ectoparasitic Isopods, *Nerocila bivittata* on benthic feeder, *Lithognathus mormyrus* at Abu Qir Bay, Alexandria is 3.13%.The percentage of infestation changed during different seasons. The highest percentage of infestation was recorded during summer (10.26%) and the lowest (2.70%) occurred during spring. It was entirely absent during autumn and winter.

Table (1): Seasonal percentage of infected *Lithognathus mormyrus* at Abu Qir Bay, Alexandria, during the period from December, 2012 to November, 2013.

Season	No of examined fish	No of infested fish	Infection %
Winter	54	0	0.00
Spring	148	4	2.7
Summer	78	8	10.26
Autumn	104	0	0.00
Total	384	12	3.13

2- Site of infection:

After examination of 12 infested hosts, the infected fish are mostly female. The ectoparasitic Isopods, *Nerocila bivittata* infect dorsal and lateral sites on the fish integument. Their attachment sites are found on lateral side of the fish towards the end of trunk and begging of tail in large number (10 specimens) of infected host (Plate 1B-D) and on dorsal fin in small number (2 specimens) of infected host. The ectoparasitic Isopods are not found inside the host. Examination of infected host under dissecting microscope revealed that, these parasites were found attached to the host with their appendages deeply embedded in the fish muscles and anchored themselves by their hooks in the terminal appendages (Plate 1B).

3- Weight and fecundity:

Data in Table (2) showed that, the infested fish were similar in length (13 -17Cm) and lighter in weight (55.36 – 84.56 gm) compared to the uninfested fish which were (13 -17Cm) and (51.2 – 112.64 gm) respectively.

The infested fish were similar in length (13 - 17Cm) and lighter in fecundity (3024.48 – 13614) compared to the uninfested fish which were (13 - 17Cm) and (2100 – 16463.04) respectively. The correlation coefficient "r" in relation between standard length and fecundity was significant for uninfected and infected fish (0.99).

Table (2): Comparison in weight and fecundity between infested and uninfested *Lithognathus mormyrus* at Abu Qir Bay, Alexandria, during summer, 2013.

Fish state	No of fish	St. L. (Cm)	Weight (Gm)	Fecundity
Uninfestation	1	13	51.20	2100.00
	2	14	67.55	7908.80
	3	15	76.79	10503.00
	4	16	90.13	15747.80
	5	17	112.64	16463.04
Infestation	1	13	55.36	3024.48
	2	14	61.77	5262.93
	3	15	66.93	8076.08
	4	16	-	-
	5	17	84.56	13614

4- Histopathology of infection:

The maximum number of parasite infections was one per hosted fish (Plate 1B). The characteristics distinguishing *Nerocila bivittata* from other *Nerocila* species were the coxae and posterolateral corners of the olanites which are posteriorly directed and not bent dorsally in *N. bivittata* (Plate 1E&F).

The skin of *Lithognathus mormyrus* is made up of usual two layers, an outer epidermis and an inner dermis. Epidermis is typically very thin, composed superficially of several layers of flattened, moist epithelial cells. The deepest layer is a zone of active cell growth and multiplication, known as a columnar germinative layer next to the dermis. Live cells of epidermis are in contact with the medium via mucous covering without cornified layer. There are unicellular mucous glands that discharge the mucus that forms the slimy outer covering of fishes (Plate 2 A).The dermal layer of the skin contains pigment cells, blood vessels, nerves and cutaneous sense organs and origin of scales. It usually is made up of a stratum spongiosum, just beneath the epidermis and a deep stratum compactum (Plate 2 B).

At the site of attachment, the skin host was recognized by batches of clear brownish in colour with have external abnormalities such as skin ulcers, scale less and discoloration (plate 1C&D). The cymothoid isopod, *Nerocila bivittata* damaged the epidermis, dermis and muscular tissue of *Lithognathus mormyrus* at the area of attachment by dactyls. At the mouth part or pereopod site of attachment, the skin (epidermis and dermis) was eroded and exposed the underlying tissue, which the isopods were then able to consume (Plate 2 C&D). The dactyls of the pereopods penetrated into the skin and anchored the isopod to the fish host. The parasite tore the epidermis layer of the fish host using their mouth parts and fed on the blood stream beneath (Plate 2 E&F).

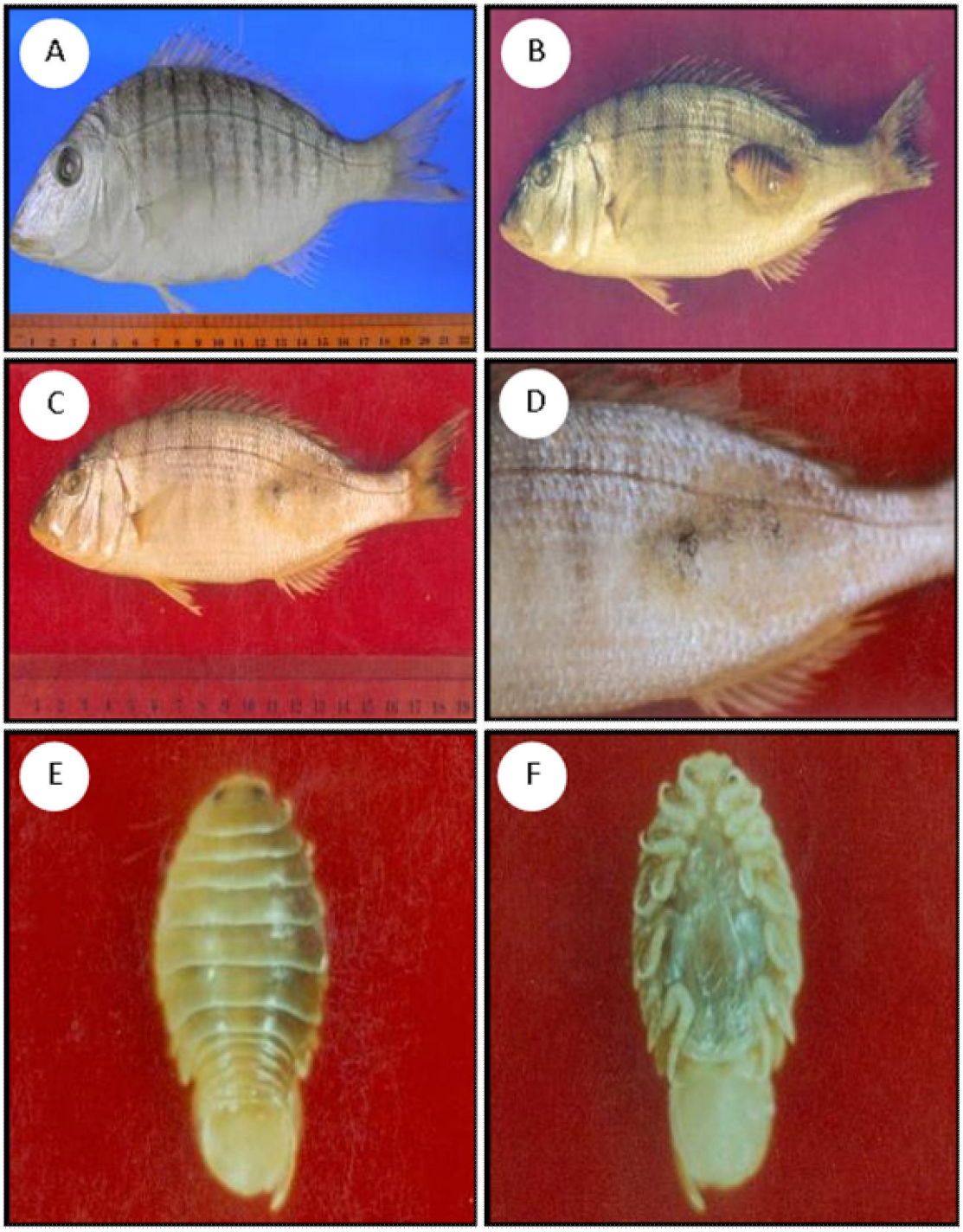

Plate (I): Light photographs showed **A**. the undamaged fish host *Lithognathus mormyrus*, **B**. the site of attachment of the isopod parasite, **C**. the damage on *L. mormyrus* resulted from the attachment by *Nerocila bivittata*, **D**. focusing on the harmed part of the end of trunk and the beginning of caudal peduncle of *L. mormyru*, **E**. dorsal view of *N. bivittata*, and **F**. ventral view of *N. bivittata.*

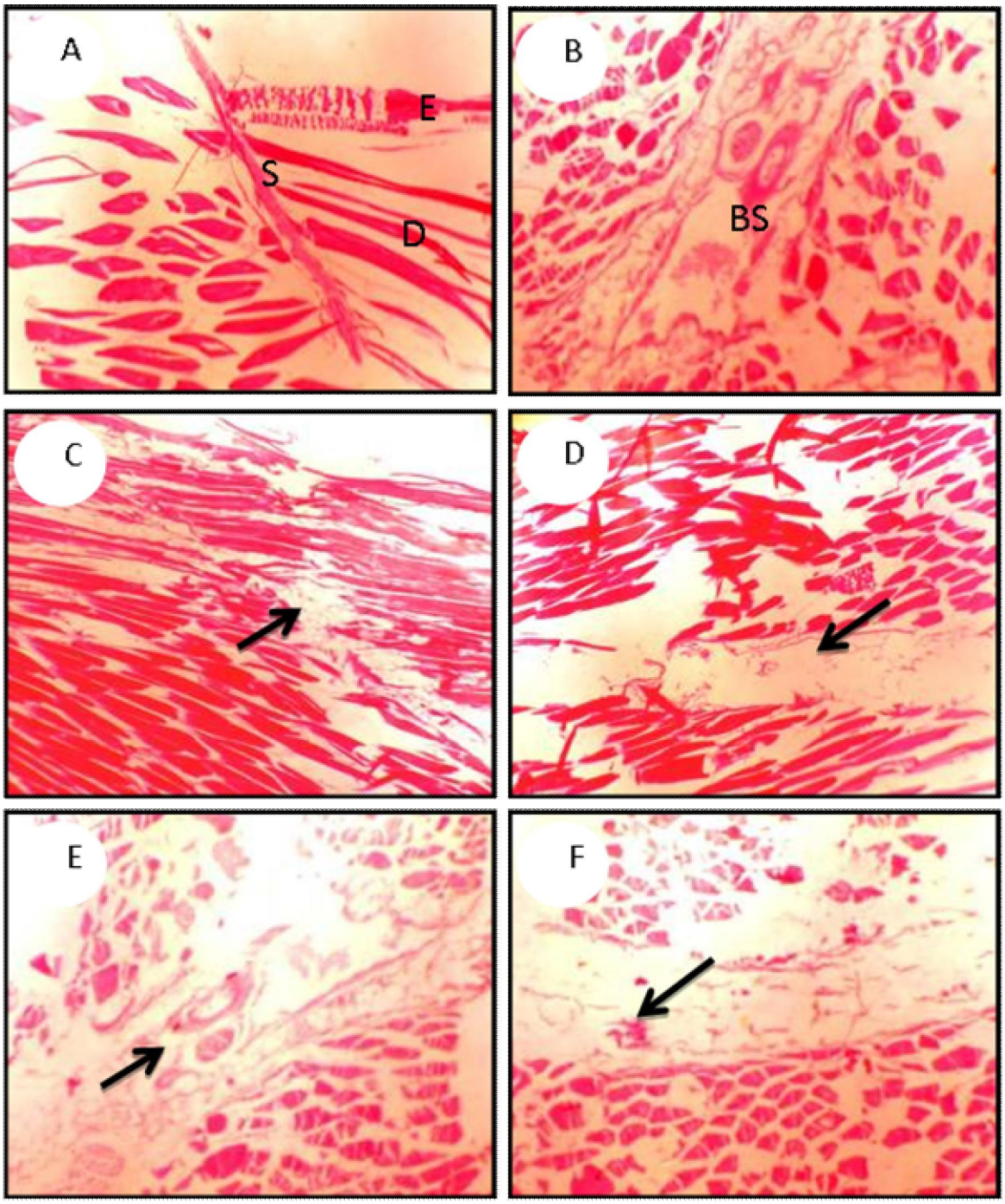

Plate (II): Vertical sections at skin of *Lithognathus mormyrus* showed histopathology resulted from the attachment by *Nerocila bivittata*. **A**. & **B**. normal undamaged skin and blood stream of *L. mormyrus*., **C**. & **D**. pathology of the skin and blood stream caused by piercing of the pereopods (arrow) and **E**. & **F**. pathology at the skin and blood stream caused by mouthparts (arrow) of *N. bivittata* (H-E x40). **BS**: Blood stream; **D**: Dermis; **E**: Epidermis and **S**: Scale

4. Discussion

Parasitic isopods are likely the dominant group of crustacean ectoparasite of fish in tropical seas (**Kabata, 1984**). Recently the parasites have been recognized as an important component of global biodiversity and research efforts directed and documented towards the parasitic species diversity has increased **(Poulin and Morand, 2004**). Parasites

affected fish health, growth, behavior, fecundity and mortality and also regulate host population dynamics and their community structure (**Marcogliese, 2004**). Isopods associate with many species of commercially important fishes around the world and cause significant economic losses to fisheries by killing, stunting or damaging these fishes. They are potentially economically important parasites as they have been shown to cause detrimental effects on fish in captivity including growth inhibition, Anaemia and death in smaller fish **(Ravi and Rajkumar, 2007** and **Ravichandran *et al.*, 2011).**

In the present study, the first record of the striped sea bream, *Lithognathus mormyrus* infestation by cymothoid isopod, *Nerocila bivittata* from Abu Qir Bay, Alexandria, Egypt. The highest percentage of infection was recorded during summer (10.26%) and the lowest (2.70%) occurred during spring. It was entirely absent during autumn and winter. This result was agreement by **Noor El-Deen *et al.* (2013)** on another cymothoid species. Home mentioned that, the prevalence of *Nerocila orbignyi* infestation in European seabass during summer and spring seasons, while infestation was disappeared during autumn and winter seasons. The result disagrees with the result obtained by **Eissa *et al.*, (2012)** in which he recorded the summer season as the highest infestation rate 19%, followed by autumn 17%, while spring 7% and the lowest was 4% in winter season. This may be attributed to differences in geographical distribution of hosts and parasites and increase of water flow in Abu Qir Bay during summer and spring seasons than that in autumn and winter which leading to solution of pollution and salinity.

In the present study**,** the overall prevalence of infestation was 10.26% during summer season. This is higher than those reported for some cymothoid species by other workers, such as 1.5% for *Cymothoa ktiskhani* in *Platycephalus insidiator*, which is a synonym of *P. indicus* (**Jayadev Babu and Sanjeevaraj, 1985**); 3.6% in *Livoneca sp.* of *Atherinomorus lacunosus* in Red Sea (**Colorni *et al*., 1997**); 5.0% for *Mothocya epimerica* infestations in *Atherina boyeri* found in the Sinop coasts of the Black Sea (**Özer, 2002**); 7.4% in *Nerocila bivittata* on *Parablennius sanguinolentus* in the Samsun coast of the Black Sea (**Alas *et al*., 2008**) and 5.9% for *Livoneca redmanii* from *Chloroscombrus chrysurus* (**Costa and Chellappa, 2010**). On the other hand, the present prevalence of *Nerocila bivittata* infect the striped sea bream, *Lithognathus mormyrus* was (10.26%) is lower than that recorded by **Ravi and Rajkumar (2007**) in case of *C. indica* from *Etroplus maculatus* and *E. suratensis*; 27.8% for *C. indica* on *Oxyurichthys microlepis* from the south-east coast of India; 15.3% and 11.76% for *Cymothoa spinipalpa* on *Lutjanus synagris* and *Caranx crysos*, respectively from Brazil (**de Carvalho-Souza *et al*., 2009**); 81.3 % in case of *C. indica* from Red Sea fish, *Myripristis murdjan* in Egypt (**El-Shahawy and Desouky, 2010**) and 40% for *Cymothoa indica* on the blue spot mullet *Moolgarda seheli* from the Yemeni coastal waters of the Red Sea (**Al-Zubaidy and Mhaisen, 2014**). The variations in infestation percentages may be due to differences in degrees of temperature according to geographical distribution of hosts and parasites.

In general, parasitic infections of fish mainly depend upon host factors such as age, size, sex, maturity stage, behavior, feeding, breeding, life cycle and particularly environmental factors.In the present study, the life cycle of *N. bivittata* involves only one host, Holoxenic cycle with a preference for sparidae from Abu Qir Bay, Alexandria, Egypt. This result not agrees with **Ramdane *et al.* (2007).** He mentioned that, *N. bivittata* is a stenoxenic species with a preference for Labridae in Algeria. The specificity of parasites may change with locality, becoming euryxenic in some areas, stenoxenic specificity in another areas and Holoxenic in third areas.

In the present study, the attachment sites of *N. bivittata* on *L. mormyrus* are rare found on dorsal fin and common present on lateral side of the fish towards the end of trunk and begging of tail. **Ramdane *et al.* (2007)** collect the *N. bivittata* from the caudal fin of *Crenilabrus pavo* in the gulf of Béjaïa. The variations in site attachment may be due to variation in type and size of host. The cause of attachment at this position may be due to easier attachment at this site by the parasite or due to easier shedding of the parasite from other areas by the host.

In the present study, *N. bivittata* was most frequently found on the lateral side of the fish towards the end of trunk and begging of tail. The position of attachment area might depend on the host's body movement. Fish swim using undulatory movements of their body and/or their paired and unpaired fins. In undulatory swimming, a backward-travelling wave is generated by the sequential activation of the segmental myotomes from head to tail (**Altringham & Ellerby, 1999** and **Printrakoon & Purivirojkul, 2011**). In the present study, the infested fish were similar in length and lighter in weight and fecundity compared to the uninfested fish. The infected specimens by ectoparasitic Isopods causes lowest in fecundity **(Khalaf-Allah, 2009**).

Histopathological studies reviewed that, the cymothoid isopod; *N. bivittata* damaged the epidermis, dermis and muscular tissue of *L. mormyrus* at the area of attachment by dactyls. The damage caused to the tissues and their subsequent exposure could have caused the death of fish, thus causing the fish population to decline. This could be

one reason for the large decline in the fish catch during that time. The same results were reported by **Printrakoon & Purivirojkul (2011)** in *Nerocila depressa* (Isopoda, Cymothoidae) on *Sardinella albella*. **Leong and Colorni (2002)** have reported *Nerocila sp.* in cultured grouper, seabass and snapper in Southeast Asia. It is likely that this cymothoid isopod could readily infect fish cultured in floating cages at the estuarine coastal region of Trat Province, Thailand. All potential aquaculturists in the region should note the presence of large numbers of this cymothoid isopod.

In the present study, *N. bivittata* tore the epidermis layer of the fish host using their mouth parts and fed on the blood stream beneath. This result is agrees with **Wood *et al.* (2007), Printrakoon & Purivirojkul (2011) and Al-Zubaidy & Mhaisen (2014)**. Like most isopods, cymothoids are considered to feed principally on host blood, but they may consume the mucus, epithelium and subcutaneous tissues of their hosts (**Ramdane *et al.*, (2007)**. This also was evident in the present study.

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