Investigation on common parasitic diseases in marine Puffer fish (*Lagocephalus lunaris*) in relation to heavy metal pollution in Lake Temsah, Ismailia Province

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**Abstract**

This study was carried out on 294 marine puffer fish (*Lagocephalus lunaris*). The fish were randomly collected from Lake Temsah, Ismailia Province at different seasons. There were no pathognomonic signs in infested fish. The recorded signs and P.M. lesions were excessive mucous secretion, congestion, marbling appearance, pale gills and destruction of gill filaments in case of crustacean infestation, pale enlarged liver with haemorrhages, in case of nematode larvae infestation. Intestine was inflated in cases with immature cestode infestation. The detected parasites were identified as 2 copepodes (*Bomolochus* sp. and *Caligus* sp.), nematode (*Cucullanus* sp. larva) and unidentified immature cestode. The total infestation rate was 40.8% and autumn displayed the highest seasonal prevalence. Crustacean parasites represented the highest prevalence (34.6%) followed by immature cestodes (6.1%) and nematode larvae (4.1%). The highest prevalence of crustacean parasites and nematode larvae were recorded in summer while the highest prevalence of immature cestodes was recorded in autumn. The relations between fish body lengths and weights with parasitic infestation were recorded. Besides, the relation between heavy metal pollution and parasitic infestation was discussed.

**Keywords:** *Lagocephalus lunaris*, *Bomolochus* sp., *Caligus* sp., *Cucullanus* larva, immature cestode, prevalence, heavy metals.

**Introduction**

Green rough-backed puffer fish, *Lagocephalus lunaris* (*L. lunaris*) is in the Tertaodontidae family which specifically have large four teeth. This marine puffer fish commonly distributed in tropical and subtropical seas (*Mohamad and Isa, 2012*). The presence of probably the most toxic poison found in nature, tetradotoxin, makes tetaodontids unmarketable. Yet, of late, it is becoming a potential item in the seafood list and parasitism is
a ubiquitous phenomenon in the marine environment and only a few parasites are known from fishes of the genus *Lagocephalus* (*Prasad and Radhakrishnan, 2010*). Crustacean parasites are common on fish hosts in marine water. Copepods distribution varies from one host species to another and one season to another (*Boualleg et al, 2010*).

Adult forms of tapeworms are not very common in bony fishes, but larval forms use bony fishes as intermediate host (*Williams and Bunkley-Williams, 1996*) while nematodes are found in all body parts of fish either as larvae or adults (*Yasmin and Bilqees, 2007*).

Pollutants might promote increased parasitism in aquatic animals, especially fish, by impairing the host's immune response. On the other hand, parasites might enhance their hosts' susceptibility to pollutants (*Khan and Thulin, 1991*). Heavy metals such as cadmium, lead, copper and more specifically mercury are potentially harmful to most organisms even in very low concentrations (*Kaoud and El-Dahshan, 2010*).

The present study reports the common parasitic diseases affecting *Lagocephalus lunaris* marine fish in relation to its body length and weights in Ismailia Province. Moreover, this work deals with the effect of heavy metals pollution on fish parasitism in different seasons.

**Fish:**
A total number of 294 rabbit fish (*L. lunaris*) with different body weights were collected alive in different seasons from Lake Temsah at Ismailia Province, (72) in autumn, (69) in winter, (60) in spring and (93) in summer. They were transported to the lab in thick plastic bags partially filled with their natural water within a short time according to *Langdon and Jones (2002)*.

**Water and tissue samples for heavy metal measurements:**
Water and fish samples were collected in different seasons of the year. Twenty water samples were collected from the same locality of fish capture in different times at a rate of five samples for each season, kept refrigerated and transferred cold to the laboratory for analysis according to *APHA (1992)*. Parasitized fish specimens were dissected freshly to obtain gills, musculature & liver, then frozen until ready for acid digestion, according to the method described in *AOAC (1995)*. The levels of Pb, Zn, Cu and Cd were determined at central lab of Faculty of Agriculture, Suez Canal University using atomic absorption spectrophotometer (Model Thermo, AA spectrometer, S series, type s4).

**Clinical picture:**
Fish were grossly examined for determination of any clinical abnormalities and any external parasites or visible cysts. The specimens were examined carefully,
externally and internally for parasitic infestation according to Conroy and Hermann (1981).

**Parasitological examination:**
The collected parasites were placed into large Petri-dish containing normal saline and washed several times to get rid of mucus and to be relaxed. The detected crustaceans were collected in a test tube, washed and cleaned using distilled water then fixed in 3% formalin and preserved in equal amount of 70% alcohol – 5% glycerin. Permanent mounts were prepared by passage in descending grades of alcohol (70, 50, and 30%), cleared in glycerin and mounted in glycerin-gelatin, according to Lucky (1977). The collected cestodes were fixed in 5% formalin, stained in Semichon’s acetocarmine, dehydrated in ascending grades of ethyl alcohol, cleared in clove oil then in xylene and mounted in Canada balsam according to Lucky (1977). The collected nematodes were kept in glycerin alcohol then washed in 70% alcohol, cleared in lactophenol and mounted in glycerol gelatin according to Meyer and Olsen (1992). The examined parasites were identified according to Yamaguti (1959), Yamaguti (1963) and Kabata (1979) and the description recorded in literature.

**Results**

**Clinical picture:**
The clinical signs of naturally infested *L. lunaris* with copepods revealed no pathognomic clinical abnormalities except excessive mucus secretion. Also, infested fish with nematode and cestode larvae showed no signs. Post-mortem examination revealed attached copepods on the gills, congestion with marbling appearance. Other cases showed pale and destruction of gill filaments. Internally, pale enlarged liver with hemorrhages were seen associated with free nematode larvae (plate,1) while in case of cestode, there was pale liver and severe enteritis (Plate,2).

**Parasitological findings:**
Microscopic examination of the collected fish revealed four metazoan parasites; one nematode from abdominal cavity (*Cucullanus* sp.) larvae (Plate, 2), one immature cestode from intestine (Plate, 2) and two crustacean copepods form gills (*Bomolochus* species Nordmann, 1832 and *Caligus* species Müller, 1785) (Plate, 3).

**Prevalence and seasonal variations:**
Table (1) shows the total and seasonal prevalence of the detected parasites in the examined *L. lunaris*. The highest percentage of crustacean infestations was in summer followed by spring, autumn and then winter. Regarding nematode larvae, the highest prevalence was in summer followed by autumn with no infestation in winter and spring. On the other hand, the immature cestodes showed the highest prevalence in autumn followed by winter with no infestation in spring and summer. Mixed infestation of crustacean
with nematode or cestode was noticed in (autumn & summer) and winter, respectively. Tables (2&3) show prevalence of the detected parasitic infestation in relation to body lengths and weights.

**Heavy metal residues and seasonal variations:**
Tables (4&5) show the residues and concentration of lead, zinc, copper and cadmium in fish tissues and water samples in different seasons. Contamination levels of heavy metals in fish and water were compared to the permissible limits recommended by Food and Agriculture Organization and World Health Organization. Also, with the Egyptian Organization for Standardization and Quality Control.

**Table (1):** Total & seasonal prevalence of the detected parasites among *L. lunaris*

<table>
<thead>
<tr>
<th>Season</th>
<th>Total No. of Exam. fish</th>
<th>Total No. of infest</th>
<th>%</th>
<th>Parasitic Infestations</th>
<th>No. of fish with mixed infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Crustaceans</td>
<td>Nematode larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>N</em></td>
<td>%</td>
</tr>
<tr>
<td>Autumn</td>
<td>72</td>
<td>36</td>
<td>50</td>
<td>20</td>
<td>27.78</td>
</tr>
<tr>
<td>Winter</td>
<td>69</td>
<td>14</td>
<td>20.3</td>
<td>12</td>
<td>17.4</td>
</tr>
<tr>
<td>Spring</td>
<td>60</td>
<td>25</td>
<td>41.7</td>
<td>25</td>
<td>41.7</td>
</tr>
<tr>
<td>Summer</td>
<td>93</td>
<td>45</td>
<td>48.4</td>
<td>45</td>
<td>48.4</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>120</td>
<td>40.8</td>
<td>102</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*N* = number of infested fish

**Table (2):** Prevalence of the detected parasitic infestation in relation to fish length

<table>
<thead>
<tr>
<th>Fish length (cm)</th>
<th>No of Exam. fish</th>
<th>Parasitic Infestations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crustaceans</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>N</em></td>
</tr>
<tr>
<td>10-15</td>
<td>123</td>
<td>12</td>
</tr>
<tr>
<td>15-20</td>
<td>135</td>
<td>72</td>
</tr>
<tr>
<td>20-25</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>&gt;25</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>102</td>
</tr>
</tbody>
</table>

*N* = number of infested fish

**Table (3):** Prevalence of the detected parasitic infestation in relation to fish body weight.
<table>
<thead>
<tr>
<th>Fish body weight (g)</th>
<th>No of Exam fish</th>
<th>Parasitic Infestation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crustaceans</td>
<td>Nematode larvae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*N</td>
<td>%</td>
</tr>
<tr>
<td>50-100</td>
<td>156</td>
<td>15</td>
<td>9.62</td>
</tr>
<tr>
<td>100-150</td>
<td>117</td>
<td>72</td>
<td>61.5</td>
</tr>
<tr>
<td>150-200</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>200-250</td>
<td>18</td>
<td>12</td>
<td>66.66</td>
</tr>
<tr>
<td>Total</td>
<td>294</td>
<td>102</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*N=number of infested fish

Table (4): Mean heavy metals concentration (ppm) in infested fish organs and tissues in different seasons.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Permissible limit (mg/kg)</th>
<th>Seasonal variation in gills (mg/kg)</th>
<th>Seasonal variation in Liver (mg/Kg)</th>
<th>Seasonal variation in musculature (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au</td>
<td>Wi</td>
<td>Sp</td>
<td>Su</td>
</tr>
<tr>
<td>Pb</td>
<td>57.4</td>
<td>57.6</td>
<td>57.5</td>
<td>57.2</td>
</tr>
<tr>
<td>Zn</td>
<td>469.6</td>
<td>469.8</td>
<td>469.7</td>
<td>469.1</td>
</tr>
<tr>
<td>Cu</td>
<td>24.3</td>
<td>24.5</td>
<td>24.4</td>
<td>24.1</td>
</tr>
<tr>
<td>Cd</td>
<td>8.73</td>
<td>8.77</td>
<td>8.75</td>
<td>8.71</td>
</tr>
</tbody>
</table>


Table (5): Mean heavy metals concentration (ppm) in Lake Temsah water from which L. lunaris were collected in different seasons.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Permissible limit (mg/L)</th>
<th>autumn (mg/L)</th>
<th>winter (mg/L)</th>
<th>spring (mg/L)</th>
<th>summer (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>0.05</td>
<td>0.0194</td>
<td>0.0196</td>
<td>0.0196</td>
<td>0.0193</td>
</tr>
<tr>
<td>Zn</td>
<td>1.0</td>
<td>0.00605</td>
<td>0.00612</td>
<td>0.00612</td>
<td>0.00605</td>
</tr>
<tr>
<td>Cu</td>
<td>1.0</td>
<td>0.00162</td>
<td>0.00166</td>
<td>0.00164</td>
<td>0.00156</td>
</tr>
<tr>
<td>Cd</td>
<td>0.01</td>
<td>0.0028</td>
<td>0.0031</td>
<td>0.0030</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

Permissible limit according to USEPA (1986)
Plate (1): A. *Lagocephalus lunaris* fish. (B-E) Gills of *L. lunaris* showing, B. excessive mucus secretion, C. marbling appearance & destruction of gill filaments, D. attached Bomolochus species with excessive mucus (arrows), E. congestion & attachment of crustaceans (arrows). F. pale enlarged liver with hemorrhages and presence of nematode larva (arrow) in the abdominal cavity.

Plate (2): A. Intestine of *L. lunaris* showing severe inflammation. B. Light photomicrograph of immature cestodes showing four cup-shaped scolex, (C-D) Light photomicrograph of Cucullanus sp. larva, C. anterior end & D. posterior end.
Plate (3): Copepode crustacea: A. Bomolochus sp. (low mag.), B. Caligus sp. (low mag.). (C-E) Light photomicrograph of female Bomolochus sp., C. whole copepode, D. anterior end showing the antennae, E. Posterior end showing egg sacs and caudal ramai. (F-I) Light photomicrograph of Caligus species, F. Female (whole copepode), G. Male (whole copepode), H. anterior end showing two eyes, second maxilla & claws, I. Posterior end showing caudal ramai.

Discussion
The clinical signs of copepod infested puffer fish revealed no pathognomonic clinical abnormalities except excessive mucous secretion which may be used to dilute the irritation and act as a defense mechanism against the infestation (Yambot and Lopez, 1997). Also, fish infested with nematode larvae and immature cestodes showed no pathognomonic clinical signs, this is in agreement with Yanong (2006). Post-mortem examination of copepod infested fish showed congestion and marbling appearance of gills with excessive mucous secretion. In some cases, gills were pale with destruction of gill filaments. These findings agreed with that reported by Eissa et al (2012). Parasitic copepods damage the gills by feeding on the delicate tissue of the gill lamellae or on the blood circulating within the lamellae, leading to a loss of respiratory surface area, extensive gill damage and severe haemorrhage, with inflammation and exsanguinations associated with the attachment and feeding of the parasite. Blood vessels in the gill filaments are blocked and this leads to atrophy of gill tips (Purivirojkul & Areechon, 2008). In nematode larvae infestation, there were pale enlarged liver with some hemorrhages, this is similar to that obtained by Yanong (2006) while pale liver and severe enteritis in case of cestodiasis may be due to presence of the parasite in the intestine which resulted in tissue alteration or destruction and
mechanical blockage leading to impaired absorption of food, this is in agreement with Eissa et al (2009). Measurements and morphological characters of immature cestodes collected from intestine, nematode larvae collected from abdominal cavity and crustacean copepods collected from gills were similar to that obtained by Yamaguti (1959), Yamaguti (1963) and Kabata (1979) so they were identified as Cucullanus sp. larva, immature cestode, Bomolochus sp. Nordmann, 1832 and Caligus sp. Müller, 1785.

Regarding parasitic infestation, the total prevalence was 40.8%. This result is lower than that obtained from Lagocephalus inermis 99.3% by Prasad and Radhakrishnan (2010). This variation may be attributed to the difference of localities, fish species and time of examination. The seasonal prevalence was highest in autumn (50%), summer (48.4%) followed by spring (41.7%) and winter (20.3%). These results disagree with Carvalho and Luque (2011) who reported that all metazoan species showed peaks of prevalence in summer. This variation may be attributed to the unequal number of fish samples in different seasons and the difference of fish species. Regarding crustacean infestation, the total prevalence was 34.6%, this result is lower than that obtained by Eissa et al (2012) (47%) from Morone labrax. The highest percentages of crustacean infestations were in summer followed by spring, autumn and then winter, this may be attributed to that crustacean gill parasites disappeared with low temperature, this agreed with Eissa et al (2012). The total prevalence of nematode, Cucullanus sp. larvae, was 4.1%, this is in agreement with that obtained by Eman Youssef and Derwa (2005) (4.8%) and lower than that obtained by Hamida Khanum et al (2011) from Macragnosthus aculeatus (36.05%). This variation may be attributed to the difference of location from which the fishes were collected and the type of searched parasitic nematodes. The highest prevalence was in summer (9.7%) followed by autumn (4.2%) and with no infestation in winter and spring, these results agree with these of Hamida Khanum et al (2011) but disagree with these of Nashwa Mohamed (2010) who found the highest prevalence in autumn then summer with no recorded infestation in other seasons.

On the other hand, the total prevalence of immature cestode larvae was 6.1%, this is nearly similar to that obtained by Hamida Khanum et al (2011) (4.09%) but less than what obtained (49.8%) by Aloo et al (2004). The highest prevalence was in autumn (20.8%) followed by winter (4.3%) which disagree with that obtained by Hamida Khanum et al (2011) who recorded the same prevalence in
summer and autumn seasons with no infestations in spring and winter. Prevalence of crustacean infestations showed positive correlation to fish length up to 20 cm. and to fish weight up to 200 g. then decreased. L. lunaris showed no clear relationship between crustacean infestation and fish lengths and weights, this agreed with Timi and Lanfranchi (2006). Prevalence of copepod increased with host size up to fish with intermediate length then decreased because changes in size of gill filaments affect their attachment and can be easily detached by respiratory currents. These results disagree with that of Aloo et al (2004) who showed positive correlation with increase in length and age.

Regarding the relationship between larval nematodes and cestodes with fish length and weight, there is an increase of prevalence at intermediate lengths and weight only. This may be due to, not only length and weight are determinants in the population parameters of parasites, but also temporal changes in diet and biology of hosts recorded during upwelling and reproduction may influence the degree of infestation of the hosts (Hamida Khanum et al., 2011).

In this study, heavy metals concentrated in L. lunaris organs were more than that detected in seawater. Certain environmental conditions such as salinity, pH and water hardness could play an important factor in heavy metals accumulation in the living organisms up to toxic concentrations and cause ecological damage (Al-Weher, 2008). The order of total abundance of the tested metals was Zn>Pb>Cu>Cd in fish tissues while in water samples, it was Pb>Zn>Cd>Cu. Javid and Usmani (2011) mentioned that Zn concentrations were the highest in all tissues analyzed in Channa punctatus and Clarias gariepinus. Gills followed by liver were the organs of the highest abundance of all tested heavy metals than musculature; this is in agreement with that of (Saeed and Abd El-Mageed, 2008). This may be due to the metallothionein proteins which are synthesized in gills and liver tissues when fishes are exposed to heavy metals and detoxify them. Moreover, seasonal variation showed higher residual values in winter, spring and autumn than in summer in both tissues and water. These results are in agreement with these of Saeed (2007).

Regarding the relationship between heavy metal and abundance of parasites, environmental change due to pollutants can influence parasitic-host interaction (Khan, 2012) as it may kill the parasite but does not kill the host. In other cases, parasitic infection may make the host more susceptible to toxins (Lafferty and Kuris, 1999). In this study, crustacean infestations were found to be negatively related to
heavy metal concentrations in different seasons. This may be attributed to the toxic effect of the heavy metals on the crustaceans which may cut its life cycle (El-Seify et al., 2011). On the other hand, no infestation was detected in liver or musculature with high accumulated heavy metals in different seasons. These results are in agreement with that of Lafferty and Kuris (1999) who recorded that pollutants may kill sensitive free living stages of the parasite or reduced survival of free-living cercariae and miracidia, leading to a lower prevalence of parasitic larvae.

References

EOSQC (Egyptian Organization for Standardization and Quality Control) (1993): Maximum residue limits for heavy metals in food, Ministry of Industry No. 2360: 5, Cairo, Egypt.


Nashwa Mohammed (2010): Studies on diseases caused by


استقصاء عن الأمراض الطفيلية الشائعة في سمكة الفهوة البحرية (لاجوسيفليس ليونارس) وعلاقتها بالتلوث بالمعادن الثقيلة في بحيرة التمساح، منطقة الإسماعيلية.

مأثور محمد منير اللعشي - * هبه إبراهيم عبد المولى
قسم أمراض الأسماك - كلية الطب البيطرى - جامعة قناة السويس
* معهد بحوث صحه الحيوان - فرع الإسماعيلية - مركز البحوث الزراعية

أجريت هذه الدراسة على 294 من أسماك الفهوة البحرية (لاجوسيفليس ليونارس). تم تجميعها عشوائيا من بحيرة التمساح بمحافظة الإسماعيلية. لم تسجل أي علامات مرضية مميزة في الأسماك المصابة. كانت أهم العلامات المرتبطة بالعمر والتاريخية (زيادة إفرازات المخاط، الاحتقان، الظهر الرخامي، شحوب الخياشيم مع تدمير الشعرات الخيشومية) في حالات الإصابة بالفصيات. أما في حالات الإصابة بيرقات الديدان الأسطوانية فكان هناك شحوب وتضخم في الكبد مع نزف، أما الأمعاء فقد كانت متلثة في حالة الإصابة بالديدان الشريطية غير كاملة النضج. تم عزل وتصنيف الثمن من الفصيات الطفيلية من نوع (الكلايسيوس و البومولوكس) ونوع من بيرقات الديدان الأسطوانية (كوكولاتس) بالإضافة إلى نوع من الديدان الشريطية غير كاملة النضج. كان معدل الإصابة الإجمالي (40.8%) وسجل الخريف أعلى نسبة إصابة. سجلت الفصيات الطفيلية أعلى نسبة إصابة (34.6%)، بينما شهدت الديدان الشريطية غير الناضجة (6.1%) ويرقات الديدان الأسطوانية (4.1%).

سجلت أعلى معدلات الإصابة بالفصيات الطفيلية ويرقات الديدان الإسماعيلية في الصيف بينما سجلت أعلى معدلات الإصابة بالديدان الشريطية الغير ناضجة في الخريف. تم تسجيل العلاقات بين الأطوال والأوزان المختلفة للأسماك والإصابة الطفيلية كما تم بحث العلاقة بين التلوث بالمعادن الثقيلة والإصابة الطفيلية.