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Some histopathological alteration of the infected blue crab *Portunus pelagicus* with parasites

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ABSTRACT

The present study was carried out to investigate parasites of one from the most important seafood, the blue crab *Portunus pelagicus*. Samples of crabs were caught seasonally fromRas El Bar, Damietta, Egypt. Results showed that crabs were infested with two different parasites (protozoa and nematode larvae). The identification of protozoa was confirmed by using the electron microscope. The current study revealed that only 30% of *P. pelagicus* were infected with parasites. Protozoan parasites showed a prevalence of 79.63%, while nematode larvae showed a prevalence of 20.37%. Histological examination of infected tissues showed disruption of tissue cells, loss of normal gill structure, interruption of lamellae, presence of several granulomas. Also, gonad follicles showed degeneration in the infected crabs. The histopathological effects of such parasites could induce host injury especially in case of heavy infection. However, the highest infection of parasites was recorded in the crabs with a medium size (carapace width 10.1-15 cm & length 6.1-9 cm). whenever the least infection was recorded in the heaviest crabs. Alternatively, the present study may lead to the conclusion that heavy crabs are believed to be healthier for the consumers as it was less susceptible to parasite infection.

INTRODUCTION

The blue crabs are valuable sea food of great demand, both in domestic markets and in the export industry. They are intertidal species with low migration that can be found throughout the year **(Robert *et al.*, 2014 ; Zairon *et al.*, 2015)**. *P. pelagicus* inhabits the Middle eastern coast of Mediterranean sea, Red sea and Suez Canal **(Mehanna, 2005 & Mehanna and El-Aiatt, 2011)**. It lives in sandy mud habitat until shallow water down to 50 m **(Fazrul *et al.*, 2015)**. **Vogan *et al.* (2001)** stated that crabs have been known to have parasites that caused histopathological alterations to their organs and tissues. Histology is the standard method for the examination of crab tissues to identify the presence of parasites and their related pathologies **(Bojko *et al.*, 2013)**.

P. *pelagicus* serves as a host for a variety of pathogens **(Shields and Overstreet, 2007)**. For example, the parasitic dinoflagellate *Hematodinium perezi* caused morbidity and mortality of the infected crabs after destroying hemocytes and hemocyanin **(Lee and Frischer, 2004)**. *H. perezi* also infected another crabs causing disease or high mortality of them **(Wheeler *et al.*, 2007; Small *et al.*, 2019)**. Parasitic disease may have a profound effect on the crab industry, including the unmarketability of infected legal-sized crabs, and the mortality imposed on pre-recruits to the fishery **(Shields *et al.*, 2005)**. Also, the rapid proliferation of the parasite and its high metabolic requirements during growth, decline protein and carbohydrate reserves of the crab host leading to host morbidity and eventually mortality **(Stentiford and Shields, 2005)**. **Shields and Overstreet (2003)** stated that protozoa, helminths and other disease causing agents might cause little or large pathological alteration in the infected crabs. The pathology includes occlusion of hemal spaces by the parasite, effects on respiratory function and gill structure and damage to muscle fibers **(Sheppard *et al*., 2003)**. **Rogers *et al.* (2015)** examined gill samples for detection of any abnormalities, such as muscle discoloration or lysis by *Lagenophrys callinectes*.

Nematodes were also isolated from the blue crab *Callinectes amnicola* (**Ekanem *et al.*, 2013)**. **Al-Behbehani (2007)** investigated the presence of nematode larvae in both sex of the blue crab *P*. *pelagicus*. Parasitic infections of crabs reduce their abundance and nutritional value. These infections, especially high parasitic infections, cause loss of colour, the appearance of dots, making the crabs unattractive and may lead to not being marketed. Parasitic infection of crabs also causes destruction of reproductive organs, deformation of nervous system and increased juvenile mortality. An increase in crab size in invasive populations was linked with the loss or reduction of parasite richness and prevalence **(Grosholz and Ruiz, 2003)**, suggesting that parasites had a significant impact on host fitness in the native range **(Torchin *et al.*, 2001)**. Support for this hypothesis was found for parasitic castrators like *Sacculina carcini*, which reduced spermatogenesis and inhibited moulting and therefore growth **(Zetlmeisl *et al.*, 2010)**, resulting in smaller crab mean size and biomass in populations with high prevalences of this parasite **(Torchin *et al.*, 2001)**. However, the presence of parasites affecting populations and ecosystems resulting in the global-scale declines of a wide range of marine species **(Lips *et al.*, 2006)**. The objectives of this study are to identify parasites in the blue crab *P. pelagicus* and describe the pathological effects on its tissues.

MATERIALS AND METHODS

P. *pelagicus* were seasonally collected alive from the shores of Ras Al Bar, Egypt. Samples were immediately transported to the laboratory for identification and examination. In the laboratory, the carapace of crabs was carefully removed and the gill filaments and gonads were removed and examined individually under microscope. Other gill tissue and gonads were preserved in Bouin fixative. Dehydration was done in ascending series of alcohol followed by clearing in xylene. Finally, the tissue is embeded in paraffin wax then cut into sections (5 μm). Slides were stained with haematoxylin and eosin (H&E). Stained sections were examined under the microscope. Photographs were taken by using a digital camera (AMCAM camera) attached to the microscope. Diagnoses of diseases and identification of parasites were based on electric microscopy and literature descriptions.

Another, specimens were prepared for scanning electron microscope (SEM). Small pieces of infected tissue of *P. pelagicus* were fixed in glutaraldehyde followed by dehydration, Critical point drying , Drying with hexamethyl disilazane (HMDS) and t- Butanol, Coating with Gold/Palladium using Sputter coater. Image processing (Software Scandium) in electron microscope unit in faculty of science in Alexandria University.

RESULTS

The present study revealed that 30% of the examined crabs were infected with parasites. The parasitic species detected were protozoa and nematod larvae. Protozoan parasites were indicated by the round and oval shape, tough hyaline cyst wall and the presence of one or more nuclei **(plate 1)**. The presence of protozoa species was confirmed by scanning electron microscope (SEM) as shown in **plate (2)**.

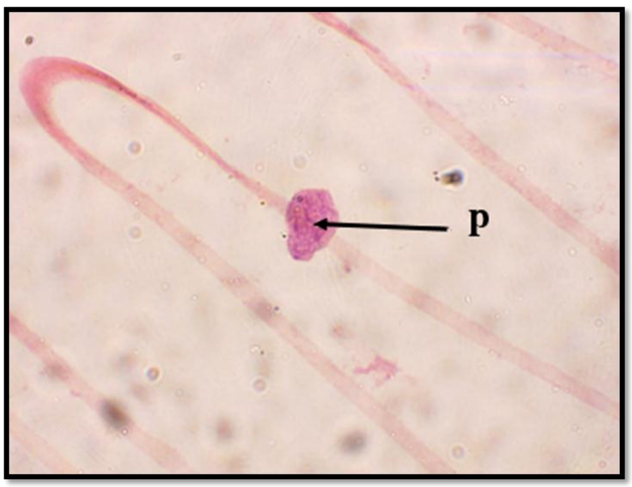

Plate 1. Photomicrographs of infected gills of *P. pelagicus* with protozoan parasites (p). (H&E, x400)

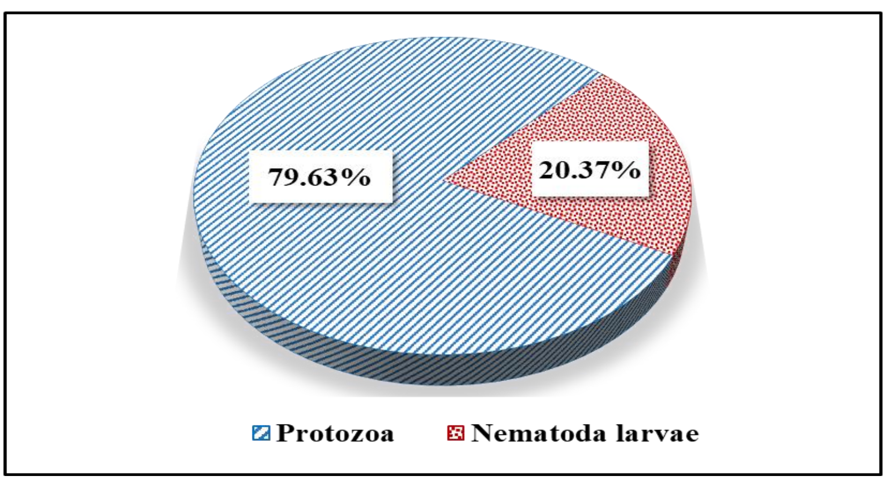

Figure 1. Prevalence of parasites in the infected blue crab *P. pelagicus*.

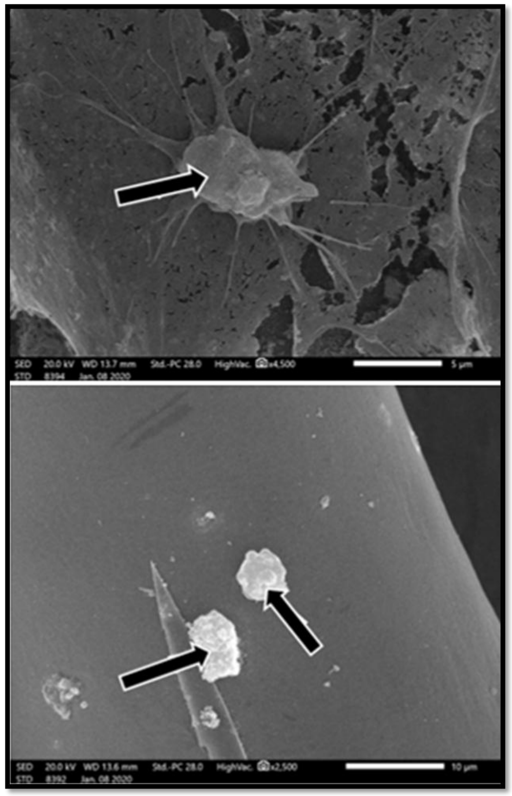

Plate 2. Scanning electron micrographs showing protozoan parasites infested gill filaments of *P. pelagicus*.

Nematode larvae were characterized by the presence of esophagus which joined to a terminal esophageal bulb by a narrow isthmus **(Plate 3)**. However, the prevalence of protozoa was higher than nematode larvae. Protozoa showed prevalence of 79.63%, while it was 20.37% for nematoda larvae **(Fig. 1)**.

Plate 3. Smear from gill region of the infected blue crab *P. pelagicus* showing nematode larva. (x400).

Abundance of parasites with respect to carapace length was shown in **Fig. (2)**. The highest infection (47 individual) was detected in the moderate size (6.1-9 cm). The number of parasites declined to 7 individual in the smallest crabs. On the other hand, parasites totally disappeared in the largest crabs.

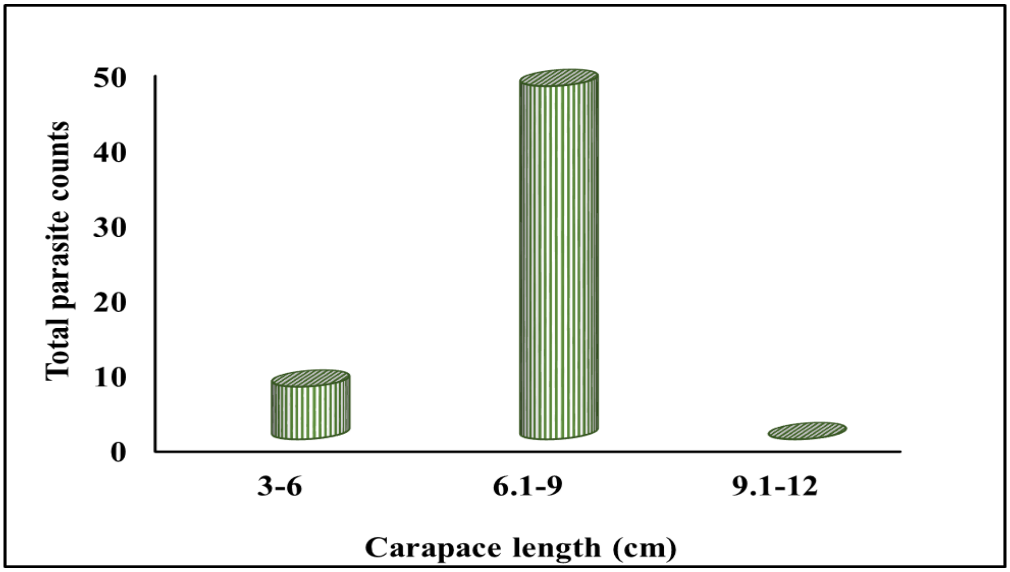

Figure 2. Abundance of parasites in relation to the carapace length.

Abundance of parasites in relation to the crab size was shown in **fig. (3)**. The highest infection (33 individual) was detected in crabs with carapace width ranged from 10.1 to 15 cm. Number of parasites declined in the smallest and larger crabs. It was 7 and 14 individuals in width classes 5.1-10 and 15.1-20 respectively.

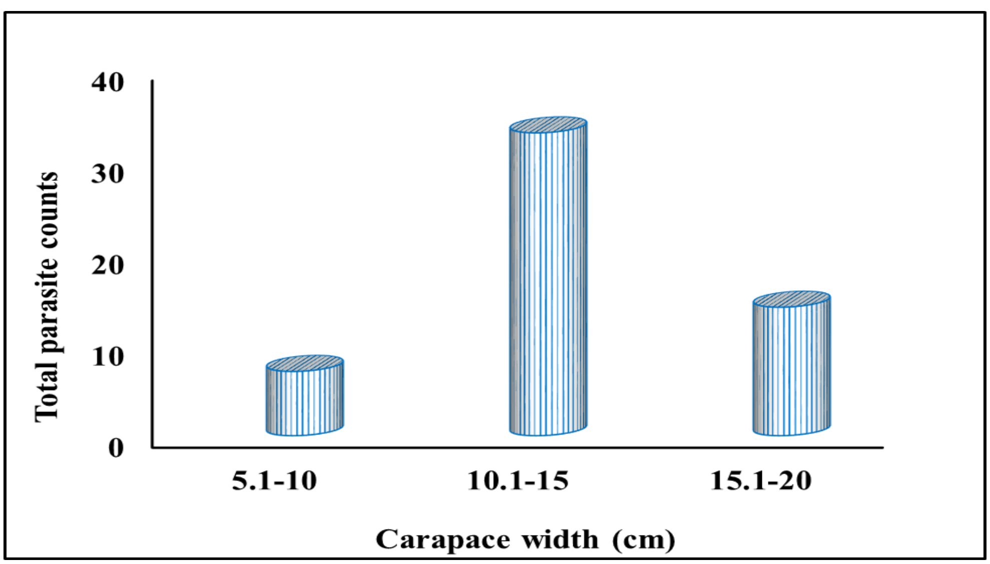

Figure 3. Abundance of parasites in relation to the carapace width.

Abundance of parasites in relation to gill weight was represented in **fig. (4)**. It was noticed that the number of parasites increased with the increasing in gill weight. Where, the highest infection (29 individual) was detected in the largest class weight. Then, it declined in the moderate and smallest weights which were nearly equal (it was 12 and 13 individuals respectively). Alternatively, there was no significant effect of parasites on the gill weight (p = 0.6).

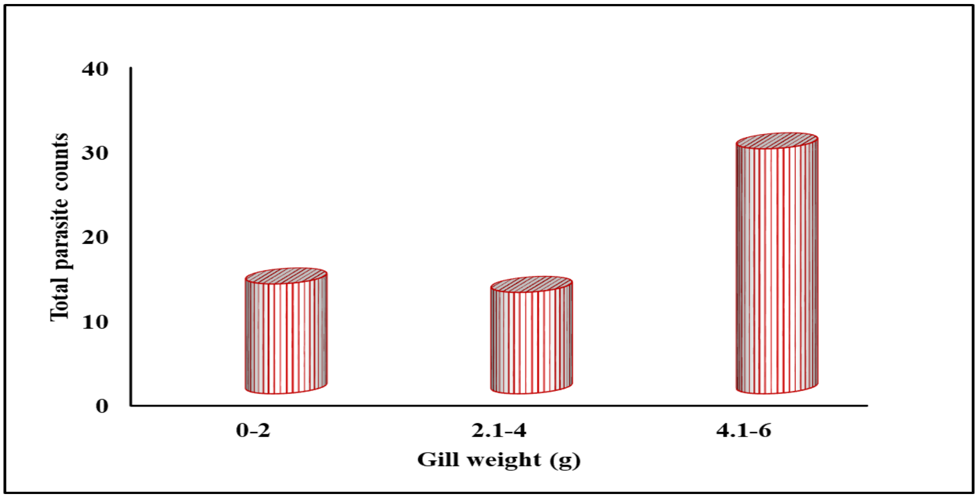

Figure 4. Abundance of parasites in relation to the crab gill weight.

Abundance of parasites in relation to gonad weight was shown in **fig. (5)**. The highest infection (29 individuals) was detected in the largest class weight. Similarly, the smallest crabs was infected by parasites (25 individuals) nearly close in number to that of the the largest crabs. Meanwhile, no parasites were detected in the moderate weight. Alternatively, it was shown that there was no significant effect of parasites on the gonad weight (p = 0.9).

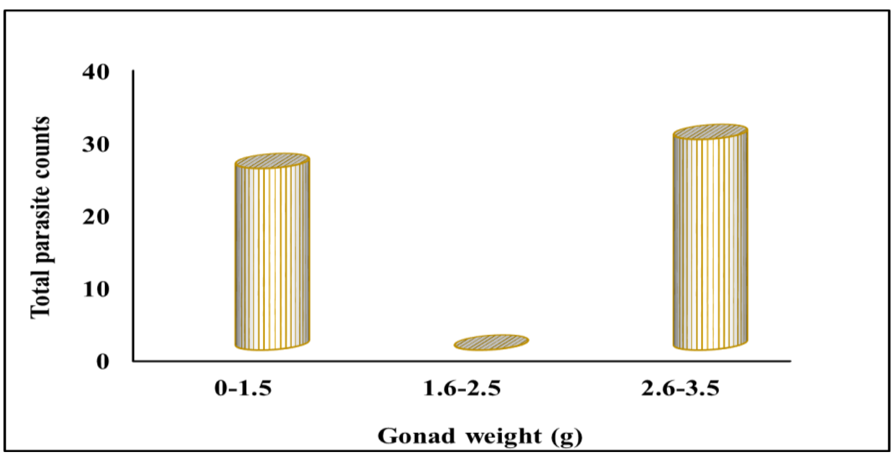

Figure 5. Abundance of parasites in relation to the crab gonad weight.

Abundance of parasites in relation to the total weight of crab was shown in **Fig. (6)**. The highest infection (23 individual) with parasites was in the smallest crabs. Then, it declined in the moderate and largest weights (19 and 12 individuals respectively). Alternatively, there was no significant effect of parasites on the crab weight (p = 0.1).

Histopathological changes in tissue of the infected crabs in the present work were shown in **plates (5&6)**. Gill and gonad showed alteration in their tissue structure as a result of the parasitic infection. Gill tissue of an uninfected crab exhibited the normal gill structure and lamellae arrangement as shown in **plate (5a)**. Whereas infected gill tissue showed some changes as shown in **plate (5 b&c)**. It elucidates curving of lamellae, presence of vacuolation and degeneration of pillar cells.

Normal gonad structure was shown in **plate (6a)**. Alterations in gonad tissues were shown in plate **(6b)**. The presence of parasitic infection in gonad tissue was associated with degeneation in gonad tissue.

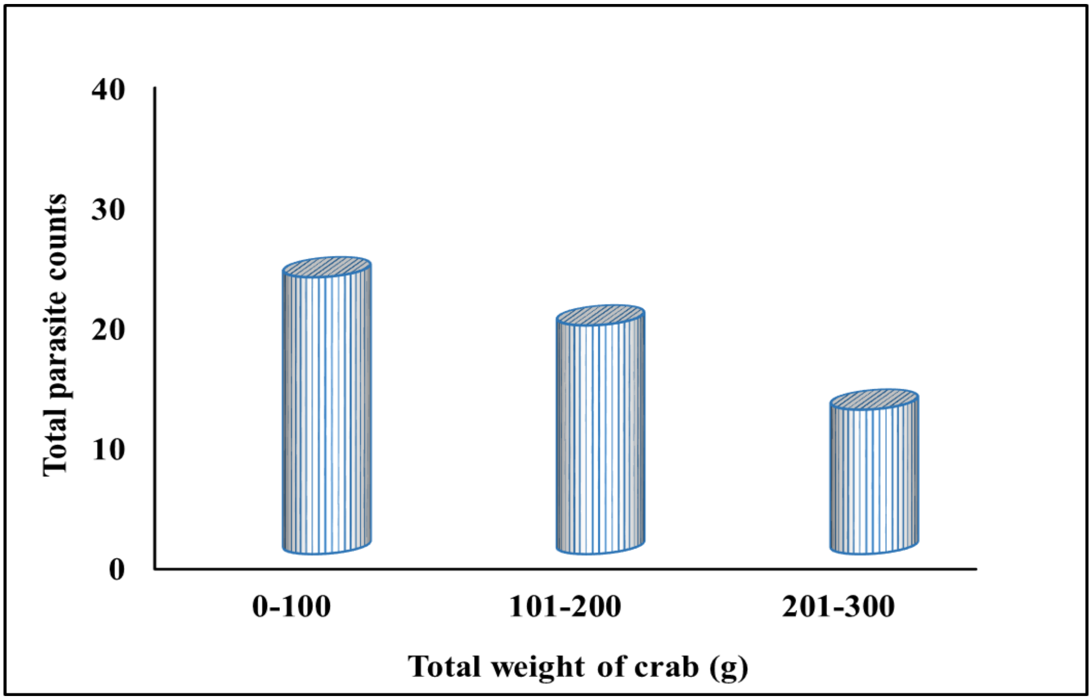

Figure 6. Abundance of parasites in relation to the total weight of crab.

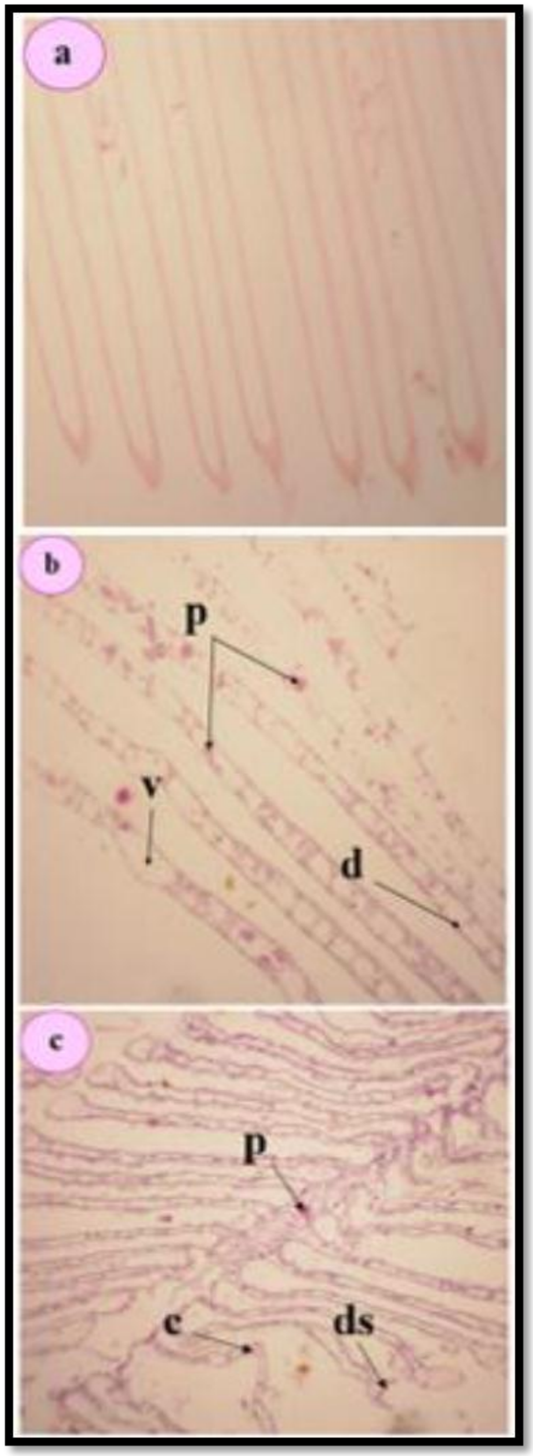

Plate 5. Photomicrographs of the gills of *P.pelagicus* stained with H&E (x100). **a)** normal gill tissue, **b&c)** infected gill tissues. *Abbreviations*: **(p)** protozoan parasite, **(v)** vacuolation, **(c)** irregular failure of lamellae, **(d)** degeneration of pillar cells & **(ds)** degeneration of secondary lamella.

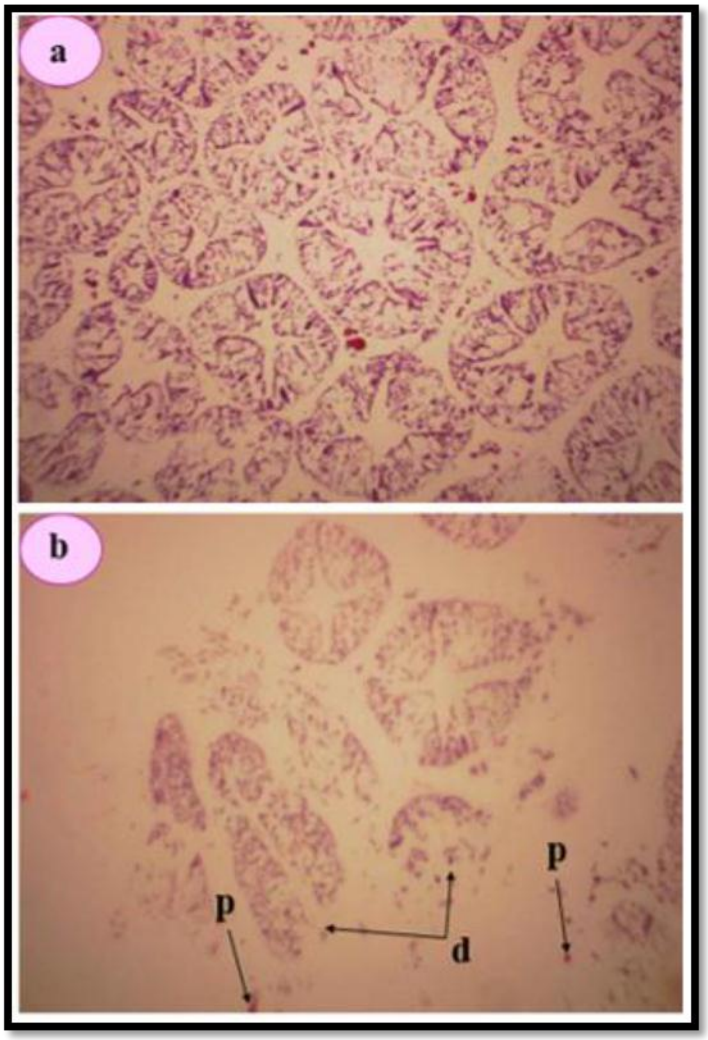

Plate 6. Photomicrographs of the gonads of *P.pelagicus* stained with H&E (x100). **a)** normal gonad follicles, **b)** infected gonad follicles. *Abbreviations*: **(p)** protozoan parasite & **(d)** degeneration of gonad tissues.

DISCUSSION

The blue swimming crab *Portunus pelagicus* represents a valuable component of coastal fisheries in Egypt **(Abdel Razek *et al.*, 2006)**. However, crabs considered as an intermediate host to a lot of parasites **(Alsaqabi *et al.*, 2010)**. It hosts a variety of protozoa and nematodes **(Poulter *et al.*, 2017)**. The current work showed the overall prevalence of parasites in crabs was 30%. Two parasitic species (protozoa and nematode larvae) were detected to infect *P. pelagicus*. The prevalence of protozoan parasites was 79.63%, while it was 20.37% for nematod larvae. A similar study on *Callinectes sapidus* found that more than 70% of the crabs were infected by protozoan **(Pagliara and Mancinelli, 2018)**. Some parasitic protozoa (*Hematodinium perezi*) was detected to be highly pathogenic in its blue crab host, with mortality rates up to 87% in naturally and experimentally infected crabs

(Messick and Shields, 2000 ; Shields and Squyars, 2000). In contrast to our results, low infection frequencies (7.1%) were reported for protozoan parasites in *Carcinus maenas* as mentioned by **Stentiford (2004)**.

The current study revealed that the highest infection of crabs was in the medium size (6.1-9 cm for carapace length & 10.1-15 cm for carapace width). A dissimilar result was reported by **Ekanem *et al.* (2013)**. They found that prevalence of the parasites was high in the smallest width class (5-9.9 cm), followed by the medium size (10-14.9 cm) while there was no record of parasites in the largest crabs (15-19.9 cm width class). We can attribute it to the difference in the crab species as well as to the difference in lattitude. On the other hand, the present study elucidated that there was not significant effect of parasite on the crab weight ($p = 0.1$).

Parasites in crabs have a great concern since they reduced reproductive success and survival rates. Alternatively, it affected on the structure and function of ecological communities **(Hatcher *et al*., 2012)**. Possible explanations for high mortality rates of blue crabs include diseases and parasites. **Wheeler *et al.* (2007)** stated that pressure necrosis and erosion of soft tissue layers are hallmarks of late stages of infection with *Hematodinium* sp. which caused significant losses in crustaceans of economic significance **(Shields, 2011)**. **Morado (2011)** found that genus *Hematodinium* caused Bitter crab disease (Pink crab disease) to large number of crab species. He added that *Paramoeba perniciosa* was also reported as a pathogen of blue crabs and a few other crustaceans. **Shields and Overstreet (2003)** mentioned that *Paramoeba perniciosa* can cause very high mortality of crabs such as blue crabs and gray crabs by destroying their connective tissue. In heavy infections, pathological changes caused by large numbers of amoebae include: tissue displacement, probable lysis of some types of tissue including haemocytes **(Anthony *et al,*. 2014)**. The present work showed a variety of histopathological alternations in the infected tissues. The infected gills loss their normal structure by interruption of filaments, presence of several granulomas and proliferation of filaments. This may affect the crabs by decreasing the surface of gill chamber and consequent low oxygen consumption. **Shields *et al.* (2003)** stated that infected blue crabs die due to malfunction of the hepatopancreas, degradation of the muscle, and loss of respiratory function. **Shields and Squyars (2000) & Wheeler *et. al*. (2007)** found large numbers of *Hematodinium* sp. congregated along the distal margins of gill lamellae causing loss of internal structural support and distention in the distal region of individual lamellae. **Wheeler *et al*., (2007)** stated that heavy infection of *Hematodinium* sp. caused lysis of the thin cuticle layer of the gill leading to an unusual fusion of adjacent lamellae, which may reflect continued suppurations of the cuticle and underlying tissues. These severe changes in the gill structure may have a direct effect on the respiratory function of diseased snow crabs.

The present study reported some alterations in gonads of the infected crabs. Hoever, it did not affect the gonad weight ($p = 0.9$). An opposite result was recorded by **Zetlmeisl *et al.* (2010)** who found that *Sacculina* sp. declined the gonad size and altered fecundity in their crab hosts.

Generally, parasites increase the susceptibility of crabs to diseases. So, the parasitic infestation had some negative effect on commercially important crabs leading to not be marketed. Morever, eating unproper cooked crabs may cause human infection with these parasites.

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