

## Biometric and histological studies of the shellfish *Paphia undulata* infested with parasites

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### ABSTRACT

The present study reported parasites and its histopathological effects on the tissue of the popular seafood clam *Paphia undulata*. Some parasites were found to have several effects on shellfish aquaculture production. Samples of clams were collected from Damietta shore, Egypt. Histopathological examination was conducted on the gills, gonads, and intestine of clams. Two types of parasites were detected in the infected clams (protozoans and nematodes). The identification of Protozoa was confirmed by using the electron microscope. This study revealed that 51% of *P. undulata* were infected with parasites. The highest infection was noticed in the clam size ranged from 2.1 to 4 cm. This infection declined with the larger clams. The current study proved that there was a negative association between the prevalence of parasites and clam size. Histopathological effects of parasites on the clam gills, gonads, and intestine were detected. Parasites not only affect clams but may also be transmitted to consumers after eating undercooked shellfish. So, this study warns against eating raw seafood as it is considered a vehicle for the transmission of human parasites.

### INTRODUCTION

Worldwide, people obtain great part of their animal protein from shellfish. Bivalves are key components of marine environments because, as filter feeders, they play a critical role in maintaining water quality and ecosystem integrity (Lee *et. al.*, 2001). Bivalves are abundant resources for the shore inhabitants (Dumbauld *et. al.*, 2009). The clam *Paphia undulata* of the family Veneridae is among the most popular eatable clam and is consumed locally in Egypt. It was noticed that clam population declined across the Egyptian fisheries in the past decade. This is mostly attributed to overexploitation, pollution and parasites (El-Gamal, 2010). Parasites infect many species of marine bivalves throughout the world (Hine and Diggles 2002). Mass mortality of bivalves was recorded to be associated with presence of parasites (Lee *et. al.*, 2001). Studies on

parasites and diseases of bivalves are important since they provide the essential knowledge for maintenance of natural resources. As reviewed by **Boehs *et. al.* (2012)**, the main records of pathogens in the bivalves included protozoa and some metazoa. Clams feed on suspended phytoplankton by trapping it in the gills. Simultaneously, the gills also filter pathogenic microorganisms from the water, concentrating them in the digestive system (**Robertson, 2007**). Some parasites cause a reactive hyperplasia of the epithelium and increased mucus production on aquatic animals. Hyperplasia appears as a cloudiness to the skin and leads to hypoxia if occur on the gills (**Purivirojkul, 2012**). Protozoan parasites within the genera *Haplosporidium* and *Perkinsus* severely affect mollusc species around the world. *Haplosporidium nelsoni* was associated with mass mortalities of oysters in the USA (**Messerman *et. al.*, 2012**). Protozoan parasites of the genus *Perkinsus* are known to infect many species of marine molluscs including oysters, abalones, clams, scallops, cockles, and mussels. *Perkinsus olseni* infected populations of *Pitar rostratus* (**Cremonete *et. al.*, 2005**). *Perkinsus marinus* and *Perkinsus olseni* cause “Dermo” disease (**Bushek *et. al.*, 2002**). On the other hand, species of *Bonamia*, haplosporidian protozoan are parasites which infect haemocytes of several bivalve species as stated by **Carnegie and Cochenneec-Laureau (2004)**. Some parasitic *Amoebae* can infect the external surface, such as gill and skin, and internal organs such as intestine. *Paramoeba pemaquidensis* caused chronic mortality (**Purivirojkul, 2012**). **Young *et. al.* (2007)** and **Bustos *et. al.* (2011)** considered *Neoparamoeba perurans* as agent of gill disease. Meanwhile, *Toxoplasma gondii* and *Cryptosporidium parvum* are widely distributed pathogens of humans and animals that may retain their infectivity in raw or undercooked mollusks (**Hohweyer *et. al.*, 2013**). **Lindsay *et. al.*, (2004)** stated that infected oysters *Crassostrea virginica* may serve as a source of *T. gondii* for marine mammals and humans, since oysters can readily acquire *T. gondii* oocysts from seawater. Oocyst stage of *C. parvum* was also detected in many oysters, mussels and clams (**Robertson, 2007**). Hepatopancreatic tubules of the heavily infected shrimp by microsporidian infection were dilated and necrotic (**Prasertsri *et. al.*, 2009**). Few species of nematodes could be found as larval stages in bivalve tissues. However, the presence of protozoan and metazoan pathogens are capable of producing significant production loses. The aim of the current study is to: 1) investigate the parasites in *P. undulata*, 2) describe the possible pathogenic effect of these parasites on the clam tissue.

## MATERIALS AND METHODS

Clam specimens were sampled from Izbet EL-Borg at the connection between the River Nile and the Mediterranean Sea in Damietta governorate, Egypt. Then samples were immediately transported to the laboratory for examination. Clam soft tissue was removed from the shell, transverse section was cut in the middle of the body to allow fixative to penetrate tissues and tissue was then preserved in fixative solution for 24 h. It dehydrated with graded ethanol, cleared with xylene, embedded in paraffin wax and sliced to 5 µm transverse sections. Sections were stained with Hematoxylin–eosin and examined under light microscope (XSZ-107BN). Photos were taken using microscope equipped digital camera (AMCAM camera).

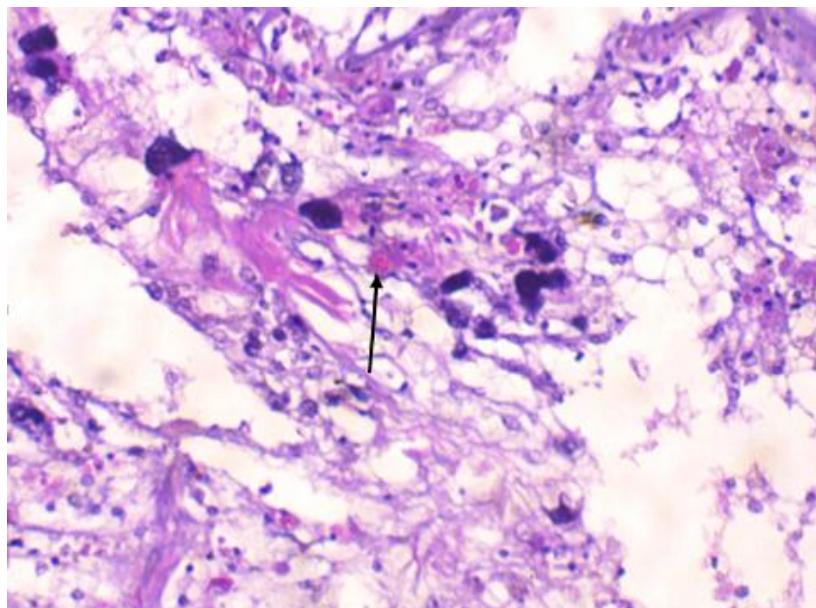
Specimens were prepared for scanning electron microscope (SEM). Small pieces of infected tissue of *P. undulata* were fixed in glutaraldehyde followed by dehydration.

Critical point drying, Drying with hexamethyl disilazane (HMDS) and tert-Butanol, coating with Gold/Palladium using Sputter coater and Image processing (Software Scandium).

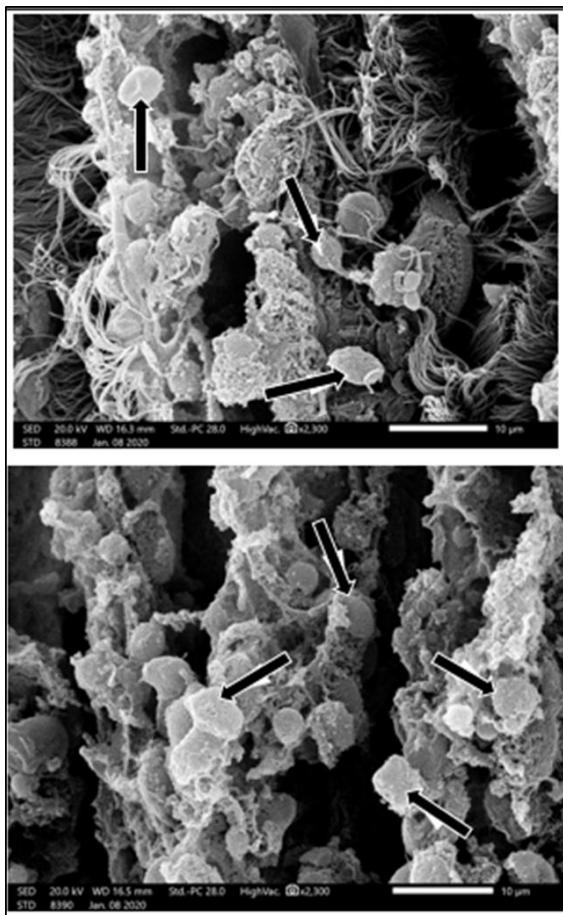
## RESULTS

The investigated clams were in the range 3.2 – 6.2 cm. The present study showed that the ratio between the infected and non infected clams was equal (1:0.96). Infection of clams was noticed by two types of parasites (protozoan and nematode parasites). Protozoa were inferred by the presence of single-celled eukaryotes, with an external plasma membrane which encloses the cytoplasm and one or more nuclei (**Fig.1**). Locomotors organelles like pseudopodia were present. Pseudopodia appear as temporary extensions of the cytoplasm which elongate the plasma membrane. Our result was confirmed by scanning electron microscope (SEM) that appeared in **Fig. (2)**

**Fig. (3)** exhibited egg of nematode that may be identified as *Trichuris trichiura* which characterized by the barrel-shaped lemon eggs. In addition to the mucus that protruded as bipolar plugs.



**Figure 1.** A photomicrograph of *P. undulata* infected by protozoan parasites. (H&E x100)

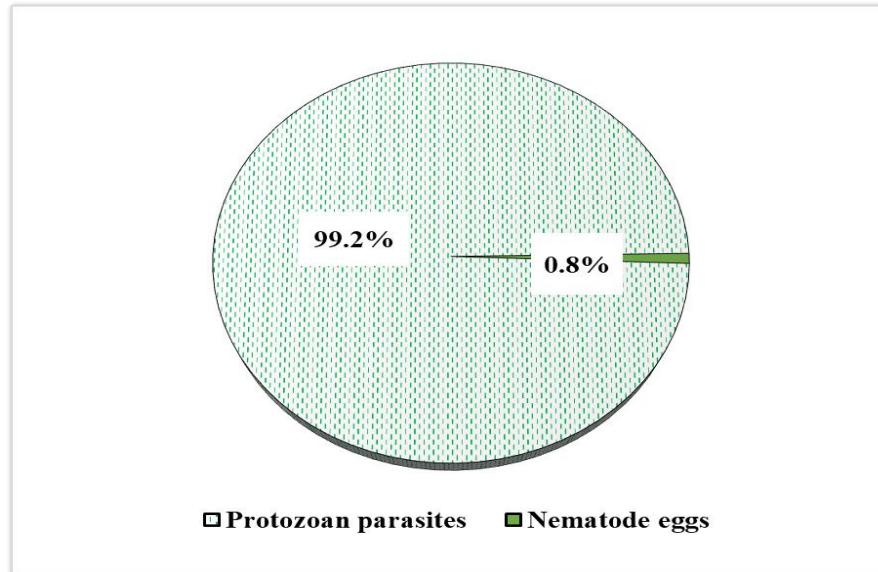


**Figure 2.** Scanning electron micrographs showing protozoan parasites infested *P. undulata*.



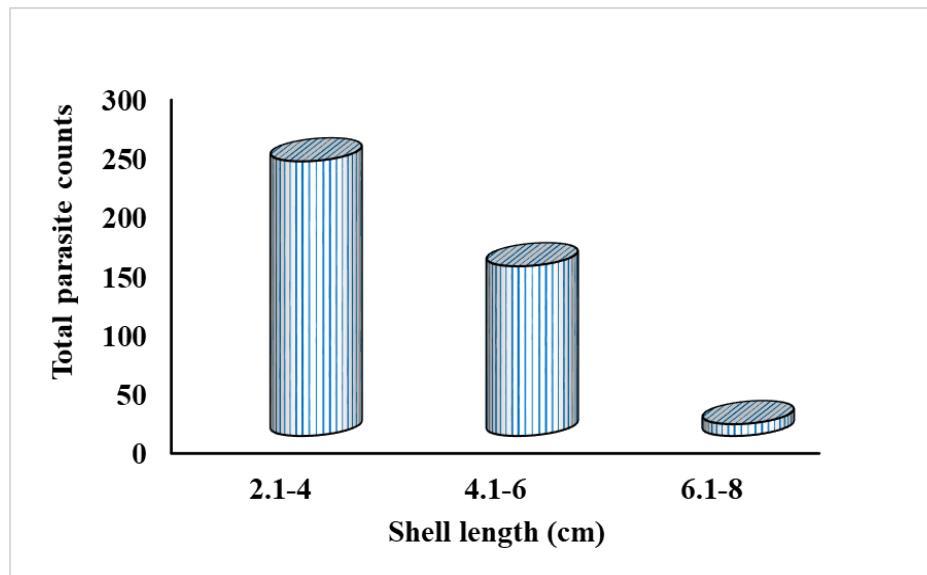
**Figure 3.** Smear from intestine of the infected bivalve showing nematode egg. (x100)

**Fig. (4).** showing the prevalence of parasites in the infected clam *P. undulata*. Protozoan parasites represente 99.2% ,while nematode eggs was only 0.8%.



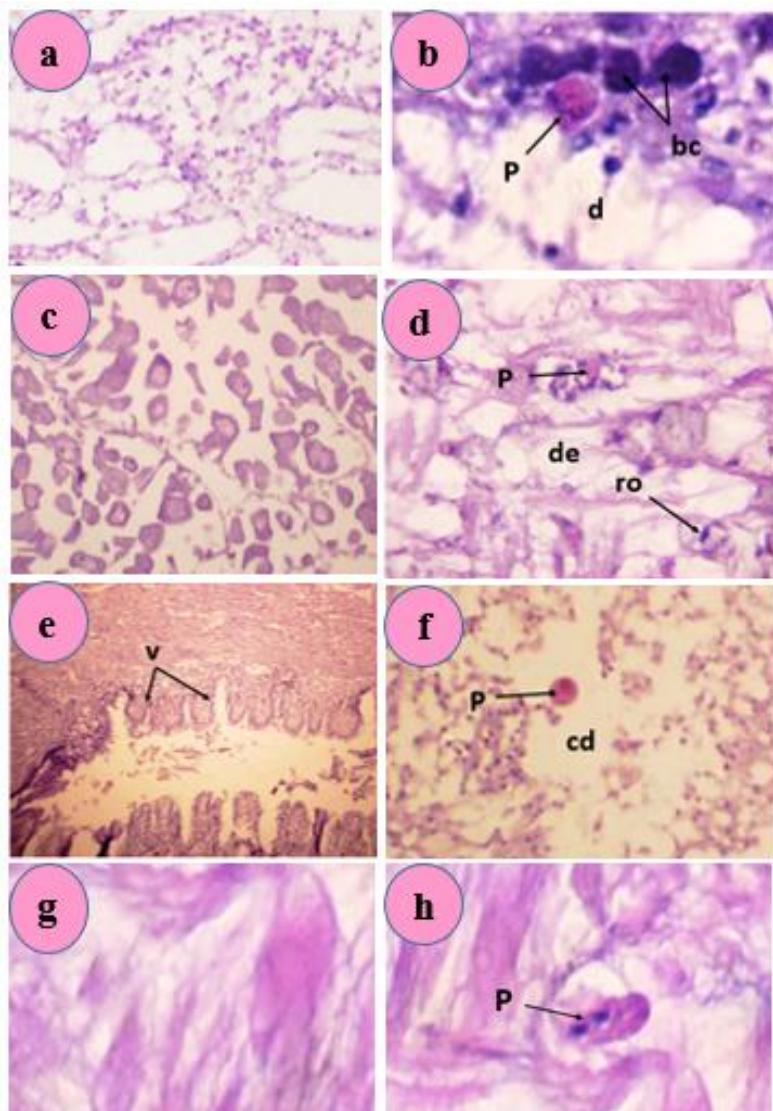
**Figure 4.** Prevalence of parasites in the infected clam *P. undulata*.

Abundance of parasites in relation to shell length is presented in **Fig. (5)**. The highest infection was in the smallest length (233 individual). Then the number of parasites declined in larger sizes ( it was 144 and 10 individual in length classes 4.1-6 and 6.1-8 respectively).



**Figure 5.** Abundance of parasites in relation to the bivalve shell length.

Histopathological alterations in tissue of the infected clams was shown in **Fig. (6)**. Gill, gonad and intestine showed changes in their tissue structure due to the infection by parasites. Gill tissue of an uninfected clam exhibited the normal organization of epithelial and connective tissues as shown in **Fig. (6a)**. Whereas infected gill tissue showed degeneration in endothelial cells. Furthermore, bacterial colonies appeared around the parasite (**Fig. 6b**).



**Figure 6.** Photomicrographs of normal (left) and infected (right) tissues of *P. undulata* stained with H&E stain (100 x). **a&b:** gill, **c&d:** gonad, **e&f:** the intestine **g&h:** connected tissue surrounding the intestine. Abbreviations: (p) protozoan parasites, (d) degenerated gill tissue, (bc) bacterial colonies around the parasite, (ro) residual ova, (v) intestinal villi, (cd) complete degeneration of intestinal cells around protozoan parasites.

The normal structure of gonad tissue with a few ova was shown in **Fig. (6c)**. The presence of protozoa in gonads was associated with gonad follicle destruction (**Fig. 6d**). Interfollicular space become very noticeable. Few residual ova in some follicles undergoing degeneration which was easily detected by the loss of its round shape. The normal structure of intestinal tissue was shown in **Fig. 6e**. Damage in intestinal wall and lysis of the infected cells were also appeared in the intestinal tissue (**Fig. 6f**). The normal structure of the connective tissue surrounding the intestine was shown in **Fig. 6g**. Degeneration in the connective tissue around protozoan parasites of the infected clam were appeared in **Fig. 6h**.

## DISCUSSION

Marine bivalves are favorite meal for most people, especially Egyptians. The Mediterranean Sea fishes and sea food especially in coastal waters of Egypt are considered as highly valued fish food. Edible bivalve species, such as *Paphia undulata*, in addition to other veneroid clams are most common to the consumers in Egypt (**Mohammad et. al. 2014**). However, it act as first intermediate hosts for many parasites (**Cremonte et. al., 2015**). The occurrence of parasites was mainly observed in the connective tissue surrounding the intestine, as well as the mantle and gill as stated by **Francisco et. al. (2010)**. The present study concerned with the detection of parasites that infected one of the most economically bivalve, *P. undulata*. Our study showed that the overall prevalence of parasites was 51%. This ratio was lower than detected by **Francisco et. al. (2010)** in another bivalve. They found that the prevalence of parasites was 70% in *Mytilus galloprovincialis*. The present work also elucidated that the prevalence of protozoan parasites was (99.2%), while it was 0.8% only for nematode. A similar high value of protozoa parasites prevalence was reported by **Vázquez and Cremonte (2017)**. They stated that *Trichodina* -like ciliates infected *Aequipecten tehuelchus* with the prevalence of 98%. A previous study of **Vázquez et. al. (2015)** assured that the prevalence of ciliate parasites was 79% in gills of the clam *Panopea abbreviata*. The current study registered that the highest infection by parasites was in the clam with the shell size of 2.1-4 cm. Then prevalence of parasites was negatively associated with the shell length. We can suggest that the negative relationship may be due to the low immunity of the small clam size. In contrast with our results, **Vázquez (2012)** stated that bigger clams were more frequently infected. The difference in the results between the two studies may be attributed to the difference in bivalve species. So, it can be concluded that the negative or positive relationship between number of parasites infected the clam and their shell length was largely depended on the genera or species. Cellular death due to necrosis result in the loss of cell membrane integrity and an uncontrolled release of products of cell death into the extracellular space (**Proskuryakov et. al., 2003**). Most of the bivalve species feed on suspended phytoplankton via a pair of specialized gills. Large volumes of water are pumped across the gills by ciliary action. Trapped food particles are transported toward the mouth by specialized cilia. *Trichodina* -like ciliates infected *Amarilladesma mactroides* and *Aequipecten tehuelchus* causing a distortion of the natural architecture of the gill filaments as stated by **Vázquez and Cremonte (2017)**. They added that mass mortalities were associated to severe infection as the case of

cockles and oysters. Several studies also reported that heavy infection of *Perkinsus* spp. in clams often results in milky-white pustule formation in clam mantle and gill tissues as a consequence of inflammation (**Choi et. al., 2002**). The histopathology of gills in the present work reported the degeneration in endothelial cells of gills. Additionally, the current study revealed the presence of bacterial colonies appeared around the parasite in gill tissues. These histopathological changes might lead to reduction or a complete loss of function as the gills lost its normal structure. Histological sections of the infected mussels with *Prosthorhynchus crucibulum* revealed a marked reduction in the reproductive tissue. This promotes a severe damage to its reproductive capacity (**Francisco et. al., 2010**). This result was similar to the result in the present study which recorded destruction of gonad follicle and degeneration of the ova in some follicles. The current work exhibited lysis of the intestinal cells and degeneration of the connective tissue surrounding it. In some cases, parasites can destroy host tissue (**Purivirojkul, 2012**). Trophozoites of *Perkinsus olseni* evoking a severe haemocytic infiltration into the surrounding tissue (**Vázquez and Cremonte, 2017**). Most of the microscopic signs of *Bonamia* spp. infection are the haemocytic infiltration in all the connective tissue (**Kroeck and Montes 2005**).

Understanding the physiology of the bivalves helps clarify why people become infected after eating raw shellfish. The shellfish are common in areas where nutrient levels are high and waters are sheltered. Unfortunately, such conditions prevail in shallow waters, which are frequently contaminated with human sewage. When pathogenic microorganisms contaminate the harvesting sites, they are filtered by the gills and are transported to the mouth and intestine. So, we can suggest that this is the reason why we found the egg of *T. trichiura* in the intestine and gill of the clam. Its low prevalence may be interpreted as few species of nematodes could infect the bivalves. This was previously assured by **Vazquez and Cremonte (2017)** who mentioned that nematodes frequently appear in low prevalence and intensities of infection. The data collected in the present study showed that the study site is considered moderately infested with parasites. So, eating unproper cooked infected bivalve may cause a zoonotic disease. If these pathogens are not cleared or inactivated by the bivalves, their consumption might pose a public health concern and impact the costal economies in the event of temporary or long-term closure of the affected areas.

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