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Potential Impacts of *Hematodinium perezi* on the Male Reproductive System of *Portunus segnis* from the Great Bitter Lake, Egypt

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Abstract. From October 2020 to September 2021, researchers examined various facets of the reproductive biology of male *Portunus segnis* crabs caught in the fishing port of the Great Bitter Lake on Egypt's Suez Canal coast. The male reproductive system of *P. segnis* consists of two testes, a commissure, two vas deferentia, and two ejaculatory ducts internally. It is bilaterally symmetrical and creamy to whitish in appearance. The three components of the vas deferentia were the anterior vas deferens (AVD), median vas deferens (MVD) and posterior vas deferens (PVD). There are three stages of male gonad development: Immature, maturing and mature. For light microscopic examinations, tissues from the testis, (AVD), (MVD), (PVD) and ejaculatory duct were fixed in Bouin's solution. The blue swimming crab *P. segnis* is an important host of parasitic dinoflagellates from the genus *Hematodinium*. The commercial supply of *P. segnis* has been harmed by outbreaks of these parasites. *Hematodinium perezi*, a dinoflagellate, had no discernible impact on the gonad growth and development of its host *Portunus segnis*. For uninfected and infected males, the size at first sexual maturity was estimated to be 66.3 and 67.3 mm carapace width (CW), respectively.

Keywords: Testes, Anterior vas deferens, Median vas deferens, Posterior vas deferens, Ejaculatory duct, Size at first sexual maturity.

1. Introduction

Portunus segnis forms the major and important component of commercial fisheries in the Suez Canal region. In the southern section of the Suez Canal, near the eastern and western coasts of the Great Bitter Lake, there is a significant concentration of brachyuran crab fishing (El-Serehy *et al*. 2015). The population structure, abundance, catch statistics, diet, reproductive biology and migration patterns of *Portunus pelagicus* have all been covered in earlier studies (Smith & Sumpton 1987; Campbell & Fielder 1988; Sumpton *et al*. 1989). A number of parasites have the potential to affect crab populations, but few researches have examined the potential effects of parasites on crab reproduction.

Unfortunately, Egyptian waterways do have the bitter crab disease. Rady (2019) made the first discovery of it in the portunid crab *Callinectes sapidus* in the Bardawil Lagoon. How this disease was introduced to Egypt is not known. However the prevalence of *C. sapidus* infection in the lake was alarmingly high, indicating that it would be crucial to investigate other commercial sources of crustaceans. Ibrahim (2022) claims that the bitter crab disease, which affects *P. segnis* by 26.85% in the Great Bitter Lake, is caused by the parasite dinoflagellate *Hematodinium perezi*.

The parasitic dinoflagellates in the genus *Hematodinium* have recently been recognized as pathogens of commercially important crustaceans (Xu *et al*., 2007). In crustacean fisheries and aquaculture systems, these parasite dinoflagellates significantly increase mortality (Wang *et al*. 2017). Although there exists a waterborne infective dinoflagellate stage, the exact mode of disease transmission is unknown (Li *et al*., 2011). A change in the colour of the shell, together with the colour and consistency of hemolymph at late stages of the illness, is an external visual marker of *Hematodinium* dinoflagellate infection in the majority of crustacean species (Stentiford and Shields, 2005). The disease can sometimes be pre-diagnosed using these symptoms, and its prevalence in wild populations can be tracked (Shields *et al*., 2005). The primary indicator of *Hematodinium* infection is the presence of parasite stages in the hemolymph and internal organs of crustaceans (Field and Appleton, 1995).

Aspects of the histopathology of *Hematodinium* infections in diverse crustacean hosts have been covered many studies. The pathology includes occlusion of hemal spaces by the parasite (Field and Appleton, 1995; Stentiford *et al*., 2002; Sheppard *et al*., 2003), effects on respiratory function and gill structure (Taylor *et al*., 1996; Sheppard *et al*., 2003; Rady, 2019; Ibrahim, 2022) and damage to muscle fibers (Hudson and Shields, 1994; Messick, 1994; Rady, 2019; Ibrahim, 2022). How *Hematodinium* infections affect the tissues and reproductive organs of *P. segnis*, as well as how they modify the male gonoduct's histology has not been specifically addressed.

For this species to be managed and exploited wisely, biological data on reproduction must be recognized. There is insufficient research on the potential effects of *Hematodinium* species on blue swimming crab reproduction. Therefore, an effort has been made in the current study to examine the reproductive biology of infected and uninfected male crabs and to reveal the microscopic specifics of the various components of the male reproductive system of *P. segnis*.

2. Materials and Methods

2.1 Study Area

The Great Bitter Lake is one of the Suez Canal Lakes (Fig. 1). It lies at 30° 14`- 30° 25` latitude and at 32° 17`-32° 30` longitude. Its greatest dimensions are 63 km in length, 13 km in width, and 20 m in depth. The Great Bitter Lake's basin was a salt marsh similar to the Sabkha that was flooded by extremely high tides of the Gulf of Suez. The majority of the lake's sides are sandy; however, there is a small rocky beach section nearby. Due to the higher sea level at Suez than at Port Said for the majority of the year, the lake's current most likely travels north; however, from July to October, it travels south because of the higher sea level at the Mediterranean Sea than at the Gulf of Suez (Gab Allah, 2001).

2.2 Collection and Processing of Samples

Portunus segnis male crabs (n = 322) were obtained monthly from October 2020 to September 2021 from the Great Bitter Lake fishing port at the Suez Canal Coast. The crabs were brought to the laboratory using a seawater-filled plastic container. The (CW) and total body weight (BW) were measured for each individual to the nearest 0.1mm and 0.1g, respectively. Infected individuals, based on visual signs of the parasitic infection caused by the dinoflagellate *Hematodinium* sp., were selected. The crabs with macroscopic signs of diseases were examined. Infected (n = 75) and uninfected (n = 247) individuals were then dissected to study the morphology and histology of the reproductive tract, stages of maturation and the size at first sexual maturity. Testes were classified into different maturity stages using the scales suggested by De Lestang *et al*. (2003): immature, maturing and matured. Small pieces of tissues from different regions of the testis, anterior vas deferens (AVD), median vas deferens (MVD), posterior vas deferens (PVD) and ejaculatory duct were processed to thin sections. Crab tissue samples were prepared for wax histology by standard methods, 5µm sections were cut and stained with haematoxylin and eosin (H&E) and observed by an Olympus compound microscope, and photographs were taken using a digital camera (LEICA ICC50 HD) with the aid of a computer program (LAC core system).

3. Results

3.1 Gross Morphology of Male Gonoduct

The male reproductive organ of *P. segnis* is an H-shaped structure, creamy to white in colour and comprises of two separate regions, the testes and relatively shorter vasa deferentia (Fig. 2). The flat and highly coiled testes are located anterior to the hepatopancreas and below the cardiac region. The central regions of the right and left testes are connected by a commissure (Co) forming an H shaped paired testicular complex located just beneath the dorsal carapace. Each posterior testis is connected with a spermatic duct, containing the (AVD), (MVD) and (PVD). Each vas deferens (VD) is located anterior to the muscle of the coxopodite of the last pair of walking legs and each PVD is connected with an ejaculatory duct, which is a smooth narrow duct extending to the base of a swimming leg.

3.2 Developmental Stages of Male Gonads

Immature: The gonads of the immature crabs are small, and creamy in colour, lying on either side of the stomach. Testes and vas deferentia are not clearly differentiated.

Maturing: Testes and vas deferentia are well developed and clearly differentiated, and creamy white in colour. Testes appear as a large coiled tube spreading laterally and posteriorly to the stomach. Anterior vas deferentia become enlarged, while the MVD and PVD are straight and opaque extending to both sides of the heart.

Mature: Testes show further enlargement, as vas deferentia are coiled and very much swollen occupying full body cavity. The AVD and MVD are enlarged and appear in milky white colour. PVD enlarged and convoluted but still opaque. Gonads obtained from males measuring above 11cm CW showed mature stage.

3.3 Histology of Uninfected and Infected Male Reproductive Tract

3.3.1 Testis

The testicular lobes were partially or fully divided into several lobules/acini bordered by a connective tissue trabecula (Tr), which was slightly stained by eosin (Fig. 3). The lobules had either direct access to the seminiferous tubule or were connected indirectly through other lobules. In addition, each lobule contains zones of distinguishing spermatocytes, a germinal zone (Gz), transformation zone (Tz) and an evacuation zone (Ez).The entire testis was covered by a double-layered wall called capsule. The germinal zone was formed by the aggregation of spermatogonial cells at the periphery of the lobules. The spermatogonial cells develope into spermatids and begin to fill the transformation zone (Tz) which connects with the evacuation zone (Ez). The evacuation zones fuse distally to form the VD. The uninfected and infected testes show normal histology with the hemal spaces containing hemocytes (Fig. 3 and 4).

3.3.2 Anterior vas deferens

Arising from the posterior end of the testis, the first part constitutes the efferent duct by which the spermatozoa are evacuated from the testis. The AVD is located median to MVD and antero-ventral to the pericardium. Separation of the coils is most difficult.

Although it is a translucent tube, the AVD appears white due to the accumulation of a mass of spermatozoa which are assembled into spermatophores. The AVD is characterized by a lining of columnar cells, especially at the distal portion. The epithelial lining of the lumen shows inward folding, where the cells became columnar with basophilic nuclei (Fig. 5). Recently formed spermatophores are found along the distal portion of the AVD. Spermatozoids without spermatophores are also present in its lumen (Fig. 6).

3.3.3 Median vas deferens

The MVD, the most massive part of the reproductive system, is a loosely coiled, opaque white tube that consists of major coils in which the tube has a large diameter and minor coils with a lesser diameter. The more prominent major coils are located anterior to the pericardium. There is no precise demarcation of the AVD and MVD. However, the former does not have white viscid and granular contents surrounding its spermatophores. The epithelial lining of the MVD is composed of columnar cells. These cells contain clusters of nuclei in their basal portion (Fig. 7). The lumen is occupied by roughly circular spermatophores bathed in the spermatophoric matrix and is devoid of sperm cells (Fig. 8).

The first substance, termed substance I (SI), is secreted by the epithelium of the proximal portion of the AVD. SI has a homogeneous appearance, which stains red with H & E, and divides the sperm mass into groups of irregular sizes. The second substance, termed substance II (SII), is secreted by the distal portion of the AVD, stains bluish to pale blue with H&E, and surrounds each sperm mass, thereby forming a spermatophore (Sph). The MVD stores large numbers of spermatophores (Sphs) embedded in a heterogeneous matrix (Fig. 7 and 8). This matrix is composed of the epithelial secretions of the MVD, and SI and SII from the AVD.

3.3.4 Posterior vas deferens

Of approximately the same width and diameter as the minor coils of the MVD, the loops of the PVD are easily distinguished by their colourless translucency. The lumen of the duct in sectioned tissue is filled with a light eosinophilic homogeneous mass similar to the secretions produced in the MVD. Sperm cells were absent (Fig. 9 and 10).

3.3.5 Ejaculatory duct and penis

The ejaculatory duct is circular in outline, with a central lumen lined by epithelial layer. The outermost layer is made up of muscle tissue, which is covered by connective tissue. The lumen is filled with eosinophilic matrix and is devoid of sperm cells (Fig. 11 and 12).

3.3.6 Size at first maturation (Lm_{50})

The CW of uninfected male crabs (*n*= 247) ranged from 62.6 to 126.6mm. Of these, 195 (78.95%) were in a sexually mature condition. The CW of infected male crabs (*n*=75) ranged from 64.1 to 128.3mm and 61 (81.33%) of them were mature. Tables 1 and 2 show the proportion of sexually mature males (uninfected and infected) within each 10mm CW size class. The estimated size for 50% sexually mature males in the Great Bitter Lake was 66.3mm CW (Fig. 13), and 67.3mm CW for uninfected and infected male crabs (Fig. 14).

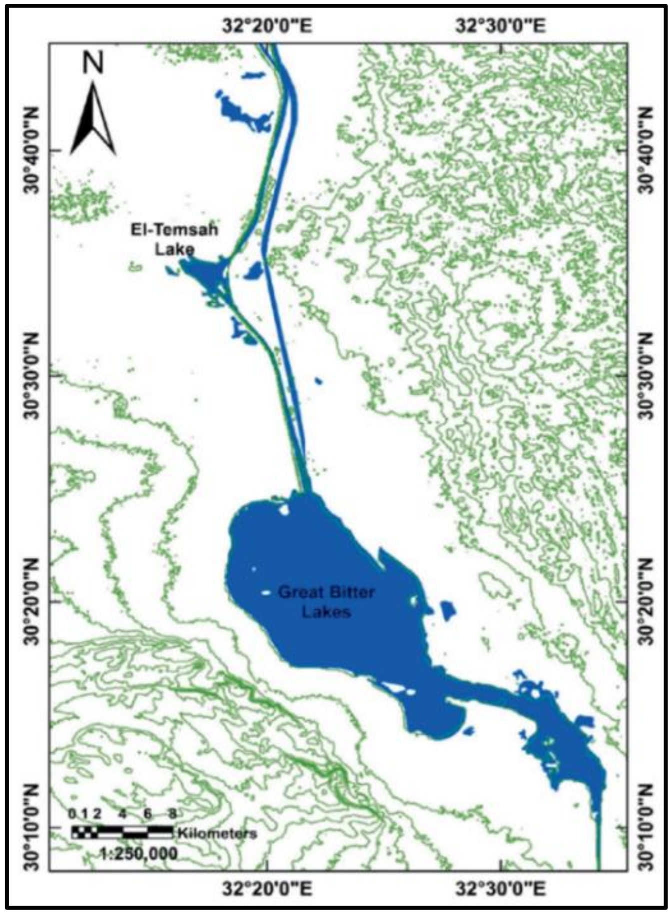

Fig. 1. Map of the Great Bitter Lake, Suez Canal, Egypt.

Fig. 2. Dissected male crab with gonad showing: Testis (a), anterior vas deferens (b), commissure (c), median vas deferens (d), posterior vas deferens (e) and ejaculatory duct (f).

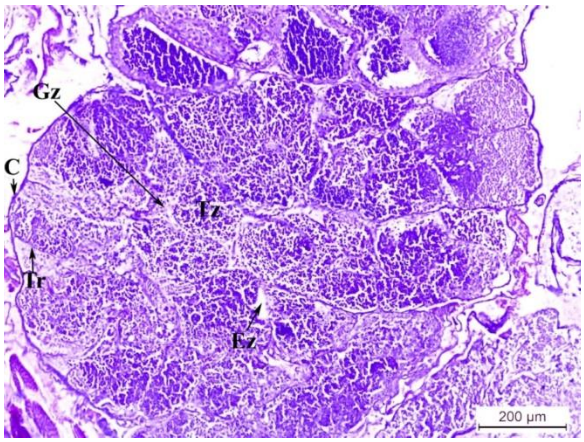

Fig. 3. Testis of an uninfected *Portunus segnis* male showing lobules, each surrounded by thin connective tissue trabeculae (Tr). Each tubule contains three distinctive zones, a pale-stained germinal zone (Gz), densely stained transformation zone (Tz) and an evacuation zone (Ez). C-capsule.

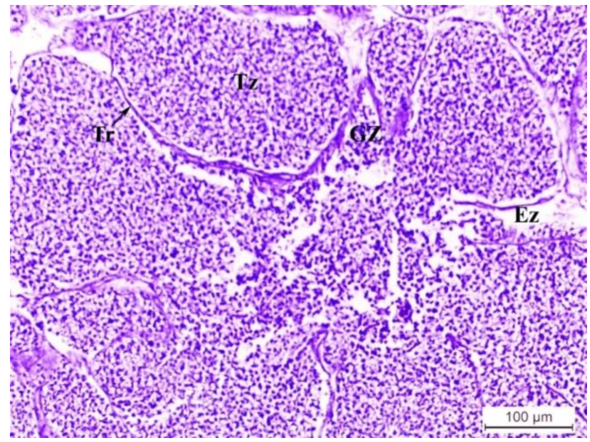

Fig. 4. Testis of an infected *Portunus segnis* male showing lobules, each surrounded by thin connective tissue trabeculae (Tr). Each tubule contains three distinctive zones, a germinal zone (Gz), transformation zone (Tz) and an evacuation zone (Ez).

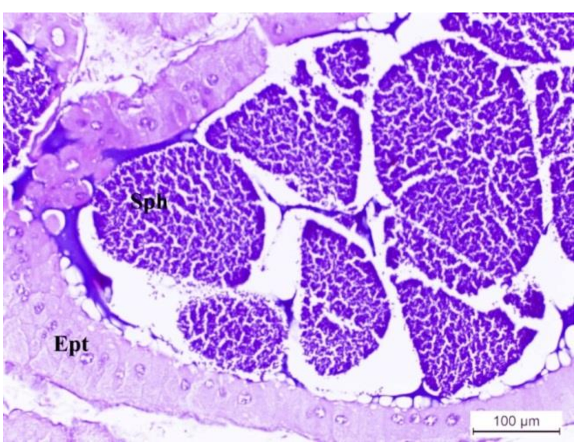

Fig. 5. Anterior vas deferens of an uninfected *Portunus segnis* male comprises of several ducts. Each is lined by a columnar epithelium (Ept) and contains several spermatophores (Sph) within the lumen.

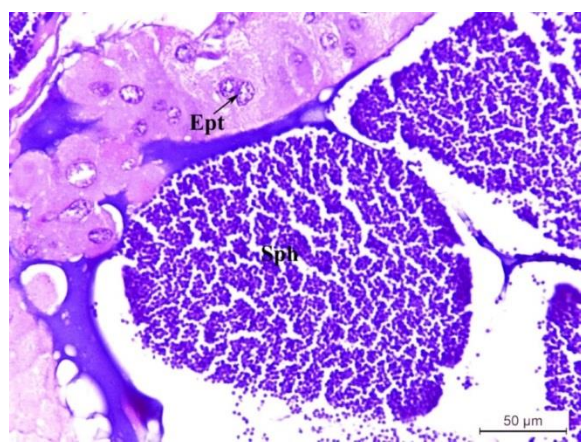

Fig. 6. Anterior vas deferens of an infected *Portunus segnis* male contains several ducts. Each is lined by a columnar epithelium (Ept), and contains several spermatophores (Sph) within the lumen.

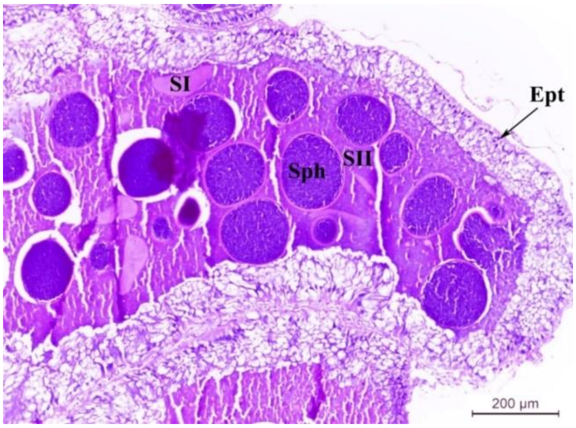

Fig. 7. Middle region of the vas deferens (MVD) of an uninfected *Portunus segnis* male comprising a single tube lined by a cuboidal epithelium (Ept). The lumen contains numerous spermatophores (Sph), each surrounded by a pink matrix, termed substance I (SI), and granular blue staining material, termed substance II (SII).

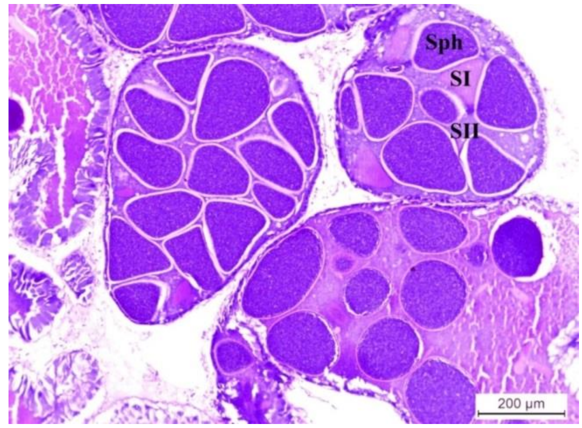

Fig. 8. Middle region of the vas deferens (MVD) of an infected *Portunus segnis* male comprising many tubes lined by a cuboidal epithelium (Ept). The lumen contains numerous spermatophores (Sph), red staining material, termed substance I (SI), and pale blue staining material, termed substance II (SII).

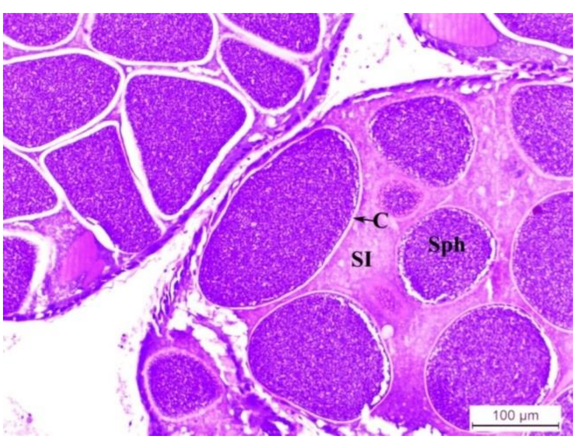

Fig. 9. Posterior region of the vas deferens (PVD) of an uninfected *Portunus segnis* male is a short duct lined by a thin cuboidal epithelium, and the Sphs within the lumen are surrounded by a thick capsule (C) and SI.

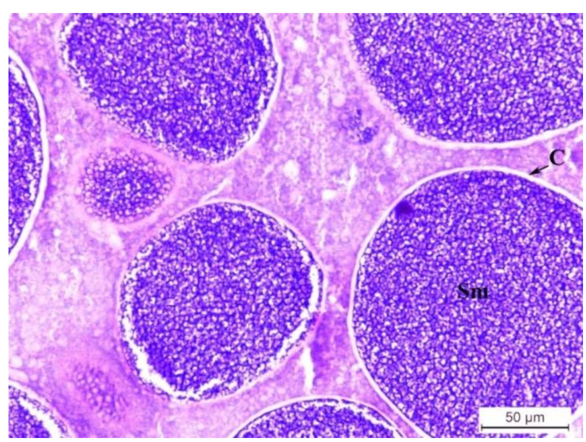

Fig. 10. Posterior region of the vas deferens (PVD) of an infected *Portunus segnis* male showing spermatophores (Sphs) containing sperm mass (Sm), surrounded by a well-defined capsule of a homogeneous substance (C).

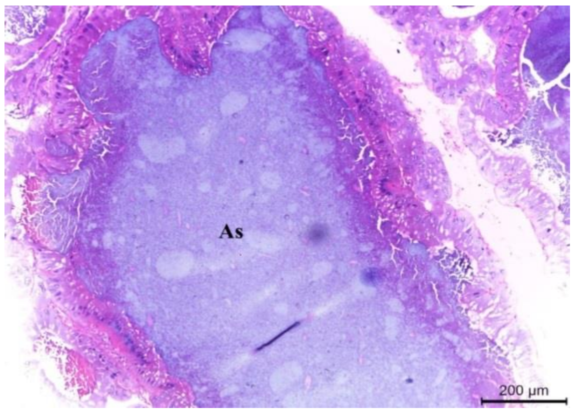

Fig. 11. Ejaculatory duct and penis of an uninfected *Portunus segnis* male showing agranular substance (As) surrounded by columnar cell lining.

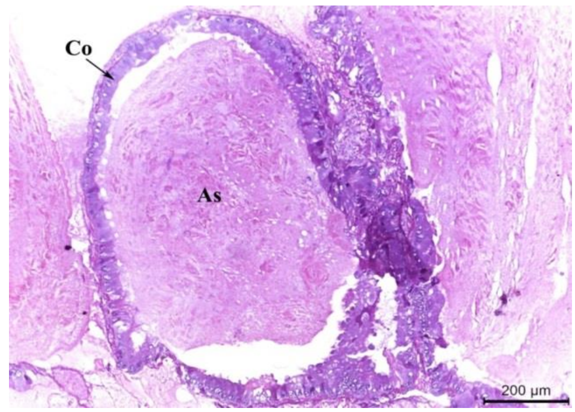

Fig. 12. Ejaculatory duct and penis of an infected *Portunus segnis* male showing agranular substance (As) surrounded by columnar cell lining (Co).

Table 1. Number of total and mature uninfected male *Portunus segnis* by size classes and corresponding proportion of mature males in the Great Bitter Lake.

Size classes Carapace width (mm)	nber of mature uninfected males	'oportion of mature uninfected males (%)
60 - 69.9	0	0
70 - 79.9	7	70
80 - 89.9	43	90
90 - 99.9	71	98
100 - 109.9	53	99
110 - 119.9	17	100
120 - 129.9	4	100
Total	**195**	**78.95**

Table 2. Number of total and mature infected male *Portunus segnis* by size classes and corresponding proportion of mature males in the Great Bitter Lake.

Size classes Carapace width (mm)	nber of mature uninfected males	'oportion of mature uninfected males (%)
60 - 69.9	0	0
70 - 79.9	5	63
80 - 89.9	11	82
90 - 99.9	20	94
100 - 109.9	16	97
110 - 119.9	7	100
120 - 129.9	2	100
Total	**61**	**81.33**

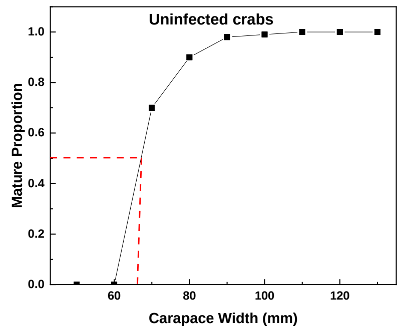

Fig. 13. Size of 50% maturity in the different size classes of *Portunus segnis* uninfected males estimated by evaluating the cumulative curve. The value of CW_{50} which corresponds to a proportion of 0.5 is indicated.

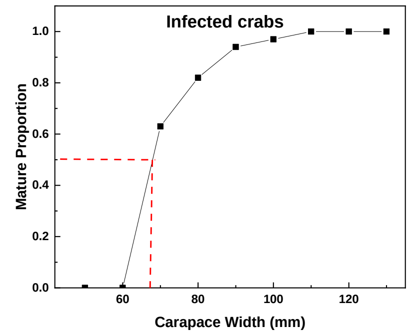

Fig. 14. Size of 50% maturity in the different size classes of *Portunus segnis* infected males estimated by evaluating the cumulative curve. The value of CW_{50} which corresponds to a proportion of 0.5 is indicated.

4. Discussion

The male reproductive system of *P. segnis* had a morphology similar to other decapod crustaceans, particularly portunid crabs, and consisted of an H-shaped structure with a pair of testes, a pair of vas deferentia, and ejaculatory ducts (Krol *et al*., 1992). Testes of *P. segnis* are tubular organs composed of numerous microscopically visible lobules connected to the seminiferous duct; consequently, they have been classified as lobular testes according to the categories (lobular and tubular) established by Nagao and Munehara (2003), and are similar to previous reports of some other brachyurans. Tubular testes have also been reported in different groups of Brachyura (Majoidea, Grapsoidea, and Xanthoidea) (McLaughlin, 1983). Adiyodi and Anilkumar (1988) stated that lobular testes are very common in portunid crabs.

In *P. pelagicus*, the testes are attached to the VD by a tiny channel known as the vas efferents (Stewart *et al*., 2010). VD is the name for a pair of longitudinally extending, convoluted tubules in the back of the body (McLaughlin, 1983). The VD of *P. segnis* is divided into three distinct regions, the AVD, MVD and PVD as has been reported in other crabs based on their morphological and functional criteria in *Portunus sanguinolentus* (Boopathi, 2011), *C. sapidus* (Johnson, 1980), *Thenus orientalis* (Burton, 1995) and *Maja brachydactyla* (Simeó *et al*., 2009). As previously mentioned in Brachyura (Adiyodi and Anilkumar, 1988), the ejaculatory duct, a smooth channel flowing between the musculature of the swimming pedals, represents the terminal element of the reproductive system.

Very few works have described the mature stages in male crabs. Diesel (1991) identified six stages of maturation in *C. irroratus* males. Based on a modified version of Diesel who said that gonad development occurred across six phases, Campbell and Eagles (1982) observed five stages of maturation in *C. irroratus*. Male crab *C. amnicola* showed five stages of maturity (Krol *et al*., 1992). Male gonads in *M. branchydactyla* were classified into four distinct morphometric categories by Haefner (1976). In *P. pelagicus* and *P. sanguinolentus*, Lawal-Are (2010) identified three stages of maturation based on the development of the testis. According to the development of the VD in *P. segnis*, three stages of maturation have been documented (De Lestang *et al*., 2003).

The testis of *P. segnis* shares a lot of similarities, on a microscopic level, with the structure described for other decapods (Krol *et al*., 1992). The seminiferous tubules that make up the testes include growing gametic cells in the centre and, like those of most Decapoda, spermatogonia in the outermost region (Mota-Alves and Tome, 1966). The accessory cells and generative cells that make up the seminiferous tubules descend from the progenitor cells that line the tubule wall. In decapods, the tubule walls are made up of one or more layers of connective tissue (Krol et al., 1992). The VD is divided into three different structural regions according to their different synthetic activities (AVD, MVD and PVD). These epithelial regions are responsible for the formation of a spermatophore, which is species-specific; the transportation of the spermatophore to the gonopore; and the maintenance of sperms until fertilization (Manjoncabeza and Raso, 2000).

In *P. segnis,* the AVD secretions play a role in the formation of the spermatophores. SI, produced by the proximal portion of the epithelium, separates the sperm mass into small clumps, and SII, produced by the distal portion of the epithelium, consolidates the small clumps into the spermatophore. This pattern has also been shown in many brachyurans (Simeó *et al*., 2009), where the production of spermatophores is caused by the secretion of two distinct chemicals in the AVD. Although they have been described differently, these two types of substances have been found in *Scylla serrata* (Uma and Subramaniam, 1979), *Chionoecetes opilio* (Sainte and Sainte, 1999), *Goniopsis cruentata* (Garcia and Silva, 2006) and *M. brachydactyla* (Krol *et al*., 1992). They typically consist of rod-shaped glycoprotein and polysaccharide chains and are thought to have originated from the columnar epithelium of the VD (Simeó *et al*., 2009). The spermatophores, which appear to be deposited in the early part of the AVD, are surrounded by a unique, single envelope layer. According to reports, *P. sanguinolentus* contains this envelope layer (Ryan, 1967). Based on descriptions of other brachyuran crabs, *P. segnis* has an ejaculatory duct with a structure that is roughly round in shape (Sherkhane et al., 2010). Spermatozoa in the hermit crab *Dardanus asper* formed a stream as they exited the testis and mingled with epithelial secretions in the VD. The surrounding muscle layer contracted throughout this mixing process, grouping and separating the spermatozoa into distinct and visible spermatophores within the coiled VD. The spermatozoa cluster together and the contractions sculpt the seminal fluids around them into the spermatophore's distinctive structure (Mathews 1953). In the current work, a similar process is anticipated in *P. segnis*. There were no changes in the histological components of the reproductive system that were associated with the infection by *Hematodinium* parasite in males. No reports of invasion of *Hematodinium* were noted in hemal spaces of reproductive organs in line with previous studies (Hudson and Shields 1994, Stentiford *et al*. 2002, Stentiford *et al*. 2003).

According to Reeby *et al*. (1990), males of *P. sanguinolentus* attain sexual maturity at 81–85mm (CW). However, Sumpton *et al*. (1989) reported that male *P. sanguinolentus* inhabiting the coastal waters of Queensland, Australia, attain sexual maturity at 83mm (CW). We found that uninfected males attained sexual maturity at 66.3mm (CW) while the size at first sexual maturity of infected males was 67.3mm (CW). This result supports previous histological studies on the male reproductive system; however, there is no effect of infection by *Hematodinium* on the sexual maturity of males. In addition parasite transmission through reproduction has become a matter of debate. The exact reason(s) for these differences is not clear. However, previous studies have shown that the size at first sexual maturity of crabs may vary with moult increment, the number of moults (Hines 1989; Rasheed & Mustaquim 2010), environmental factors such as temperature and salinity (Fisher 1999).

The present study on *P. segnis* helps in understanding the microscopic structure of various parts of the male reproductive system. A clear understanding of the histological details for both uninfected and infected males and size at first maturation is envisaged to help in framing this commercially important species.

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التأثيرات المحتملة للطفيل *هيماتودينيم بريزي* على الجهاز التناسلي الذكري لسرطان البحر السابح *بورتيونس سيجنيس* القاطن بالبحيرة المرة الكبرى، مصر

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المستخلص. فحص الباحثين جوانب مختلفة من بيولوجيا التكاثر لدى ذكور سرطان البحر السابح *بورتيونس سيجنيس*، الذي تم تجميعه شهريًا في الفترة من أكتوبر 2020 إلى سبتمبر 2021 من البحيرة المرة الكبرى بقناة السويس في مصر. وقد وجد أن الجهاز التناسلي الذكري فى سرطان البحر السابح *بورتيونس سيجنيس* يتكون من خصيتين، واختناق، ووعائين ناقلين، وقناتين قاذفتين داخليتين. ولهذا الجهاز تماثل جانبى ومظهر أبيض كريمي. ويقسم الوعاء الناقل إلى ثلاثة مناطق هم: الوعاء الناقل الأمامى، والوعاء الناقل المتوسط، والوعاء الناقل الخلفي. كذلك تقسم مراحل تطور المناسل الذكرية إلى ثلاث مراحل: غير ناضجة، وقبيل النضج، وناضجة. تم تثبيت عينات من الخصية، والوعاء الناقل الأمامي، والوعاء الناقل المتوسط، والوعاء الناقل الخلفى، والقناة القاذفة، فى محلول بوان، بغرض الفحص النسيجى بالمجهر الضوئى. ويعتبر سرطان البحر السابح *بورتيونس سيجنيس* عائل مهم للطفيليات ثنائية الأسواط من جنس *هيماتودينيم*. ولقد تضرر العائد التجاري لسرطان البحر السابح *بورتيونس سيجنيس* بسبب تفشي هذه الطفيليات. وتبين من الفحص النسيجي ان الطفيليات ثنائية الأسواط ليس لها تأثير ملحوظ على نمو المناسل وتطورها فى سرطان البحر السابح *بورتيونس سيجنيس*. ووجد ايضًا ان عرض الدرقة فى بداية النضج الجنسي فى الذكور غير المصابة والمصابة كان 66,3 و67,3 مم على الترتيب.

الكلمات المفتاحية: الوعاء الناقل الأمامى، الوعاء الناقل المتوسط، الوعاء الناقل الخلفي، القناة القاذفة، الحجم عند بداية النضج الجنسي.