



## Spatio-temporal population and reproductive responses in *Hediste diversicolor* (Polychaeta: Nereididae) from the Damietta Harbor Barge Canal, Egypt.

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### ABSTRACT

The distribution and reproduction of *Hediste diversicolor* were studied in the Barge canal of Damietta Harbor by taking monthly samples at three stations for one year. Temporal and spatial variations of some water quality parameters and sediment conditions were analyzed. The highest densities occur in the middle of the canal at station II, establishing it as a suitable zone for worm production. The seasonal pattern of density showed peaks during the summer and autumn months with significant differences between stations. Females were a preponderance giving an annual sex ratio of 2.5(females):1(males). The maximum body length (9.8 cm) and weight (0.79g) appeared at station II. The formula of the length-weight relationship indicated allometric growth. The spawning season was long, the worm can breed all the year-round, with however a maximum during spring. Mature oocyte stages were limited to certain seasons at the station I with increased pollution impact, whereas continuous mature oocyte occurrence was observed at stations II and III. The results reflected differences in population and reproductive responses among Barge canal parts, suggesting that pollution and ecological status should be considered in the evaluation of polychaetes population dynamics.

### INTRODUCTION

Anthropogenic impacts on harbors and shallow bays could induce severe effects on benthic communities (Guerra-García and García-Gómez, 2004). Damietta Harbor is one of the most important harbour in the southern Mediterranean. The harbor has a barge canal that connects it to the brackish Nile river estuary. (Fig. 1). The Barge canal receives considerable amounts of anthropogenic discharges (Eltohamy *et al.*, 2017). Due to the effects of runoffs, estuarine waters, and seawater intrusions from the harbor, the canal is a complex aquatic environment, the continuous water mixing establishes clear environmental gradients of salinity, nutrient concentrations, and other factors. The sedimentary nature of this manmade canal is affected mainly by seawater current, mud, and sand moved downstream from the Nile river and adjacent runoffs. The isolation of riverine fresh water from the estuary brackish water by the permanent Farskour dam makes the entire area tidally influenced, thus the canal is dominated by brackish to saltwater (El-Tohamy *et al.*, 2018).

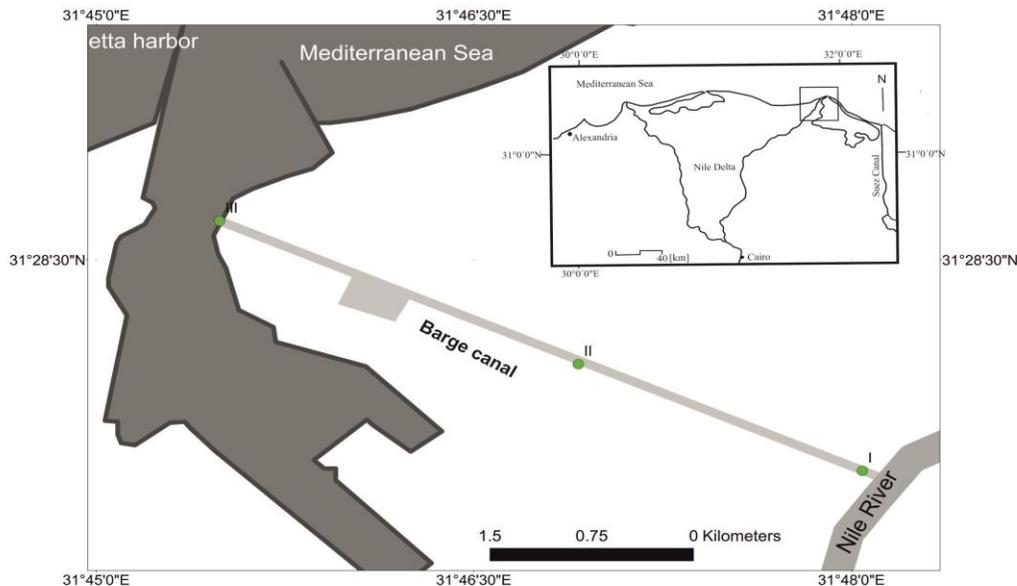
Polychaetes usually showing dominancy among marine benthic communities in both soft and hard bottoms (**Musco, 2012**). The changes in sediment texture and algal cover are significant factors affecting polychaetes distribution patterns (**Tena *et al.*, 2000**). Because of high levels of adaptation to environmental changes, polychaete assemblages can be used as an efficient tool as a biological indicator for ecosystem health (**Dorgham *et al.*, 2014**). Nereididae is one of the most diverse and abundant polychaete families found in marine and brackish water ecosystems (**Çinar and Ergen, 2001**). Its representatives constitute important elements of trophic chains within a given ecosystem as consumers of some plants and smaller invertebrates, or as prey for Crustaceans and juvenile fish (**Kies *et al.*, 2020**). In Egypt, Nereididae is widely used by humans as bait for fish. In the Mediterranean waters, the taxonomy, ecology, and population dynamics of this family have been discussed by several authors (e.g. **Gillet, 1990; Arias and Drake, 1995; Çinar and Ergen, 2001; Giangrande *et al.*, 2003; Virgilio *et al.*, 2009**). As for the Egyptian waters, little has been published mainly on the ecological distribution (**Abd-Elnaby, 2009a; Abd-Elnaby, 2009b; Selim, 2009; Dorgham *et al.*, 2014; Abdelnaby, 2020**) and reproduction (**Hamdy *et al.*, 2014**).

*Hediste diversicolor* (O.F. Müller, 1776) is a member of the family Nereididae, it has been described as a cosmopolitan species by **clay (1967)**. It is widespread in shallow marine and brackish waters of the temperate zone of the North Atlantic (**Einfeldt *et al.*, 2014**), Mediterranean, Baltic Black, and Caspian seas (**Scaps, 2002; Vasileiadou *et al.*, 2016**). This omnivorous infaunal ragworm lives in mud, sand, gravel, and also occurs under stones where the U- or Y-shaped burrows are usually adjacent to the stones (**Scaps, 2002; Durou *et al.*, 2007; Einfeldt *et al.*, 2014**). *H. diversicolor* is ecologically important as euryhaline bioturbates (**Kristensen, 1983; Cuny *et al.*, 2007**) and also as prey items for many species of birds, fishes, and invertebrates (**Rosa *et al.*, 2008**). Compared to other Nereididae, this species is characterized by the lack of a pelagic phase (**Vasileiadou *et al.*, 2016**); the female incubates their larvae immediately after emergence, resulting in limited dispersal (**Einfeldt *et al.*, 2014; Scaps, 2002**). In Egypt, although several ecological studies listed this worm as an important item of the species checklist (e.g. **Dorgham *et al.*, 2014; Hussian *et al.*, 2019; Mitwally and Abada, 2008**), the worm reproductive response is mostly ignored. According to **Scaps (2002)**, *H. diversicolor* exhibited morphological and biological differences across populations from different geographical ranges, for example, the populations in southern Europe reached maturity earlier than that of northern Europe. Most studies on estuarine Nereididae greatly favored females to determine the time and prevalence of spawning periods throughout the examination of oocytes' sexual maturity stages during the year (**Mettam and Santhanam, 1982**). So, this study was conducted to obtain information on the sex ratio and the cycle of reproduction. Also, the study aims to determine the Spatio-temporal populations and reproductive response (oocyte stages) in the Damietta Harbor Barge canal.

## MATERIALS AND METHODS

### 1. Study area and sampling stations

During the period from January 2019 to December 2019 specimens of the polychaete, *H. diversicolor* were collected monthly from the Damietta Harbour Barge Canal. It is located in the eastern part of the Nile Delta on the southeastern Mediterranean Coast at 31°27′-31° 28′ N and 31°45′ - 31°48′ E, with 4.5 km long and 5-7 m in depth. The study was carried out in the shallow water zone on the eastern side of the Barge canal. This zone has a width of about 1.5-2 m and a depth of a maximum of 50 cm at normal water level whereas, during low tide, a large portion of this zone is usually exposed to air. According to **El-Banna and Frihy (2009)**, along the Nile delta coast, there is a semidiurnal tidal regime with an average tidal range of about 0.5 m which affects the water level in the canal. As shown in figure one, three stations were selected: station (I) was located near the Damietta Nile estuary, station (II) nearly at the middle of the canal, and station (III) occurred near the seaside.

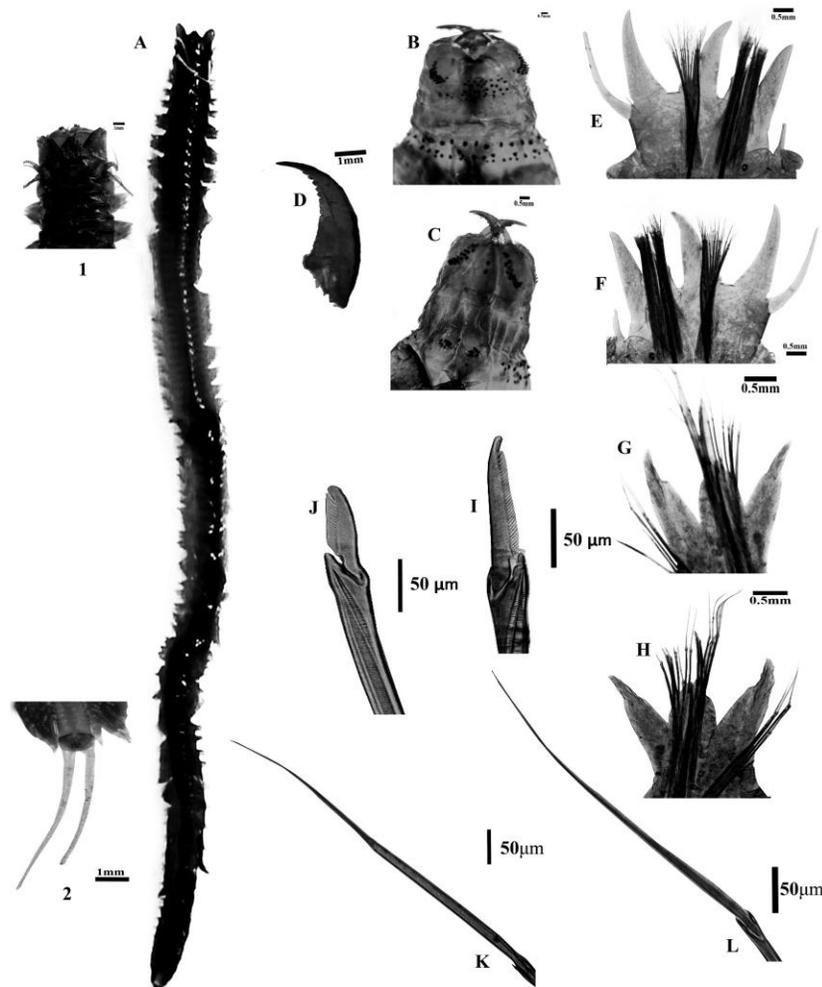


**Fig. 1.** The area of study and sampling stations

### 2- Sampling procedure

Ragworms were obtained at each station from an area of 1 m<sup>2</sup> quadrat at depth of about 10 cm in the substrate, and three replicates were collected at a distance of five meters from each other. Each sample was sieved to 1 mm, and the sampled organisms were fixed in 10% buffered formalin. The detailed morphological features of *H. diversicolor* is shown in Figure 2. In order to characterize the sediment's physicochemical properties, samples were collected monthly (n=2, per sampling site). Sediment samples were kept at -20°C until analysis. In the meantime, in situ measurements of physicochemical properties of water (temperature, salinity, dissolved oxygen, and pH) were made using a YSI temperature/ salinity/ dissolved oxygen probe

(model 85), and digital pH meter (Orion research model 201). Chlorophyll-a was determined according to the method described by **Wetzel and Likens (2013)**.



**Fig. 2.** The parts of *H. diversicolor*: (A) The whole worm, (1) Head region, (2) Tail region. (B) Ventral view of the anterior end with an everted proboscis. (C) Proboscis dorsal view. (D) Jaw lateral view. (E) Dorsal view of anterior parapodium. (F) Ventral view of anterior parapodium. (G) Dorsal view of posterior parapodium. (H) Ventral view of posterior parapodium. (I) Homogomphous falcigers. (J) Heterogomphous falcigers. (K) Homogomphous spinigers. (L) Heterogomphous spinigers.

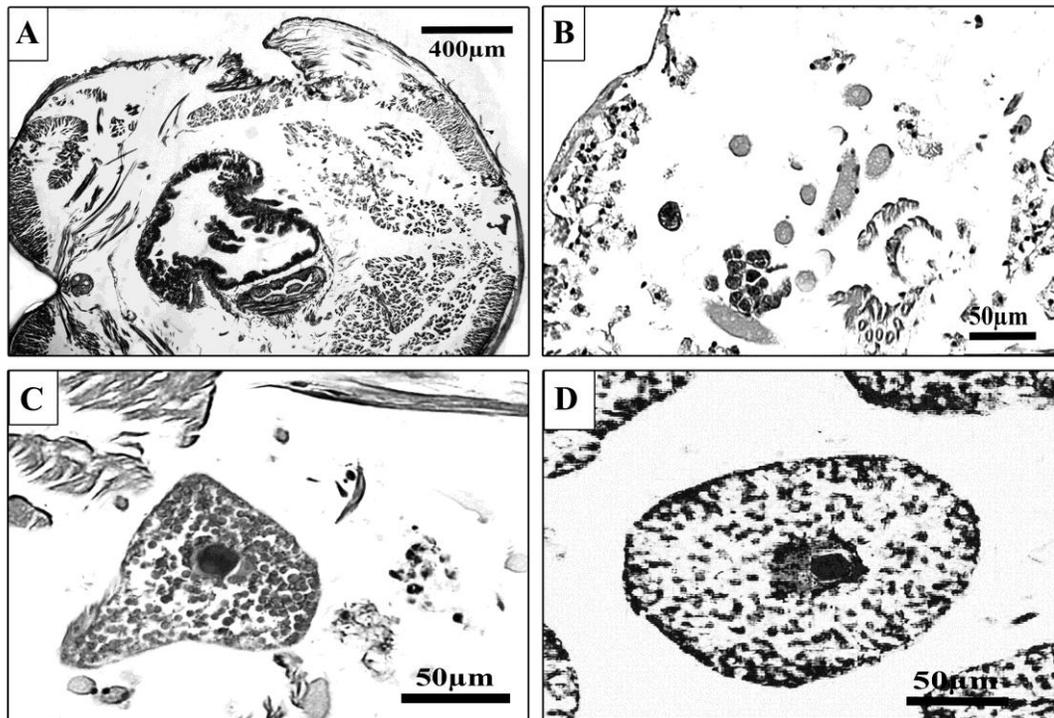
### 3- *H. diversicolor* population and reproductive responses

#### 3.1. Density and Biometry

The numerical density of *H. diversicolor* at different stages (males, females, and juveniles) was performed under a stereomicroscope. The total body length (cm) and weight (g) were measured monthly for some male and female worms. The length-weight relationship was determined according to the allometric equation  $W = aL^b$  (**Beckman, 1948**), where **W** is the total body weight (g), **L** is the total body length (cm), **a** is a constant, and **b** is the growth coefficient.

### 3.2. Oocyte sexual maturity stages

Female worms obtained from sediment were examined in each station from all months. The oocyte stages were obtained by the incision of females using a scalpel was performed. The contents of three segments behind the head were placed on a glass slide, and the coelomic fluid was examined under a microscope (Díaz-Jaramillo *et al.*, 2015). As shown in figure 3, four stages were defined in worms: worms at stage A correspond to sexually undifferentiated specimens (absence of the germ cells), stage B (primary oocytes), oocyte diameter ranges from 30 to 80  $\mu\text{m}$ ; stage C (growing oocytes), diameter ranges from 90 to 180  $\mu\text{m}$ ; stage D (mature stage) oocytes were large, spherical and ready to be spawned (>200  $\mu\text{m}$ ) (Olive and Garwood, 1981).



**Fig. 3.** Illustration of the oocyte development observed in the *H. diversicolor*. (A) Anterior region transverse section of undifferentiated sex, no sperms, and no oogenesis existed in the coelom. (B) Primary oocytes (previtellogenic stages) are in clusters (arrow) and distinguished from other coelomic cells by their relatively large size and highly stained chromatic nuclei. (C) Growing oocytes (vitellogenic stage) increased in size as yolk accumulates. Oocytes are acidophilic. Irregular in shape. Nucleus not centered. Oocytes have a hairy surface (microvilli). (D) Mature oocytes approaching a spherical shape. The nucleus was centered, large lipid granules accumulated around it.

### 4. Histological sectioning

According to the protocol described by Tzetlin and Filippova (2005), monthly cross-sections of some worms were examined. The sections were stained with hematoxylin and Eosin. This is the most common staining procedure used in histology, and it is primarily used to display structural features (Ross and Pawlina, 2006).

## 5. Statistical analysis

One-way ANOVA with Tukey's-b test was employed to test the differences between variables. Correlations analysis was used to define the relationship between some selected variables. The data were tested for normality before analysis, and non-normal were transformed using  $\log(x+1)$ . ANOVA and Correlations were performed using SPSS 18.

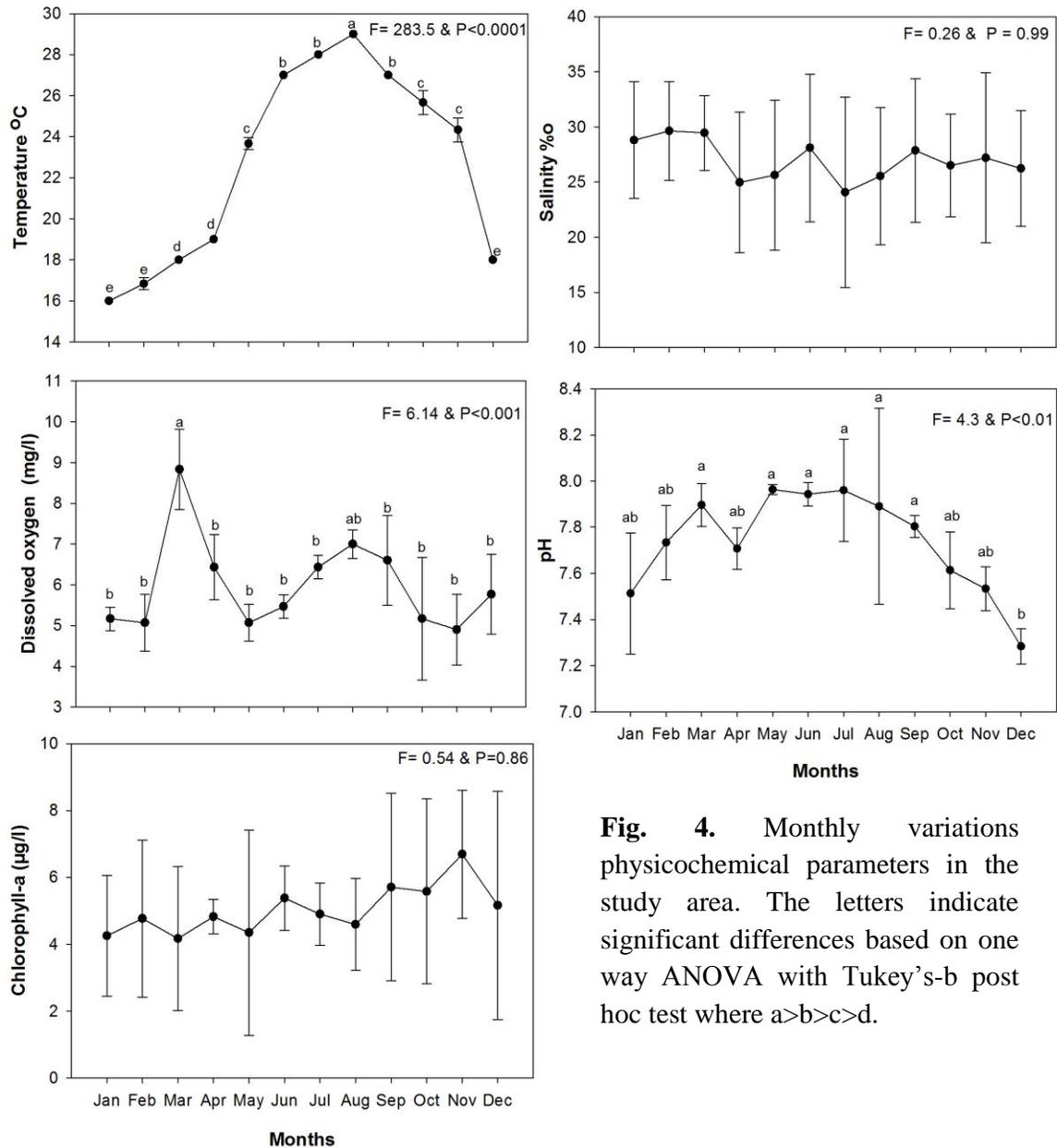
## RESULTS AND DISCUSSION

### 1. Water and sediment physicochemical variables

One-way ANOVA showed significant temporal variability ( $p < 0.05$ ) except for salinity and phytoplankton biomass (Fig. 4). Spatially, water physicochemical properties showed a similar pattern in terms of, temperature, pH, and dissolved oxygen (Table 1). Chlorophyll-a values at station II were significantly higher than those of the other stations. Salinity showed the most obvious spatial gradient with a trend of increasing toward station III near the sea. This is attributed to the prevalence of low saline water flow from the Nile estuary at station I (Eltohamy *et al.*, 2017). The particle size of sediment displayed gradual variation along the barge canal, whereas fine sand dominated at station III (66%), the medium sand was higher at station II (26%), while at station I coarse sand formed 18.9%, gravel (3.3%), and clay (15.6%). The organic matter (17.8%) sustained the highest value at station I and was accompanied by lower dissolved oxygen concentrations. The organic matter at the station I was related to the direct impact of estuarine water mixed with domestic and agricultural wastes at this location (Eltohamy *et al.*, 2017).

**Table 1.** Average values  $\pm$  stdev of the physicochemical parameters and sediment size composition percentages at the sampling stations. The letters indicate significant differences based on one-way ANOVA with Tuckey's-b test.

| Water/sediment                     | I                           | II                          | III                         |
|------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| <b>Water</b>                       |                             |                             |                             |
| Temperature ( $^{\circ}\text{C}$ ) | 22.7 $\pm$ 4.86             | 22.7 $\pm$ 4.8              | 22.7 $\pm$ 4.7              |
| Salinity (PSU)                     | 21.1 $\pm$ 2.7 <sup>c</sup> | 27.4 $\pm$ 3.7 <sup>b</sup> | 32.5 $\pm$ 0.6 <sup>a</sup> |
| Dissolved oxygen (mg/l)            | 5.2 $\pm$ 0.9               | 5.8 $\pm$ 1.5               | 5.9 $\pm$ 1.5               |
| pH                                 | 7.7 $\pm$ 0.2               | 7.4 $\pm$ 0.3               | 7.8 $\pm$ 0.3               |
| Chlorophyll-a ( $\mu\text{g/l}$ )  | 4.6 $\pm$ 1.8 <sup>b</sup>  | 6.5 $\pm$ 1.3 <sup>a</sup>  | 4.1 $\pm$ 1.9 <sup>b</sup>  |
| <b>Sediment</b>                    |                             |                             |                             |
| Organic matter (%)                 | 17.8 $\pm$ 4.1 <sup>a</sup> | 10.2 $\pm$ 2.1 <sup>b</sup> | 6.5 $\pm$ 1.5 <sup>c</sup>  |
| Sediment types                     |                             |                             |                             |
| Gravel sand                        | 3.3%                        | 1.1%                        | 3%                          |
| Coarse sand                        | 18.9%                       | 15.6%                       | 12%                         |
| Medium sand                        | 22.2%                       | 26.7%                       | 14%                         |
| Fine sand                          | 40%                         | 46.7%                       | 66%                         |
| Clay                               | 15.6%                       | 10%                         | 5%                          |



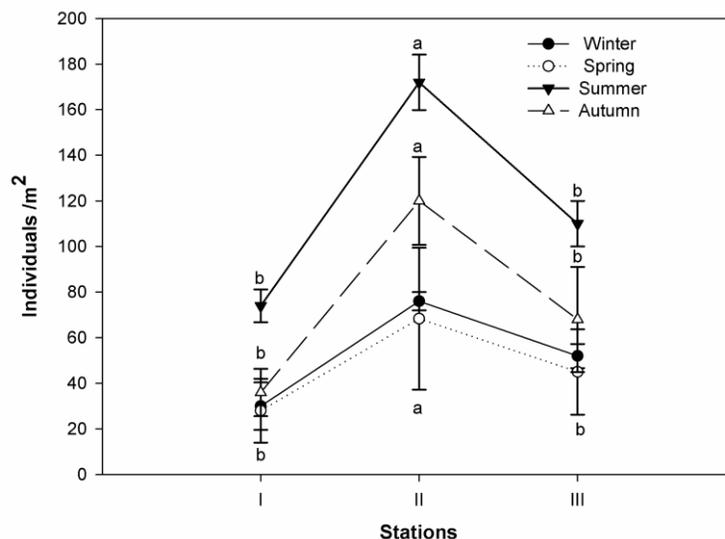
**Fig. 4.** Monthly variations physicochemical parameters in the study area. The letters indicate significant differences based on one way ANOVA with Tukey's-b post hoc test where  $a > b > c > d$ .

## 2. *H. diversicolor* population and reproductive responses

### 2.1. Numerical Density

The spatial abundance of *H. diversicolor* showed an inverted U-shaped pattern of distribution in the Barge canal where the middle zone reflected the highest densities (Fig. 5). The animal U-shaped distribution pattern is typically found in habitats with estuarine characteristics (Díaz-Jaramillo *et al.*, 2015), and the middle area of an estuary is the proper site for population development (Hough and Naylor, 1992). These observations support our finding in the Barge canal. Seasonal population densities showed significant differences between locations (Fig. 5), with a significant increase in summer and autumn

densities in contrast to spring and winter. This indicated that the summer and autumn months represent the main recruitment periods when a great number of juvenile individuals are incorporated into the population. In warm areas, the spawning and recruitment periods are long and continuous throughout the year (Arias and Drake, 1995) but with different seasonal intensities (García-Arberas and Rallo, 2002). The positive correlation of worm density with temperature ( $r = 0.66$  &  $P < 0.001$ ) support the previous confirmation. The considerable variation of worm density between sampling stations can be attributed to tidal levels, salinity variations, and the nature of the substratum (Abrantes *et al.*, 1999). According to E costa (1999), the growth rate of *H. diversicolor* was higher in the sand than in mud. This may be due to the resistance of sediment containing high percentages of clay to dig burrows by the worms (Galasso *et al.*, 2018). Also, at high levels of organic enrichment, muddy sediments accumulated more organic matter than sandy sediments (Martinez-Garcia *et al.*, 2015), which may explain the low density at station I that has the highest percentage of clay compared to other stations. On the other hand, another unfavorable condition may result from the highly tidal wave at station III leading to disturbance of the surface sediments inhabited by the worms. So, the species density increased at station II which has moderate conditions and more important fewer disturbance conditions.

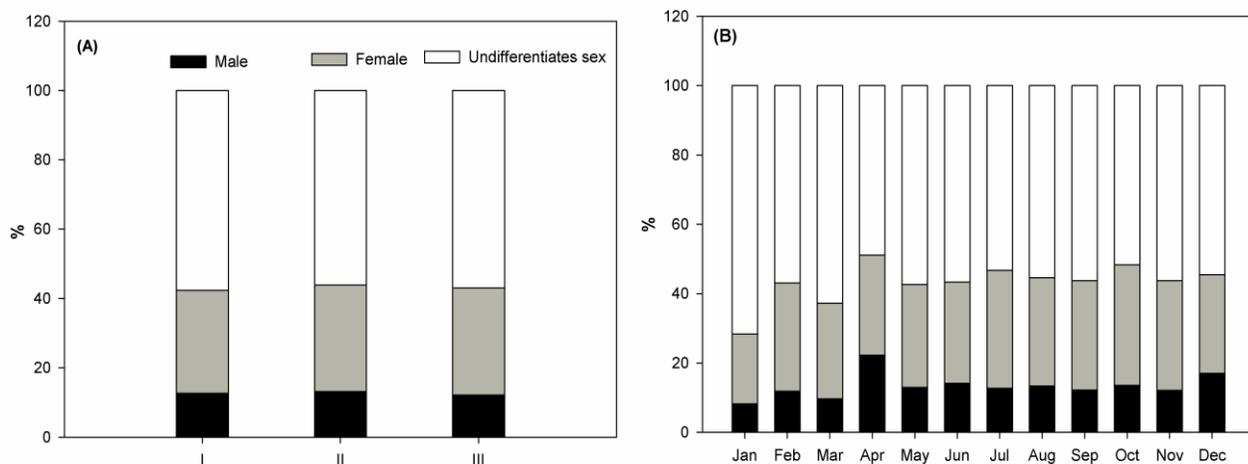


**Fig. 5.** Seasonal density (Mean  $\pm$  S.D) of *H. diversicolor* at the sampling stations. Different letters indicate significant differences ( $p < 0.05$ ).

## 2.2. Sex ratio

Sexual difference in *H. diversicolor* was apparent only at maturation, when males colour turned from a reddish-brown to a bright-grass green, whereas females have a much darker green. According to Olive and Garwood (1981), the germ cells can be recognized in males only in the last six months before spawning in comparison with females oocytes which found some 18 months before spawning. The sex ratio is determined by comparing the relative proportion of males to females, over a period when both sexes are recognizable (Olive and Garwood, 1981). The population percentages of mature *H.*

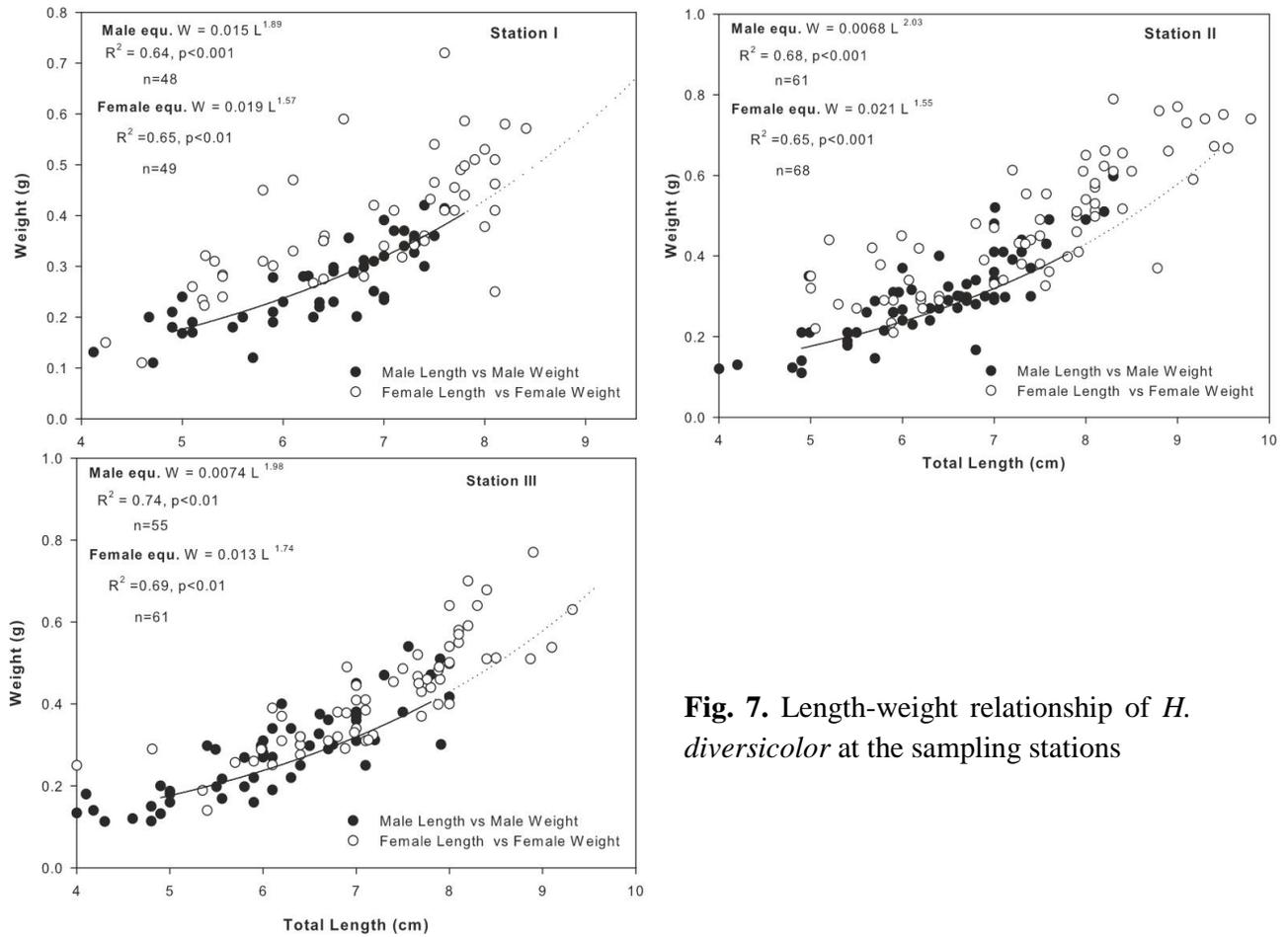
*diversicolor* exhibited similar values among stations, males comprised 12-13%, whereas females made up 29-31%. The monthly maturity (males and females) varied mostly between 28-44%, with the highest maturity of 48.2% (males-13.5% & females-34.7%) and 51.1% (males-22.2% & females-28.9%) in October and April, respectively (Fig. 6). The immature individuals were the major component of the worm population at all sampling stations. The number of females outweighs that of males along the barge canal over the year, giving an annual sex ratio of about 1(males):2.5(females). Several authors (e.g. Abrantes *et al.*, 1999; García-Arberas and Rallo, 2002; Olive and Garwood, 1981; Scaps, 2002) studied the breeding populations of *H. diversicolor* in different locations, observed the preponderance of females. Olive and Garwood (1981) confirmed that the age of sexually mature *H. diversicolor* was variable and influenced by environmental conditions. The gametogenesis was not initiated in the first year of life and the maturity takes between 1 and 2 years before spawning (Scaps, 2002).



**Fig. 6.** Spatial (A) and temporal (B) sex ratio percentages in *H. diversicolor*

Males body size ranged from 0.11 to 0.60 g weight, 3.69 to 8.3 cm total length, without spatial significant differences. On the other hand, females body size that ranged from 0.12 to 0.79 g, 4.1 to 9.8 cm total length showed significant spatial variability (Table 2). Accordingly, females were the largest individuals in the study area, attained their maximum length (9.8 cm) at station II in January, against (9.32 cm) at station III, and (8.41 cm) at station I in February. According to Mouneyrac *et al.* (2009), the body size of *Nereis diversicolor* from the sediment of polluted water is smaller than the body size in the opposite conditions. This is consistent with the present study, as the body size of worms at the station I was the lowest. Biometric relationships (Fig. 7) showed that males and females weight was significantly correlated with total length with higher determination coefficients ( $R^2$ ) for males than females at all stations. The established size-weight relationship was similar to those observed by Durou *et al.* (2008) at the Authie estuary of the English channel and Galasso *et al.* (2018) at the Arnel lagoon of the French Mediterranean coast. The growth coefficient (b) of both sexes at all stations being smaller than 3, which means that the worms have positive allometric growth. Such

growth pattern was also observed in another Nereid worm species, i.e. *Pseudonereis anomala* (Hamdy *et al.*, 2014) on the Alexandria coast.



**Fig. 7.** Length-weight relationship of *H. diversicolor* at the sampling stations

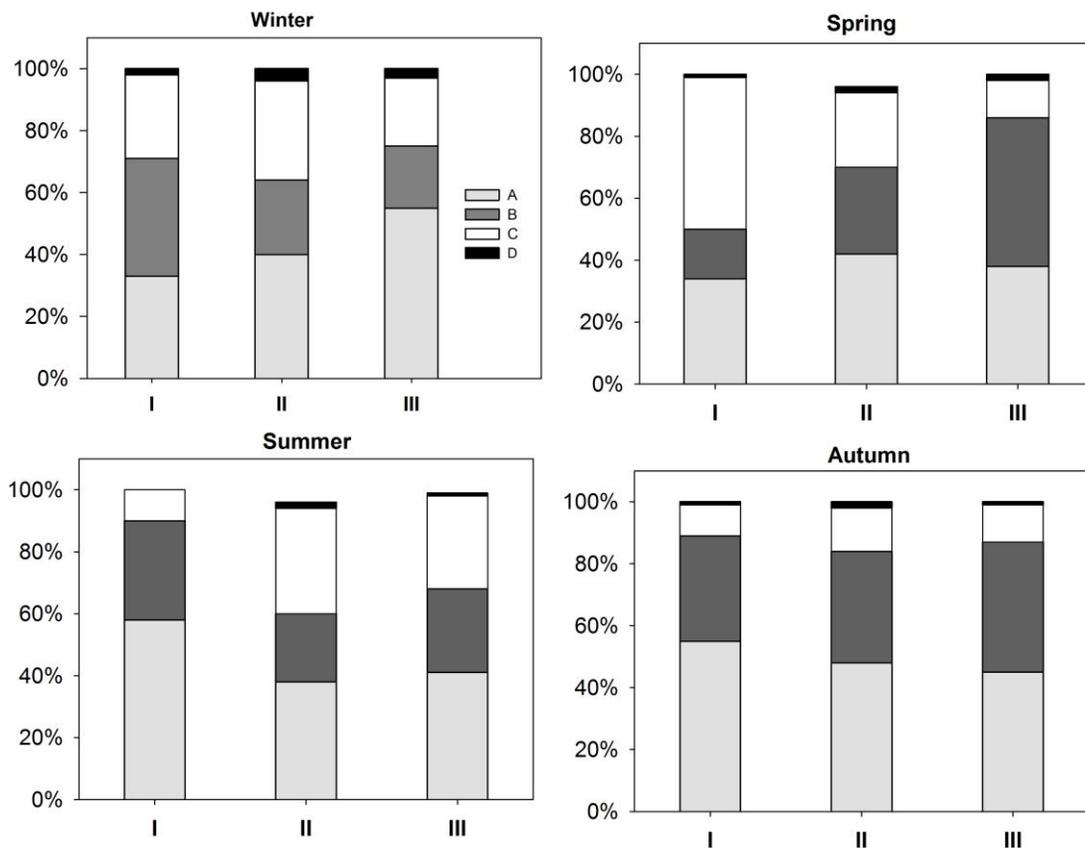
**Table 2.** Max, min, and mean values  $\pm$  stdev of total length and weight of *H. diversicolor* at the sampling stations. The letters indicate significant differences where a>b.

| Stations | Sex | Total length (cm) |      |                               | ANOVA |       | Weight (g) |      |                               | ANOVA |       |
|----------|-----|-------------------|------|-------------------------------|-------|-------|------------|------|-------------------------------|-------|-------|
|          |     | Max               | Min  | Mean $\pm$ SD                 | F     | P     | Max        | Min  | Mean $\pm$ SD                 | F     | P     |
| <b>I</b> | ♀   | 8.14              | 4.24 | 6.81 $\pm$ 1.11 <sup>b</sup>  | 3.9   | <0.05 | 0.72       | 0.12 | 0.39 $\pm$ 0.12 <sup>b</sup>  | 6.24  | <0.01 |
|          | ♀   | 9.8               | 5    | 7.40 $\pm$ 1.23 <sup>a</sup>  |       |       | 0.79       | 0.21 | 0.47 $\pm$ 0.16 <sup>a</sup>  |       |       |
|          | ♀   | 9.32              | 4.1  | 7.18 $\pm$ 1.17 <sup>ab</sup> |       |       | 0.77       | 0.14 | 0.42 $\pm$ 0.42 <sup>ab</sup> |       |       |
| <b>I</b> | ♂   | 7.42              | 3.69 | 6.29 $\pm$ 0.87               | 0.802 | 0.45  | 0.42       | 0.11 | 0.26 $\pm$ 0.08               | 2.1   | 0.13  |
|          | ♂   | 8.3               | 4.1  | 6.35 $\pm$ 0.93               |       |       | 0.61       | 0.12 | 0.30 $\pm$ 0.11               |       |       |
|          | ♂   | 8                 | 4    | 6.13 $\pm$ 1.03               |       |       | 0.54       | 0.13 | 0.28 $\pm$ 0.13               |       |       |

## 2.2. Oocyte sexual maturity stages

The oocyte maturity stages of *H. diversicolor* is shown in figure 8. Stage (A) of sexually undifferentiated worms and stage (B) of primary oocytes were observed in all seasons at all sampling stations. Growing oocytes (stage C) and mature oocytes (stage D) were observed in all seasons at stations II and while mature oocytes were absent during summer at station I (Fig. 8). The precise timing of spawning is variable, it is affected by environmental conditions especially temperature (Olive and Garwood, 1981). Along the Barge canal, mature and growing oocytes appeared most of the year. This observation is in agreement with several studies (e.g. Heip and Herman, 1979; Mettam, 1979; Olive and Garwood, 1981. Moreover, Mouneyrac *et al.* (2010), observed an extended occurrence of mature oocytes in impacted habitats, suggested active gametogenesis during the year which nearly similar to that reported in clean areas.

Percentage of mature oocytes at station I was significantly different ( $P < 0.05$ ) from those at stations II and III in most seasons. These differences indicated short breeding periods of the impacted station I worms when compared to non-impacted populations. The exposure to pollutants could disruptor the polychaetes endocrine chemicals (Díaz-Jaramillo *et al.*, 2015) resulted in delayed reproductive maturation.



**Fig. 8.** Seasonal sexual maturation percentage of the four sexual maturity stages; A–undifferentiated sex, B– primary oocytes, C–growing oocytes, and D–mature oocytes in *H. diversicolor* collected from the Barge canal.

## CONCLUSION

Population and production dynamics of the polychaete *Hediste diversicolor* from the Damietta Harbor Barge canal (Southeastern Mediterranean, Egypt) were studied from January 2019 to December 2019. Temporal variations of density were closely related to environmental conditions with a summer density peak; minimum densities were found during spring and winter. The percentage of mature oocytes appeared to be affected largely by the pollution status which affected adult survival. So, it's important to take into consideration the effect of pollutants on polychaetes production since impactful responses were found in individuals exposed to polluted sediments.

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