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Biological Structure and the Effect of Temperature on Embryonic Stage of the *Spirobranchus kraussii* (Baird, 1865) (Annelida: Serpulidae) in Bandar Abbas in the Persian Gulf

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Abstract

A Serpulidaepolychaetae, *Spirobranchuskraussii*, was newly recorded in the Persian Gulf, Iran. It is the first record of Serpulidae species in Iran. Serpulidaeforms dense aggregations in rocky shores in Bandar Abbas. This research investigated the biological structure and the effect of temperature on embryonic stages of *S. kraussii*. Sampling was conducted with a quadrant of 10×10 cm^2, in rocky shores from January to May. The largest specimen of *S. kraussiimeasured* to a total body length of 36.4 mm, body width of 2.3 mm, total tube length of 48mm, and body wet weight of 0.11mg. Outer surface of tube is white and triangular in cross-section, inner surface colored blue or violet. Body color in ventral surface of thorax is dark violet, blue and pink. The sex ratio in *S. kraussii* was 1:2 and 1:0.81 in winter and spring respectively. Length-weight relationship was exponential and growth seems to be allometric for *S. kraussii*. *A linear* relationship was found between total length and other parameters. Correlation coefficients between parameters were positive and significant ($p<0.05$). The specimen of *S. kraussii* in this study was larger than other specimens from different regions. The highest fertilization value and embryonic developmentwere observed at 28 ºC while at 34 ºC lowest fertilization occured and embryonic stage did not develop and cells denatured. The smallest eggs are produced at 34 ºC, whereas the largest are produced at 28 ºC. Results of this study suggest that temperature is an effective environmental factor which influences *S. kraussiilife* cycle, offsprings and survival of larvae.

Keywords: *Spirobranchuskraussii; Life cycle; Persian Gulf.*

1. Introduction

The Family Serpulidae (Rafinesque, 1815), is a member of fouling communities and resident polychaetes attached to hard substrata from intertidal to abyssal (5200 m) zone (Rouse and Pleijel, 2001; Ten Hove and Kupriyanova, 2009; Kupriyanova and Nishi, 2010; Kupriyanova et al., 2011). Serpulidae are easily recognizable by several characters such as, their calcareous tube, operculum, colorful radiolar

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crown, chaeta and uncini (Sun et al., 2016; Sanfilippo et al., 2017). They are able to form dense populations on fouling structures such as quay, mariculture equipment and ship hulls, which provide shelter, food and substrate for many coastal organisms (Cinar, 2006; Hill, 2013). Pores between tubes in Serpulidae polychaetae collect water, serve predators andare breeding place and nursery area for coastal organisms (Vinn, 2013). Serpulidaepolychaetae produce planktonic larvae which, settle to the sea bed after a while and metamorphosize (Bowden et al. 2009; Gosselin and Sewell, 2012). These tubeworms generally undergo four main stages of development in their life cycle, embryonic stages, larval stages, settlement and metamorphosis, growth and maturation (Asgari and Jahangard, 2012). Marine invertebrates release their gametes and sperms directly into water column for sexual reproduction which after fertilization, the negatively buoyant zygotes sink to sea floor, and divide into the blastula stage (Caldwel et al., 2002). Geographic situation and environmental (physical and biological) factors control many phases of the life cycle and embryonic development stages in Serpulidspolychaetae (Asgari and Jahangard, 2012). The role of temperature as an abiotic factor, in the growth, survival, and regulation of life cycle of many polychaetae species is well known (Olive et al., 2000; Simoniand Prevaleni, 2003). The effect of temperature in Serpulid species is dependent to the tolerance of species and can be a serious threat for offspring fitness, adaptability and persistence in this species (Byrne et al., 2010; Byrne, 2012).

The Persian Gulf is a relatively shallow body of water and temperature fluctuations of this Gulf waters have created unique marine and coastal ecosystems (Bayani, 2016). The Strait of Hormuz connects the Persian Gulf with Gulf of Oman and it provides the only sea passage from the Persian Gulf to the open ocean. The Persian Gulf and its coastal areas serve many organisms. It is likely that some species inhabiting the Persian Gulf coastal may be transported from other parts of the world through Strait of Hormoz, entering thePersian Gulf.

Spirobranchuskraussii (Baired, 1865) is a fouling Serpulidaepolychaetae that is newly recorded in the Persian Gulf and covers vast surface of rocky shores of Bandar Abbas (the Persian Gulf, Iran). However, this species is a fouling organismhas and has important ecological role in rocky shores of Bandar Abbas. This species is reported as a species widely distributed in the Indo-Pacific region forming large intertidal aggregations (Belal, 2012). It appeared in Indo-pacific Ocean, first and is now present in most regions of the world (Belal and Ghobashy, 2012).

Physical and biological factors control many phases of the life cycle in marine invertebrates (Simonini and Prevedelli, 2003). The impact of abiotic factors in early stages of life cycle of *S. kraussii* (as an important ecological species) will help us to understand and determine whether abiotic factors, such as temperature fluctuations can change the existence and resistance of this fouling organism in rocky shores of Bandar Abbas. The aims of this study are determining biological structure of newly recorded polychaetaeand the effect of temperature fluctuations on the embryonic stages of *S. kraussii*, in rocky shore of the Bandar Abbas.

2. Materials and Methods

2.1. Study Area and Sampling

Samples were taken from the rocky shore in Bandar Abbas (27̊ 10'53.26 ''N and 56̊19' 08.91'' E) located in Hormozgan province, in the Persian Gulf coast line, Iran (Figure 1). Sixty specimens were collected from 3 stations in $10\times10cm^2$ quadrats at during low tide from January to May (Belal, 2012). Small samples of rock were collected with a hammer and chisel. In the laboratory, all samples were washed with freshwater and preserved in 70% ethanol until further analysis.

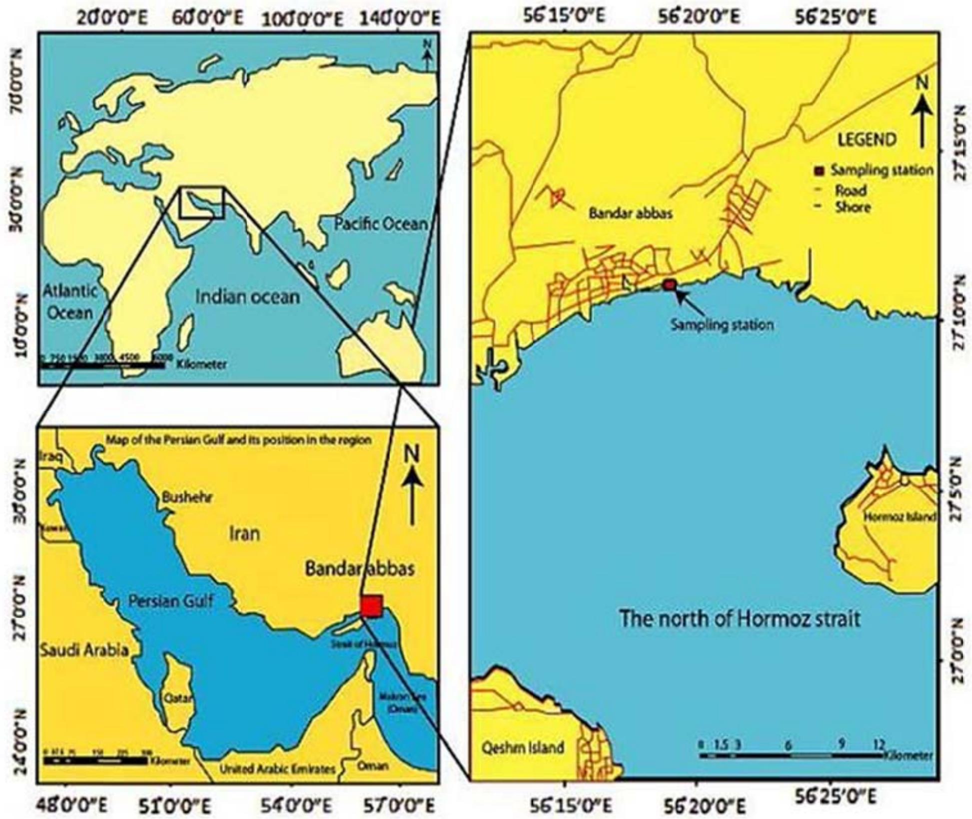

Fig.1: Map of the studied area with sampling station.

2.2. Taxonomic Analysis, Biometrical Measurements and Sex Ratio

To enable measurements of the animals, tubes were broken with a sturdy forceps. Then samples were sorted under a stereomicroscope and the numbers of specimens counted and identified. For identification the family and species of polychaete, we used taxonomic keys and/or descriptions form by Baird (1865), Mohammad (1971), Crisp (1977) and Wehe and Fiege (2002). Serpulids images were taken by a digital camera (Dino-lite, AM-423X) attached to stereo and compound microscopes and equipped with an ocular micrometer. Biometrical measurements of samples such as body weight and length and width and length of tube were recorded with vernier calipers (± 0.05 mm accuracy). In the present study, body length was as an independent variable and body width and length of tube were dependent variable. The “b“represents the relative growth constant (b=1 means isometric growth; b>1 means positive allometeric growth; and b<1 means negative allometeric growth). The statistical significance of “b “was tested by One- way ANOVA, adopting a significance level of 5% (Negreiros- Fransozo et al. 2003) and noting the confidence interval of the regression line (Masunari et al., 2005). The population size structure of *S. kraussiiwas* analyzed in Excel2007. Total length of10 worms/month was measured.

The sex identification in *Spirobranchus* sp. was determined based on the type of gamete (coelomic fluid and immature oocytes) spawned by each individual (Gosselin and Sewell, 2012). Female to male ratio was also calculated.

2.3. Experimental Design

In order to assay the effects of temperature on embryonic stages of *S. kraussii*, adult specimens were collected from rocky shore of Bandar Abbas. Spawning was induced by removing whole animals from their tubes without harm by carefully breaking the ends of the tubes (Asgari and Jahangard, 2012). Then,each worm was placed in a separate vial containing 20 ml of 19ºC seawater (Kupriyanova et al., 2001), to stimulate gamete release after 10- 15 minutes. Coelomic fluid and immature oocytes were removed by decanting off the sea waterafter the eggs had settled. The eggs were washed several times in filtered seawater and fertilized with a small amount of dilute sperm suspension (Kupriyanova and Havenhand, 2002). Later, step embryonic development stages were identified (Anderson, 1973) and images taken by Dino- light camera attached light microscope. Then, Cultures were maintained in 100-ml beakers of filtered seawater in a controlled temperature at 17ºC, 25ºC, 28ºC and 34ºCand light (1000 ± 200) lux in 12/12 light/dark regime. Size of fertilized cells and age at birth (Time or days elapsing between the zygot and hatching from the capsule) were documented.

3. Results and Discussion

3.1. Description and Remarks

The polychaete species were identified as *Spirobranchus kraussii* (Fig. 2) and confirmed by Elena Kupriyanova.

The largest specimen of *S. kraussiiin* this study was total body length 36.4 mm, body width 2.3 mm, total tube length 48mm, body wet weight 0.11mg. The posterior part of body is sharp in male and rounded with small depression in female (Fig. 3). Outer surface of tube is white and triangular in cross-section, inner surface colored blue or violet (preserved specimens). Body color in ventral surface of thorax sometimes is dark violet, blue and pink (Figs 3 and 4). Colorful ciliated feather-like tentacle or radiolar crown and operculum are shown in Figures 3 and 4. The species of polychaetae have a different radioles color. In Serpulid, tubeworms are typically red, pink, or orange in color, with white transverse bands because of astaxanthin (a carotenoid pigment) that is responsible for the bright red color of the crown of *Serpula vermicularis* (Pamela et al., 2000). Primary role of radiol in *S. kraussiiis* to function as an organ for filter feeding, radioles also serve as respiratory organs. The role of operculum is when animal threatened or disturbed, it withdraws rapidly into its protective calcareous tube and used the operculum as a plug to fasten the entrance to the tube (Hanson, 1949).

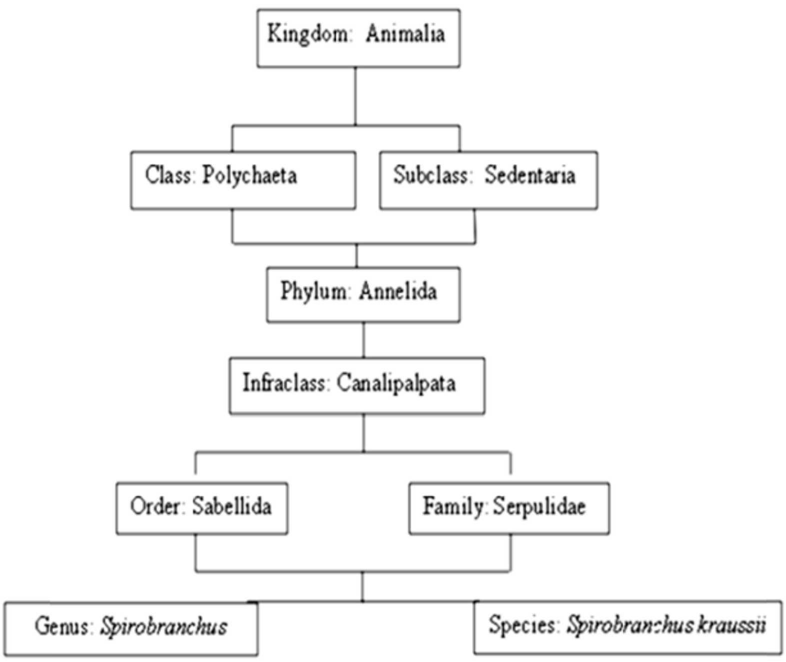

Fig. 2: *Spirobranchus kraussii* classification.

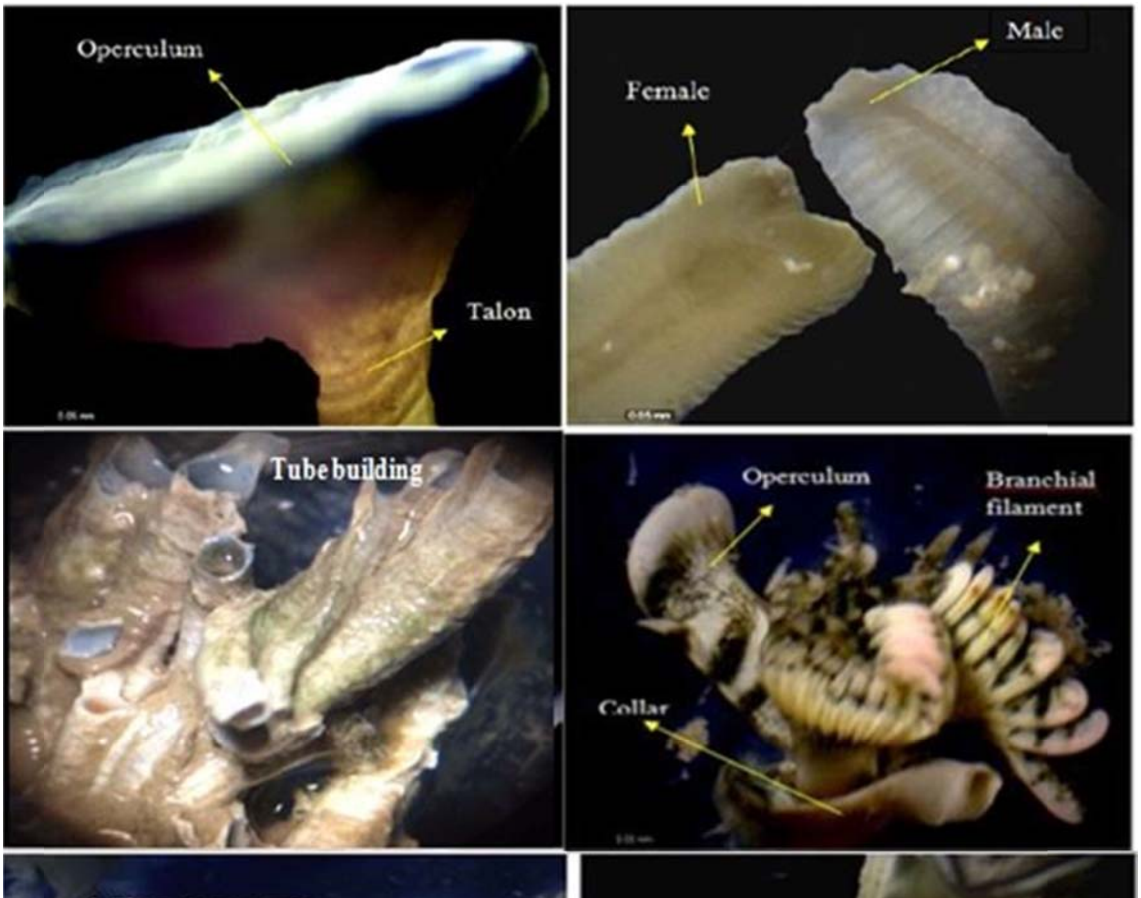

Fig. 3: Parts of *Spirobranchus kraussii body* and tube-building. Scale line 0.05 mm. Photographed by Fatemeh Lavajoo.

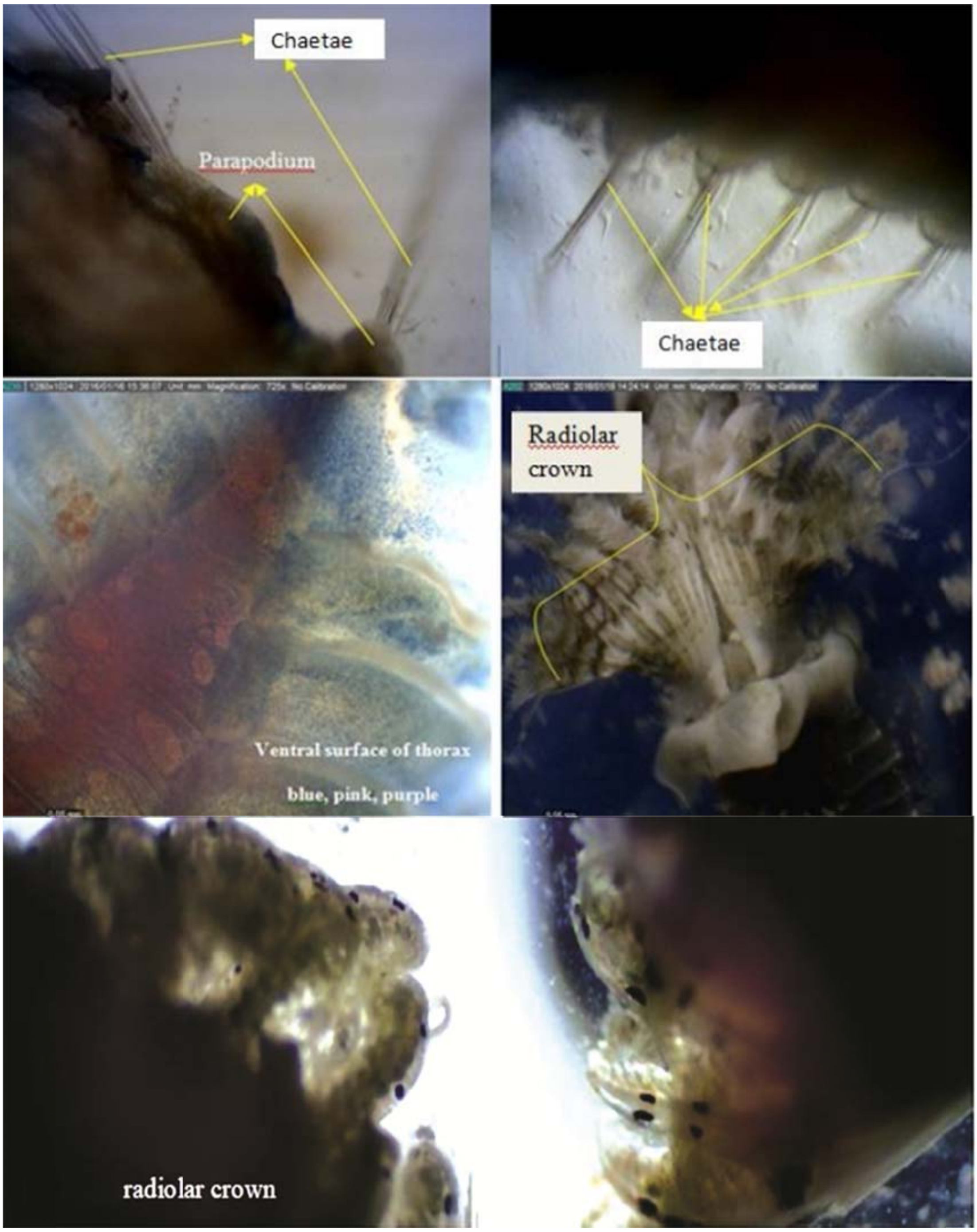

Fig. 4: Parts of *Spirobranchus kraussii body*. Scale line 0.05 mm. Photographed by Fatemeh Lavajoo.

Body length of *S. kraussiiwas* larger in this study than that reported by Crisp (1974) at 25mm, Cinar (2006) from the Levantine coast of Turkey at 10.2 mm and Belal (2012) from Suez Bay at 23mm .

3.2. Biometrical Measurements and Sex Ratio

Cinar (2006) reported the biometrical relationship between body length and wet weight (Fig. 5) is exponential with allometric growth (b= 2.48) as well as linear ($p<0.05$) with other parameters. Results of this investigation, fully confirms Cinar (2006) findings with a smaller allometric growth index (b= 2.015) and positive significant correlation coefficients ($p<0.05$) between parameters. Results of this study show slightly larger range of total length and tube length of *S. kraussii* (8 to 36.4mm and 10 to 48mm, respectively) compared with Belal and Ghobashy (2012). at 7 to 35 mm and 19 to 42 mm, respectively. Simalrly, the 26 mm total length of *S. kraussiiin* the present study was larger than that reported by Pixell (1913) at14 mm, Day (1961) at 25mm and Shalla (1985) at 17mm and Belal (2001) at nearly 26 mm. Wet weight of *S. kraussii* specimens ranged from 0.002 to 0.11mg. There is a direct correlation between the lengths of the worm and its tube, as the worm elongates, its tube length elongates accordingly (Fig. 5). There is no significant difference between the length of body and length of tube (p>0.05). This is a mechanism for saving energy as small specimen does not need building tube (Crisp, 1976). The frequency of total body length in *S. kraussiiin* current study showed the highest frequency occurred in 7-14 mm category during January to May (Figure 6).

The female to male sex ratio of *S. kraussii* was 2:1 and 0.81:1 in winter and spring, respectively, much smaller than reported by (Belal, 2012) for this species from Suez Bay. Smaller sex ratio can be related to reproductive season, weather condition and beginning of spawning season in spring. Spring is the period of female ovary development. The higher sex ratio was probably due to recruitment and increasing the immature stage (63.4%) in winter as observed by Costa, (2003) andBelal (2012). Temperature, day length and lunar cycles are other environmental factors that influence reproductive activity in polychaetae (Belal, 2012; Minetti et al., 2013).

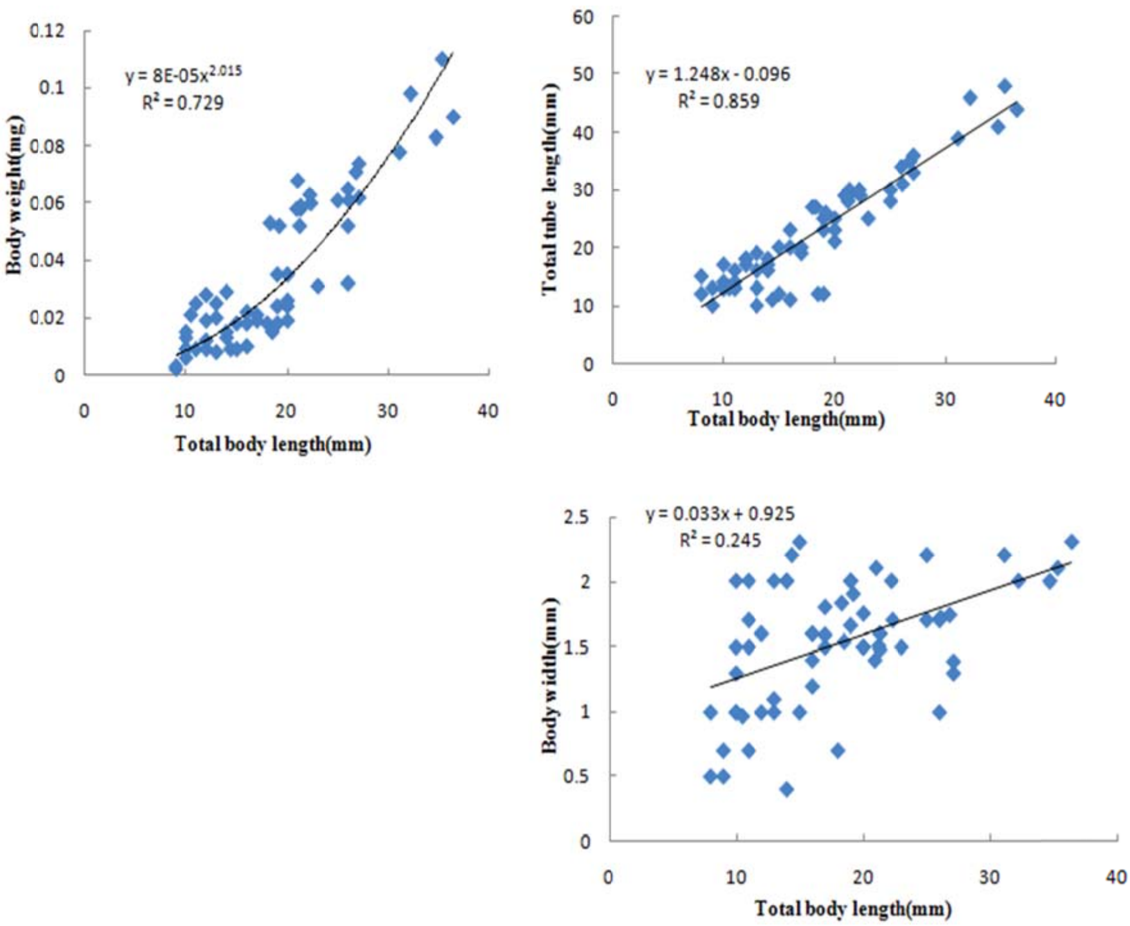

Fig. 5: The relationship between biometrical measurements of *S. kraussii*

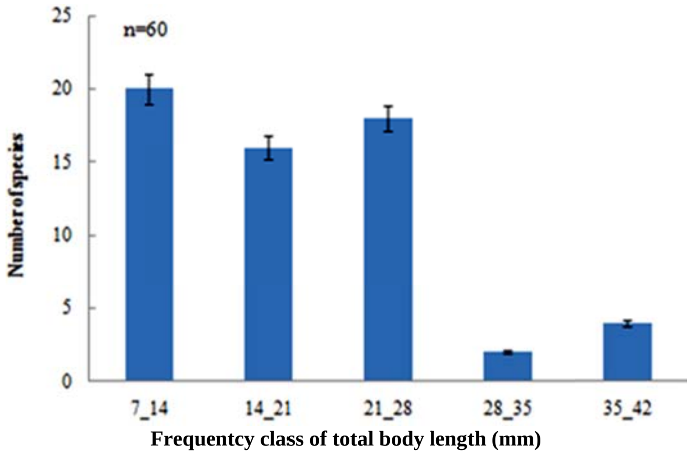

Fig. 6: The frequency class of total body length in *S. Kraussin* Iran

3.3. Effects of Temperature on Embryonic Stage

The specimens of *S. krausiiin* in the current study exhibited broad differences in fertilization index, egg size and timing of development in first stage of life cycle in relation to temperature.

Embryonic stage starts with fertilization and formation of the single-celled enveloped zygote (Fig. 7 A, B and C) followed by first and second cleavage stages (Fig. 7 D and E) to producing a cluster of cells called cleavage (Fig. 7F, G and H). Then, cells rearrange themselves to form a hollow ball called the blastula (Fig. 7 I). Next, the single-layered blastula is reorganized into a three-layered structure known as the gastrula. At the end of gastrula, larva hatch.

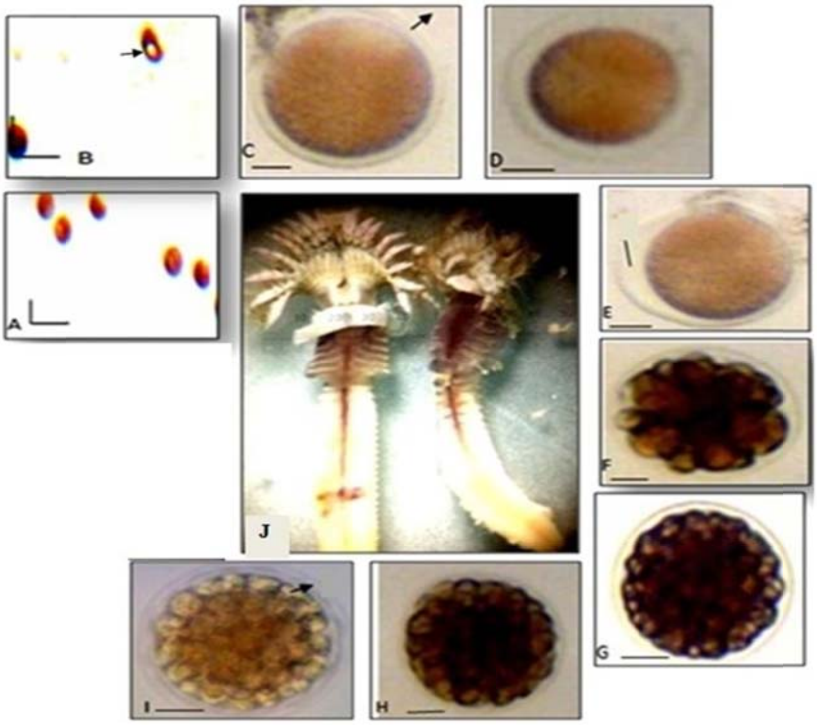

Fig. 7: Early embryonic development of *Spirobranchus kraussii.* (A and B) Newly fertilized zygote, (open arrowhead is polar head),Scale bar: 0.05mm (C) Pre- cleavage stage (open arrow head is fertilization envelope), Scale bar: 0.05mm (D) First cleavage stage, Scale bar:0.05mm (E) Second cleavage stage, (open arrowhead is fertilization envelope) Scale bar:0.05mm (F) >16-cell stage, Scale bar: 0.02mm(G) >32-cell stage, Scale bar: 0.05mm(H)Side view of 64-cell stage, Scale bar: 0.02mm(I) The cells rearrange themselves to form a hollow ball called the blastula(open arrowhead is rearrange cells), The size of egg is 70–80µm, Scale bar: 0.02mm. Total Incubation time is 40h at 25ºC (J) Adult male and female *S. kraussii*.

At low temperature, egg cells do not develop. At higher temperatures and in particular at 28 ºC, however, the development of the embryo is faster (even faster than) at 25 ºC (Fig. 8) while at 34ºC the lowest fertilization and embryonic development occurred and cells denatured. The duration of first phases of the biological cycle at 17ºC is generally longer than those at 25ºC and 28ºC. The time required for the embryos to hatch is reduced significantly as temperature rises ($p< 0.05$) up to 28 ºC. It is reported thatthe best temperature for fist stage of life cycle of *S. kraussiiin* Bandar Abbas occurs at 28 ºC.

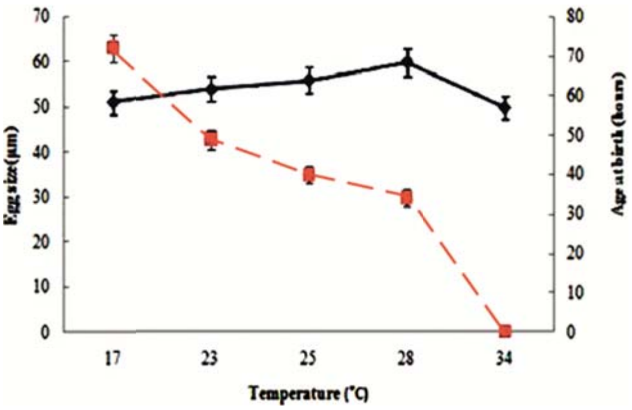

Fig. 8: Effect of temperature on egg size (ϕ) and age at birth (ϯ) of *S. kraussii*. (Error bars represented standard error).

Increasing sperm speed affects fertilization success and is enhanced under warmer water temperatures (Schlegel et al., 2014). In many other species of opportunistic polychaetes, environmental factors influence many phase of the biological cycle (Prevedelli and Simonini, 2000). Temperature has a strong influence on fecundity, size of the eggs and of the ovigerous capsules and also reproductive investment. Simonini and Prevedeli (2003) reported the time required for the embryos to hatch reduces significantly with temperature hikes. In some polychaetes, such as and *Capitella* sp., environmental conditions especially temperature, play a crucial role in controlling the duration of development, growth and reproduction (Mendez et al., 2000; Prevedelli and Simonini, 2000). It is obvious that variability in the size of eggs reflects adaptation to different and fluctuating environmental characteristics (Simoni and Prevaleni, 2003).

4. Conclusion

In the present study, *S. kraussii*, a newly recorded fouling polychaetae species is reported from rocky shore of Bandar Abbas, Iran. We consider *S. kraussii* an important ecological species in the Persian Gulf which is distributed vastly on the surface of rocky shores. It is likely that this species is transported to the Persian Gulf from the Indo- Pacific through attaching to hulls of ships crossing the Pacific Ocean or Indian Ocean towards the Persian Gulf via the Strait of Hormuz. In this study, *S. kraussii* recorded larger biometrics than specimen reported from other regions. This can be because of more stable, favorable and different ecological and climatic (particularly temperature) conditions in the Persian Gulf.

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