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Spawn and Early Larval Development of Spanish Dancer Nudibranch *Hexabranchnus sanguineus* (Rüppell and Leuckart, 1828) (Gastropoda: Nudibranchia)

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ABSTRACT

Hexabranchnus sanguineus is best known as the Spanish dancer was found predominantly on the shallow fringing reef platforms all around Hurghada. Egg ribbons, fecundity and growth of larval stages of four specimens of *H. sanguineus* were studied in the laboratory. It lays a rose shape egg ribbons, attached to suitable hard substrate, varied in lengths and breadths. The egg ribbons lengths ranged from 19.68-20.28 mm. Eggs are clustered together in clear and transparent spawn jelly capsules. Eggs are spherical in shape with 100-113 microns in diameter. The number of eggs in the capsules was not the same in all ribbons, ranged between 8-34 eggs/capsule. The estimated total fecundity of *H. sanguineus* ranged from 1.5×10^6 - 3.6×10^6 eggs. The small, translucent, slow rotary movement trochophore larva was developed within capsules on the 6th day and reached 120-150 μ m. The active mobile veliger larvae were released successively into the surrounding medium like red fumes with 150-190 μ m from 8-9 days. The shell possesses only a single spire. It measures 170-200 μ m in length and 130-150 μ m in height.

Key words: Spanish dancer, nudibranchia, hexabranchnus sanguineus, larval development

INTRODUCTION

Global distribution of opisthobranch (third large group of snail) is a specialized group of phylum mollusk (Bouchet and Rocroi, 2005). Marine habitat of opisthobranch is illustrated by two pairs of tentacles and a single gill located behind the heart. Morphologically diverse group of opisthobranch represents over 6000 species in all over the world (Yonow, 2008) and engage the great variety of ecological niches (Kristof and Klussmann-Kolb, 2010). As a defense mechanism it secretes strong acids or toxins and follows the camouflage characters (Gosliner, 1987; Wagele and Klussmann-Kolb, 2005; Dhivya *et al.*, 2012).

The nudibranchs are amongst the most beautiful and flamboyant of the marine gastropod mollusks as well as the most highly evolved. Because of their attractive color many of them are offered for sale in pet shops (Kasamesiri *et al.*, 2014). They lack a shell as an adult although many planktotrophic species hatch with a larval shell, which is lost at metamorphosis when the animal assumes its adult body form. All species have lost the shell as adults (Yonow, 2008; Sreeraj *et al.*, 2012). Some nudibranch species have secondary metabolites which can be used as an antitumor, antimicrobial and inhibitor for barnacle larval settling (He *et al.*, 2014).

Hexabranchnidae is a monotypic family of colorful nudibranchs (often called "Sea slugs") which contains only a single genus hexabranchnus, with two species, *Hexabranchnus morsomus* and *Hexabranchnus sanguineus* and has no subfamilies (Valdes, 2002).

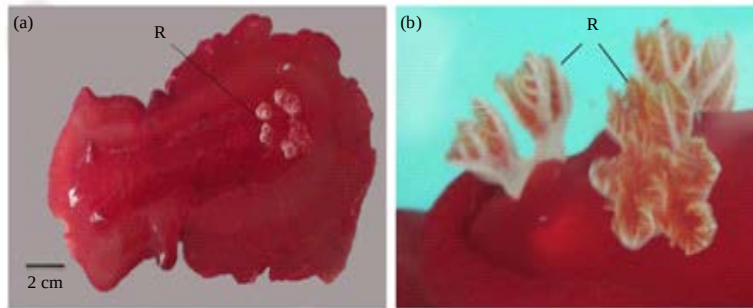


Fig. 1(a-b): (a) Dorsal view of Spanish dancer *Hexabranhus sanguineus* and (b) Rhinophores of the animals

Hexabranhus sanguineus (Rüppell and Leuckart 1828) is best known as the Spanish dancer and is endemic to the red sea, it has a deep red colour with white markings just inside the mantle edge, which is held curled up when the animal is crawling. The rhinophores (Fig. 1b) are deep red and the gills are paler with white axes. Owing to its large size, extraordinary colors and striking forms, it is particularly conspicuous and easily obtained (Gohar and Soliman, 1963; Valdes, 2002).

The Spanish dancer nudibranch, *H. sanguineus* provides a well-documented example, reviewed in Pawlik (1993) of sequestration of chemical defenses by a specialist consumer. It feeds on sponges in the genus *Halichondria* that contain oxazole macrolides that deter predators. The nudibranch modifies these compounds somewhat and concentrates them in its dorsal mantle and in its egg masses where they serve as defenses against predators. Concentrations of the macrolides are little in the sponge, higher in the nudibranch and highest in the egg masses but even the lowest natural concentrations result in strong suppression of feeding by fish (Hay and Fenical, 1996; Debelius, 1998).

Hexabranhus sanguineus is hermaphrodite, the gonad is located on the right-hand side of the body and the gonopore, where exchange of sperm is effected. Individuals are capable of fertilizing each other after fertilization, the animals separate and crawl away to lay their spawn on suitable substrata (Ramakrishna *et al.*, 2010).

The breeding season extends the whole year round but mainly from April to November and attains its climax in June and July. This activity gradually declines with the onset of the cold months of December and January, though it does not cease entirely (Gohar and Soliman, 1963; Yonow, 2008).

Little work has been done on the biology or ecology of hexabranhus as the comprehensive study is that of Gohar and Soliman (1963) on Red Sea, Hurghada and Francis (1980) on Tongatapu Island.

The main aim of this study is to determine and document the spawning habits, egg ribbons, fecundity and growth of larval stages of *H. sanguineus* under laboratory conditions.

MATERIALS AND METHODS

Hexabranhus sanguineus (Fig. 1) were found predominantly on the shallow fringing reef platforms all around Hurghada such as Abu-Galawa and Abu-Sadaf reefs all over the year but they tend to be less active during the winter season. Eight mature specimens of *H. sanguineus* were collected by snorkeling at depths up to 2 m from Abu-Sadaf reefs 900 m from the seashore in

front of National Institute of Oceanography and Fisheries (NIOF), Red Sea Branch, Hurghada, Red Sea (latitude 27°17'37"N, longitude 33°47'10"E), during May 2014.

Collected specimens were taken immediately to the wet laboratory at NIOF, Red Sea Branch and placed in a 1.0 m³ capacity cylindrical fiberglass tank then in continuous observation in rearing glass aquaria. Continuous flow of well aerated sea water was used.

During the first few days after hatching, the water in the tanks was partially changed daily but when the larvae showed positive geotaxis, the still living ones were daily removed by a pipette to new aquaria with fresh filtered sea water.

Although, the aerated sea water was used water temperature was controlled in the tanks using heaters. Water temperature (°C), salinity (‰), pH and dissolved oxygen were measured daily inside the tanks using multiparameter to prevent over-stress. Developmental stages from egg to hatching larvae were observed daily with a stereoscopic microscope.

Animals and egg ribbons dimensions were measured in millimeters using 30 cm soft plastic ruler to prevent tissues damage. Two aspects of the spawns were observed, size of egg ribbons and number and size of eggs. Eggs, pre-larval and larval stages of development were examined and photographed alive with USB camera linked to a binocular stereoscopic microscope and their dimensions were measured in microns using micrometer. Standard Deviations (SD) are given in parentheses.

For the study of fecundity in *H. sanguineus*, the number of eggs per millimeter was counted with an ocular micrometer under an optical microscope. The total number of eggs were calculated based on 30 parts with 1 mm² were taken randomly from the initial, middle and last portion for each ribbon related to the ribbon dimension.

RESULTS

The collected animals of *H. sanguineus* (Fig. 1) had a large body in size, dorso-ventrally compressed, especially when they extended, with generally oval outline. The dimensions body was 17.99 cm (±1.87) and 12.19 cm (±1.41) in length and breadth, respectively.

Immediately after transportation of the animals to the aquarium, they showed their remarkable mating activities that will last for long periods may be several hours or even several days.

Four specimens only laid their spawn ribbons, rose shape in the laboratory (Fig. 2) after bringing from the sea, the 1st and 2nd animals laid their ribbons in the wall of the fiber glass tank within one day, the 3rd animals laid the ribbon in the middle tube which used for exchange water after two days and the 4th animals laid the ribbon in the small glass aquaria after five days.

Water temperature (°C), salinity (‰) and pH were measured daily inside the tanks using multiparameter, where the water temperature was controlled in the tanks using heaters and the water temperature was 24°C, salinity was 41.1‰ and the pH was 7.9.

The four egg ribbons shape as the same, generally, spirally coiled in anticlockwise direction into up to 4 unequal rounds and its dimensions varied from animal to another depends on the animal size, the average length was 731.5 mm (±85.5) and the average breadth was 21.9 mm (±2.3), for the four ribbons, as shown in Table 1. The color of the newly deposited egg ribbon ranged from reddish orange to rose-red or dark red (Fig. 2) but by time the color been dark orange as a result of eggs growth and metamorphosis process.

Eggs are clustered together in clear and transparent spawn jelly capsules which varied in shape and dimensions (Fig. 2). Generally, eggs are spherical in shape, with 100-113 microns in diameter and the red yolk globules are concentrated at the vegetative pole (Table 1 and Fig. 3). The number of eggs in the capsules was not the same in all ribbons, ranged between 8-44 eggs/capsule according

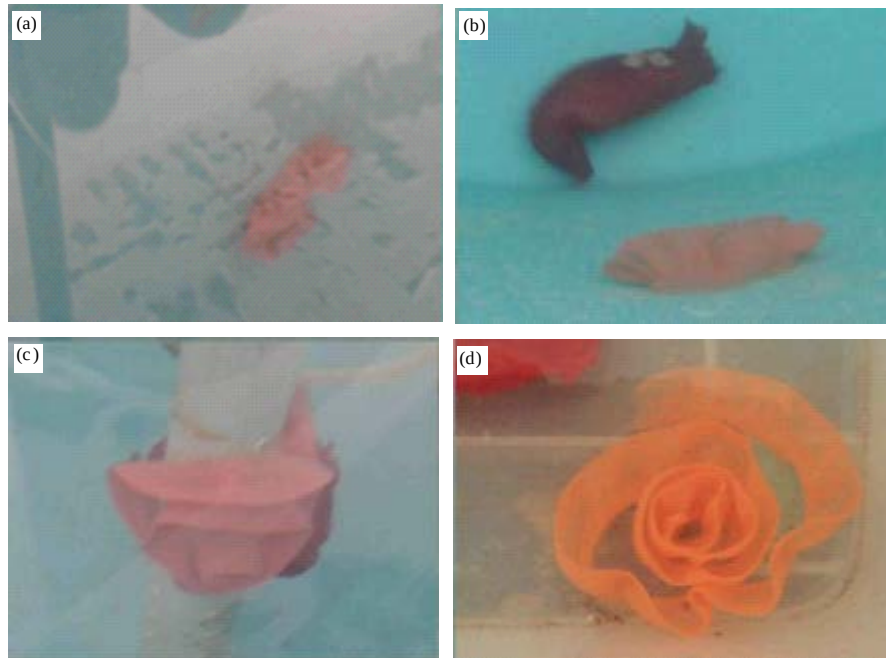


Fig. 2(a-d): Four spawn ribbons of the four *Hexabranhus sanguineus* which laid their spawn ribbons in the laboratory (a, b) Two specimens laid their ribbons in the wall of the fiberglass tank within one day, (c) One specimen laid the ribbon in the middle tube which used for exchange water after two days and (d) One specimen laid the ribbon in the small glass aquaria after five days

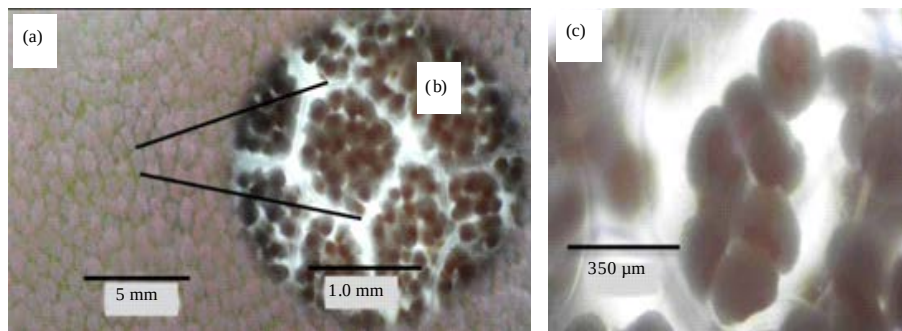


Fig. 3(a-c): (a) Part of egg ribbon (b) Eggs capsules and (c) Eggs from a portion of egg ribbon of *Hexabranhus sanguineus* in different magnification

Table 1: Average size of specimens, egg ribbons and egg diameters

Samples	Animal dimensions		Egg ribbon		Egg diameters	
	Length (cm)	Width (cm)	Length (mm)	Width (mm)	Length (μm)	Width (μm)
1st	15.6	10.8	652	19.5	110±2.65	102±4.03
2nd	18.8	12.3	703	21	105±5.45	101±3.99
3rd	19.3	13.1	715	22	112±1.02	102±2.87
4th	19.9	13.8	852	25	111±1.09	099±5.69
Average	18.4±2.0	12.4±1.5	730.5±85.5	21.9±2.3	110±3.12	102±1.61

to the size of the animal and the position of the capsule in the beginning or in the last portion in egg ribbon. The estimated total fecundity of *H. sanguineus* based on number of eggs per one square millimeter ranged between 1,510,423-3,621,000 eggs (Table 2).

Development begins immediately after oviposition within egg ribbon up to veliger larvae in about eight days. Hatching starts along the whole ribbon almost at the same time because the embryos of one spawn (same ribbon) are nearly of the same age and due to the short period of oviposition, the embryo (Fig. 3) reached 100-130 μm on 4th day after hatching within capsules. The small, translucent, slow rotary movement trochophore larva was developed within capsules on the 6th day and reached 120-150 μm .

From 2-3 days before hatching Ribbon loses its flexibility and cohesion and become flaccid and soft moreover the colour of the ribbon loses its brightness and become dirty red blessed. The developmental larvae become in active circling movement within their capsules, searching for an exit from the ribbon. The water movement effects on larvae liberation from egg ribbons (Fig. 4b). From 8-9 days the active mobile veliger larvae (Fig. 4) were released successively into the

Table 2: Average numbers of egg ribbons, egg clusters and eggs in each animal

Samples	No. of capsules		No. eggs per capsule	No. of eggs (mm^{-2})	Fecundity
	1.0 mm^2	Ribbon			
1st	6.6 $\pm$ 1.3	83,912	18.2 $\pm$ 9.6	120.2 $\pm$ 10.8	1,510,423
2nd	7.1 $\pm$ 1.5	104,817	16.7 $\pm$ 7.8	118.6 $\pm$ 8.7	1,677,076
3rd	6.1 $\pm$ 2.2	95,953	22.6 $\pm$ 4.4	137.9 $\pm$ 15.6	2,110,966
4th	6.8 $\pm$ 1.9	144,840	25.1 $\pm$ 2.9	170.1 $\pm$ 20.2	3,621,000
Average					2,229,866

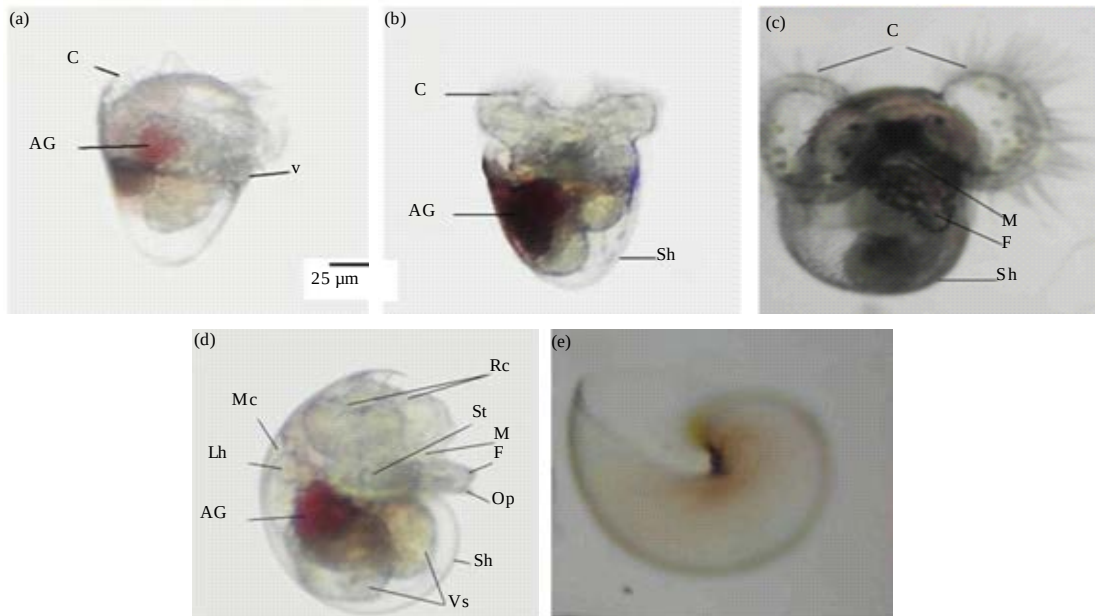


Fig. 4(a-e): Showing larval development stages of *Hexabranhus sanguineus* (a) Dorsal view of 6 day old embryo, (b) Ventral view of 8 day old embryo, (c) Ventral view, (d) Lateral view of newly hatched veliger larvae with 10 day old showed two nearly 8 shaped lobes with markedly long and active cilia and (e) Colorless larvae shell which appeared in the larval stages only, Mc: Mantel cavity, Lh: Larval heart, AG: Anal gland, Rc: Retractable cilia, St: Statocyst, M: Mouth, F: Foot, Op: Operculum, Sh: Shell, Vs: Visceral mass, C: Cilia, V: Velum

surrounding medium like red fumes (Fig. 4c and d) with 150-190 μm and swim actively upwards then it moves down towards the bottom at the corner of the aquarium. In the newly hatched veliger larvae, the mantle, operculum and shell cavity were observed which were in a reddish color.

The shell (Fig. 4e) possesses only a single spire. It measures 170-200 μm in length and 130-150 μm in height. It is nearly colorless and growth lines are moderately visible.

DISCUSSION

Nudibranchia is the largest group in opisthobranchia with more than 3500 described species. The gills can be used for identification. Some species wave their gills as they move or feed. Nudibranchs live in marine environments from Antarctica to the tropics and dwell at virtually all depths of sea but reach their greatest size and variation in warm, shallow waters (Ramakrishna *et al.*, 2010).

Nudibranchs lay their eggs in flat ribbons attached to rocks or other objects (dorids) or in tangled masses attached either to the sea bottom or to algae and other objects (aeolids). The eggs, often multiply enclosed in capsules (as opposed to singly enclosed) are embedded in a mucous matrix that both supports them and protects them (Goddard and Green, 2013). *Hexabranhus sanguineus* as a dorids, laid their eggs in flat ribbons attached to the wall of the fiberglass tank, in the middle tube which used for exchange water and in the small glass aquaria in the laboratory. Nudibranch egg masses can be identified by placement, color, dimensions, number of eggs per capsule and capsule spacing (Hurst, 1966). The color of the newly deposited egg ribbon of *H. sanguineus* ranged from reddish orange to rose-red or dark red as mentioned in Gohar and Soliman (1963).

The number of eggs is depending on the availability of food and the environmental influences, which may be considered as controlling factors such as water temperature, salinity and pH (Mahmoud *et al.*, 2013) and also dependent on size ranging from 3×10^6 - 140×10^6 (Hadfield and Switzer-Dunlap, 1984). The present study was agreed with the study of Gohar and Soliman (1963), which showed that the period which the embryos spend within the capsules before hatching depends mainly upon the temperature of the surrounding water while it lasts only 6 days at 27°C, it extends up to 12 days at 22°C. In the present work the period extended to 10 days at 24°C. The number of eggs ranged between 1.5×10^6 - 3.6×10^6 eggs was same as that of Gohar and Soliman (1963) in the same species sizes (1.9×10^6 - 4.0×10^6 eggs).

Spawn is characteristic for each species in nudibranch species, in shape and form, colour size and arrangement of eggs within. The eggs hatch either into planktonic veligers, a swimming larval phase which then settles and begins benthic life or into a minute crawling juvenile (Yonow, 2008; Mahmoud *et al.*, 2013). Most nudibranch species produce planktonic larvae that remain in their larval phase for periods of minutes to months, sometimes dispersing over great distances before settling on suitable substrata (Todd *et al.*, 2001). The larvae of *H. sanguineus* hatches as a swimming veliger and metamorphosis will occur after a few days in the plankton.

Various life-cycle stages of opisthobranch mollusks have served for research in such diverse areas as behavior, development and ecology (Faucci *et al.*, 2007). In particular, adult opisthobranchs have become premier models for neurobiological investigations, because neurons in their central nervous system are large and easily identifiable and manipulated (Cohen *et al.*, 2006; Schlesinger *et al.*, 2009).

Hexabranhus sanguineus veliger larvae as nudibranch veligers are all relatively similar anatomically. Some features do different color of digestive gland, possession of eyes, behavior

within egg capsule, behavior after hatching and dimensions of the shell and sculptural patterns of the shell (Thompson, 1966). The hatching veligers swim upwards after hatching and after a few hours they begin to swim randomly over the bottom. This corresponds to the two phases of veliger behavior found for the nudibranchs studied by Hadfield (1963) and the same species by Gohar and Soliman (1963).

CONCLUSION

The present data showed that the perfect conditions to spawn *H. sanguineus* in the laboratory must be in suitable conditions; 24°C for water temperature, 41.1‰ for salinity and 7.9 for pH. Additional food is not required up to veliger larvae. Water renew is very important for larval survival. The information obtained from this study may help to support enhancements in nudibranch ecological and commercial culture for future utilization and conservation.

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