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Intracapsular embryogenesis and larval development of *Chicoreus ramosus* (Linnaeus, 1758) and *Dendropoma platypus* (Mörch, 1861) (Gastropoda, Prosobranchia) under normal and treated conditions

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ABSTRACT

Samples of *C. ramosus* and *D. platypus* were collected from Hurgada, the Red Sea during June-August 2018. Samples were transported to the lab at the University of Alexandria, Egypt. This study aims to describe the embryogenesis of *C. ramosus* inside the capsules and test the effects of signaling factors and neurotransmitters on the *in vitro* transformation of larvae of *D. platypus* and identify some bioactive inducers that increase larval metamorphosis. Capsules of *C. ramosus* were fixed in 10% saline formaldehyde and capsules of *D. platypus* were placed in plastic aquaria for immune-histochemical test. The sequence of the embryonic transformations and the duration time of each step of *C. ramosus* were identified. The importance of the following experiment is to provide a suitable medium for the developing larval stages and improve eugenic manner of the new generations. Undertreated conditions of *D. platypus* larvae, MF 2.5 mM, C8:0 1 μM, ADMA 2.5 mM, and Acetylcholine 0.5 mM larval transformation from trochophore to veliger to early juvenile stages was accelerated than larvae of control in seawater. Serotonin 10 μg/ml and Acetylcholine 1 mM exerted a negative effect while C8:0 0.1 or 10 μM, MF 300 μM, ADMA 1 mM and 1.5 mM did not affect larval transformation. Atorvastatin (5, 20 and 40 mg), Pravastatin (50 mg and 100 mg), Cetirizine hydrochloride (10 mg), Nebivolol (2.5 mg, 5 mg. and 10 mg), Atenolol (25 mg and 100 mg) and Amlodipine Besylate (2.5 mg, 5.0 mg, and 10 mg) exerted a negative impact on larvae in all stages and mortality predominated.

Introduction

Chicoreus ramosus belongs to Clade Neogastropoda, Superfamily Muricoidea, Family Muricidae, Subfamily Muricinae. The shell has an impressive size 20–30 cm long, evokes thick and thorny leaf like a murex with twigs, usually white. Bright and orange edge is present at the shell aperture. Large brown operculum made of a horn-like material. This chicory is large and fleshy, grayish-beige. It is a predatory carnivorous species, usually feeding on bivalves and other gastropods, found in Red Sea, from east to South Africa, the Gulf of Oman, Aldabra, the western central Pacific Ocean and the eastern Polynesia, southern Japan, New Caledonia and Queensland in Australia (Poutiers, 1998, Mostafa et al., 2013, Salas & Chakraborty, 2020). *Dendropoma platypus* belongs to Clade Caenogastropoda (Cox, 1960), family Vermetidae. It is sessile and suspension-feeder, found in Hawaiian Islands, French Polynesia, Australia, Red Sea and the Mediterranean Sea (Gohar & Eisawy, 1967, Mostafa et al., 2013, WoRMS, 2017). These worm-snails grow very irregularly, given its gregarious nature. Isolated specimens usually adopt a circular arrangement on rock, with a tendency for the shell to grow turning to the right. The sculpture and its external color can only be seen in isolated specimens. The shell last whorl diameter to 21 mm; shell length to 40 mm, Aperture diameter to 9 mm, four coiled whorls. The external color is usually more or less light grayish and irregular. Internally, the shell is smooth, shiny, and variable in color, usually dark wine-colored near the opening (Golding et al., 2014). The embryonic and larval stages of *Chicoreus ramosus* are described (Mostafa et al., 2013). Reproductive Biology of the Gregarious Mediterranean Vermetid Gastropod *Dendropoma Petraeum* and *D. irregular* were studied (Calvo et al., 1998, Spotorno-Oliveira et al., 2015).

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The Muricidae and Vermetidae include marine species of internal fertilization that deposit their eggs inside benthic capsules where embryos develop partially or completely (German et al., 1998). Development patterns of these families are pelagic type, demersal and direct (Mahmoud et al., 2013; Mostafa et al., 2013). Easy acclimatization of Muricidae and Vermetidae in ecosystems is associated with the low mortality of embryos during their intracapsular development (Bandel, 1976). This mode of development has made this group an interesting research material (Spight et al., 1974, Gallardo, 1979). Two main types of larval development of marine invertebrates were identified (Bandel, 1976), referred to as Planktonic and non-planktonic feeders. This second type can be subdivided into: Lecithotrophic and intracapsular metamorphosis when metamorphosis occurs before hatching (Jagadis et al., 2013). Many gastropod species provide their embryos with extra-embryonic sources of nutrients, such as nutritious eggs and nutritive fluids (Gallardo, 1979). Among the neogastropods there are many species that have lecithotrophic larvae and develop entirely inside capsules (Bandel, 1976; Saglam & Duzgunes, 2007; Jagadis et al., 2013). Encapsulation is quite common among gastropods as being gelatinous mass, or consistent, or lenticular capsules (Naegel & del Prado-Rosas, 2004). These egg capsules have a complex chemical structure (Bandel, 1976) and its formation requires morphological, physiological and behavior of adults (Hyman, 1967). According to Vasconcelos et al. (2004) and Jagadis et al. (2013), indirect development involves a morphological stage between the embryo and the juvenile. In this way, protected larvae would be considered with indirect development. In direct development, according to these authors, the embryonic stages are followed by the direct formation of the juvenile without an intermediate larval stage. The types of larval development were reviewed by (Bandel, 1975, D'asaro, 1988). Hatching as veliger larva was found in Vermetidae as *D. platypus* (Mörch 1861) (the gastropod under study) and *D. maximum* (Sowerby, 1825) (Kloppel et al., 2013). The Complete development within the capsule is found in some Muricidea as *C. ramosus* (Linnaeus, 1758) (the gastropod under study). Veliger larva and the capsule of *Chorus giganteus* (Lesson, 1831) were histologically described (German et al., 1998). According to these authors, larval development occurs within the capsule with a free veliger stage.

The nervous system forms the basis for information transfer and responsible for metamorphosis within invertebrates (Lemaire & Marcellini, 2003). By immunohistochemistry (Pennati et al., 2013) investigated the transformation of the peptidergic (GLW-amide and RF-amide) larval neuroanatomy at different stages of metamorphosis and the subsequent development of the primary polyp nervous system. Authors focused to study the role of neuropeptides on the larval metamorphosis (Pennati et al., 2013). Other authors interested to study the messenger nitrogen monoxide (NO) and cyclic nucleotides on neuronal outgrowth and larval metamorphosis (Jungmann et al., 2004). Asymmetric dimethylarginine (ADMA) is an endogenously competitive inhibitor of NO synthase (Shibata et al., 2008). Several studies have shown the effect of 1,2-dioctanyl-*rac*-glycerol (C8), Serotonin (5-HT), and acetylcholine in signaling systems, neuronal control (neurotransmitters) or control of morphogenetic and behavioral reactions on metamorphosis of marine invertebrates (Urrutia et al., 2004, Bishop & Brandhorst, 2007). Methyl Farnesoate and serotonin were proven to accelerate metamorphosis in hydroids, ascidians, barnacles and molluscs (Glebov et al., 2014).

This study aims to follow the embryonic development of *C. ramosus* inside the capsules; larval metamorphosis under normal condition, characterize the main transformations undergone by the embryo during this process. Our study tried to identify some bioactive inducers that increase larval metamorphosis of *D. platypus* and some water toxicants that hinder the cultivation in aquaculture. In the present study, we investigated the effects of signaling factors and neuro-transmitters on the *in vitro* transformation of larvae of *D. platypus* to provide a suitable medium for the developing larval stages and improve eugenic manner of the new generations.

Material and methods

Samples collection

Samples of *C. ramosus*, intertidal species and *D. platypus,* subtidal species were collected from rocks, reefs and iron objects of six sites within the northern lagoon of Hurgada, the Red Sea from June-August 2018. Identification of both species were carried out according to (WoRMS, 2017). Snails were collected from different patch reefs and rocks across each site. Live samples of *C. ramosus* and *D. platypus* were placed in coolers, covered in seawater with air pumps and transported to the Invertebrate lab, Faculty of Science, at the University of Alexandria, Egypt.

Characterization of embryonic and larval phases in C. ramosus

Recognition and characterization of *C. ramosus* embryonic and larval developmental phases were performed using capsules collected from the aquaria. Samples were taken using a rectangular dredger with dimensions of 40 x 15 cm blades. The retained sediment in the dredger bag containing the biological material was sieved using a 1-mm mesh being deposited in labeled plastic aquaria and fixed in a 10% saline formaldehyde solution. Other live capsules of *D. platypus* were placed in aquaria for an immune-histochemical test under constant aeration, salinity and temperature always close to those measured in the environment, respectively, 35 and 25–26 °C. The samples were sorted and the capsules were separated and preserved in 70° GL alcohol. Subsequently, measurements and observations were made regarding diameter and height, coloration, presence of egg in the stage, and type of adhesion substrate, respectively. Dimensions were obtained through a stereomicroscope (Zeiss, Germany) with a micrometer eyepiece and precision millimeter caliper 0.05 mm. The capsules were opened through an incision in the operculum suture. The photographs were made by a clear camera stereomicroscope.

Immunohistochemical test in D. platypus larvae

After ≈ 12 days post-fertilization, opercula were incised from 120 capsules. Viable veliger larvae were collected with micropipettes. Larvae were always fed on soft parts of bivalves. Using glass beakers of 50 ml and a micropipette, ≈ 80 veliger larvae were placed in each beaker with seawater. The first beaker was left with seawater as control and the seawater in the other beakers was sucked and replaced with a particular bioactive inducer with a definite concentration to test for its effect on larval transformation from pre-veliger to veliger to juvenile stages according to (Bishop & Bates, 2002; Bishop & Brandhorst, 2007). Each beaker was tested at intervals for 12 days. Post-fertilization to 25 days post-hatching. The bioactive inducers were: seawater (control) (FSW, mesh size ¼, 0.5–1.0 mm); MF (Methyl Farnesoate) (2.5 mM – 300 μM), claimed to induce larval molting and growth; 1,2-dioctanyl-*rac*-glycerol (C8) (10 μM – 1 μM – 0.1 μ M) claimed to be involved in signaling systems; Serotonin (10 μg/ml); Acetylcholine (1 mM – 0.5 mM); Asymmetric dimethylarginine (ADMA) (1 mM – 1.5 mM −2.5 mM), Acetylcholine (1 mM – 0.5 mM – 30 g/ml) and cGMP (1 μM); claimed to serve in neuronal control (neurotransmitter).

The main purpose of this technique was to provide a suitable medium necessary for the transformed larval stages and to avoid larval mortality. This study investigated the impact of lipid-regulating agents Atorvastatin (5, 20 and 40 mg) and Pravastatin (50 mg and 100 mg). The protocol used was according to Bishop and Bates (2002) and Bishop and Brandhorst (2007). The antihistaminic Cetirizine hydrochloride (10 mg), the β-blocker Nebivolol (2.5 mg, 5 mg. and 10 mg) and Atenolol (25 mg and 100 mg) and the calcium-channel blocker Amlodipine Besylate (2.5 mg, 5.0 mg, 10 mg) on larval transformation from trochophore to veliger (according to (Ali et al., 2016). All chemical agents were dissolved directly in Phosphate buffer saline (PBS) and poured in the cultures. Each trial was in triplicate; the mean of the larval and juvenile stages were calculated and subjected to a One-way analysis of variance (Dijana et al., 2012).

Results

Laboratory observations of C. ramosus

Snail characterization; grayish-white solid shell; well-developed spines; spiral furrows; white opening with a denticulate edge; pink columella and horny operculum (Fig. 1a). *C. ramosus* fed in the aquaria by pressing its foot onto the shells of its prey until opening them enough to enter its long proboscis. The copulatory activity was observed sporadically involving a single couple to the time. Oviposition time varied in relation to the number of capsules laid and it took place continuously and uninterruptedly. The average time to complete oviposition of each capsule was 35–45 min. The capsules, after being laid, they were molded by the female's foot and were immediately adhered to the substrate by their peduncles.

Intracapsular embryogenesis of C. ramosus

The snail is characterized by the complete absence of external dimorphism. Anatomy of gonads indicated that females bear a penis while ovary was mature. It seems that this species is a protandric hermaphrodite exhibits gonochoric and broadcast spawning. During the study period (June – August 2018), the total number of collected egg capsules were 857 (Fig. 1b) (262, 256 and 139 capsules respectively). Of these, 514 were intact with the embryo in perfect condition, 201 ruptured and 142 showed signs of predation. 209 egg capsules with diameter 120.28 ± 3.82 mm were adhered to the side walls of aquaria representing 40.66%, 115 ones with diameter 119.75 ± 7.25, on rocks representing, 137 ones 26.65% on molluscan shells with diameter 120.12 ± 6,11 and 53 ones with diameter 119.68 ± 9.16 representing 10.31% were dropped. The molluscan shells used to fix the capsules were generally elongated, flattened and with frayed edges. The capsules were often adhered close to the edge of these shells. The capsules were spherical, flexible, opaque white in color and with parchment texture. Presented broad adhesion base (Fig. 1a). On average, capsule measurements were: diameter 318.95 ± 0.33 mm; height 10.52 ± 0. 25 mm. The wall of each capsule was composed of several layers, the outer one being interrupted, forming a suture where the operculum comes off. The operculum was located in the lateral- dorsal portion of the capsule with a diameter of 10.13 ± 0.37 mm and height 9,5 ± 0.14 mm. Through this structure, the juvenile ruptured the capsule at the time of hatching.

Characterization of developmental phases of C. ramosus

In the ovigerous capsules, three externally differentiated structures can be recognized. The peduncle, a thin and resistant fila-

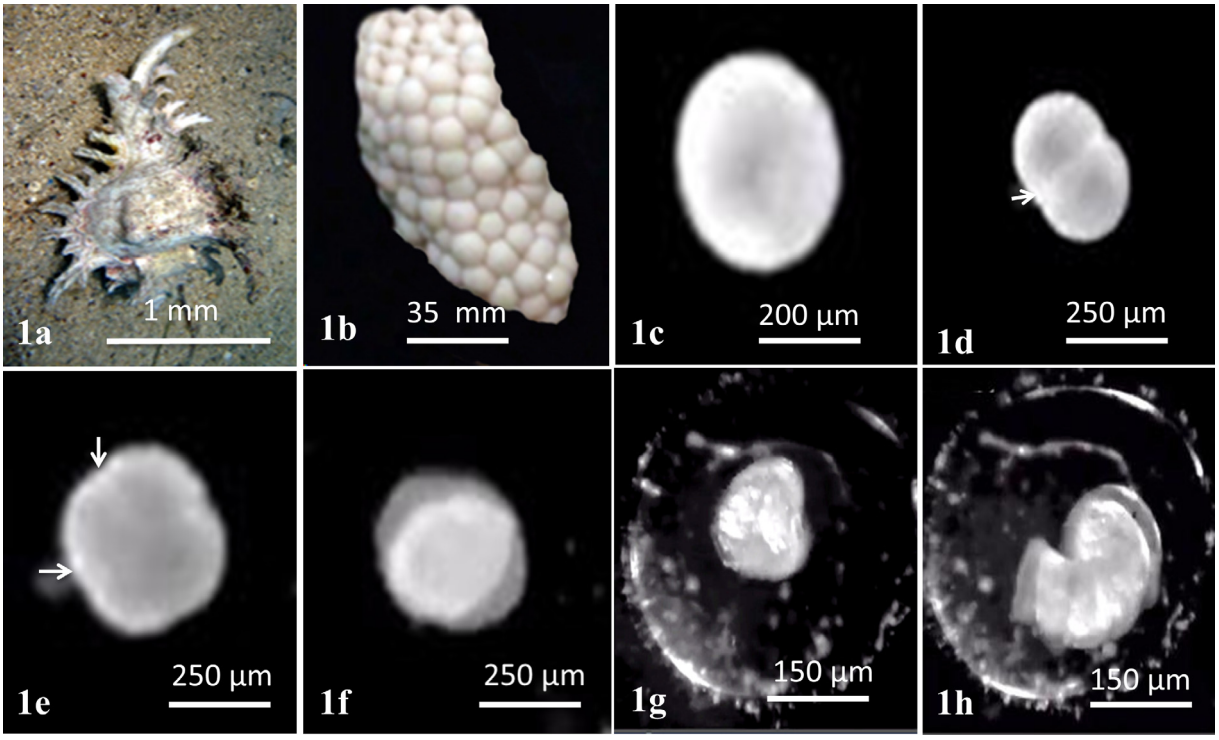

Fig. 1. Phase contrast photomicrograph of *Chicoreus ramosus* embryonic stages: **a.** WM shell. **b.** egg capsule. **c.** eggs develop inside the albumen and intracapsular fluid. **d., e. and f.** egg cleavage. In steps C1 and C2, egg transformations were observed. **g and h** pre-veliger and veliger larvae. **i.** advanced stage of veliger larva. **j.** larval heart beats were observed. **k.** a developed foot, two ciliated trochs and spiral shell. **l.** bilobulated velum, operculum, cephalic region and a hardened globular protoconcha. **m.** the shell of embryonic stem of the veliger. **n.** the juvenile ruptured the suture of the capsule.

ment of pale yellow color attached at its base to a small fixing disc, which kept the capsules solidly adhered to the substrate. The capsular body was semi-transparent structure, slightly curved and resistant very similar to the peduncle, in which they stand out two longitudinal sutures that divide it into two symmetric halves. Inside, the eggs develop inside the albumen and intracapsular fluid (Fig. 1c). The apical end of the capsular body presents a hole of app. 1 mm in diameter, which was sealed by a mucous plug, known as operculum. As the time of hatching, it lost its hardness and consistency, which would facilitate the exit of the embryos from inside the capsule.

From the analysis of the 514 intact capsules collected during the study period, it was possible to characterize the main stages of embryonic and larval development of *C. ramosus*. The sequence of the transformations and the duration time of each step were identified: egg, cleavage (C1, C2, C3), pre-veliger, veliger and intracapsular juvenile. Within each capsule there were 15–32 viable eggs, measuring 122 ± 3.62 μm (Fig. 1c), and rich in vitelline and bright viscous material. Egg cleavage was spiral. Three main phases were characterized: C1, C2 and C3 (Fig. 1d, 1e, 1fc). In steps C1 and C2, egg transformations were observed without any noticeable sign of movement. In phase C1 (118 ± 3.67 μm), cleavage and early cell divisions were observed. At this stage, the split egg measured app. 282 μm. In phase C2 (125 ± 4.13 μm), macro- and micromers were differentiated with nutrient reserves concentrated in the macromers. In phase C3 (130 ± 2,31 μm), gastrula stage was formed, with the beginning of the torsion and the formation of a thin organic matrix on the vegetative pole of the embryo, which give rise to the embryonic shell (protoconcha). The next two steps, pre-veliger and veliger had distinct larval characteristics (Fig. 1g and1h). The emergence of the thin transparent embryonic shell and larval structures were observed such as the velum, the operculum and differentiation of the cephalic region (Fig. 1i). There was still a large amount of vitelline in these two larval stages. App. nine days after the beginning of cleavage, the appearance of the pre-veliger was observed. In this phase, the embryo had started to make slow rotational movements, contractions and movement of the operculum, larval heart beats were recorded (Fig. 1j). The pre-veliger measured app. 140 ± 6,60 mm (Fig. 1g). The veliger 150 ± 1. 74 mm appeared 15 days after the beginning of the cleavage, being possible to visualize a distended foot, two ciliated trochs and spiral shell (Fig. 1k). Veliger presented larval structures such as bilobulated velum, operculum, cephalic region, larval heart which were well developed and with a hardened globular protoconcha. A reduction in the amount of vitelline was observed especially in the second stage of veliger (Fig. 1l). In the third stage of veliger larva, metamorphosis took place, which was characterized by the resorption of transient larval structures and the appearance of muscular foot, digestive system, heart and palliative organs. The shell of embryonic stem of the veliger presented an average of 16.675 ± 2.68 mm in total length (Fig. 1m). In the intracapsular juvenile, the shell became opaque when it had 1.5 turns. the siphon and the well-developed feet were observed (Fig. 1i and 1j). The intracapsular juvenile (165 ± 5.31 mm) appeared app. 23 days after the beginning of the cleavage, showing quite active, crawling on the walls of the capsule. At a later stage, due to the increase in its size, it no longer moved much, however it made movements with the foot, forcing it against the operculum over the rupture line (suture). After an average of 33 days from the beginning of the cleavage, the juvenile ruptured the suture of the capsule (Fig. 1n); showed great mobility, seeking refuge in the sediment where quickly buried. This stage had consumed all the vitelline reserve and was observed alive in the aquaria while feeding on planktotrophic.

Immunohistochemical test in D. platypus larvae

Undertreated conditions of veliger larvae (Fig. 2a), MF 2.5 mM and 300 μM, C8:0 1 μM, Asymmetric dimethylarginine (ADMA) 2.5 mM, and Acetylcholine 0.5 mM and cGMP 1 μM transformation

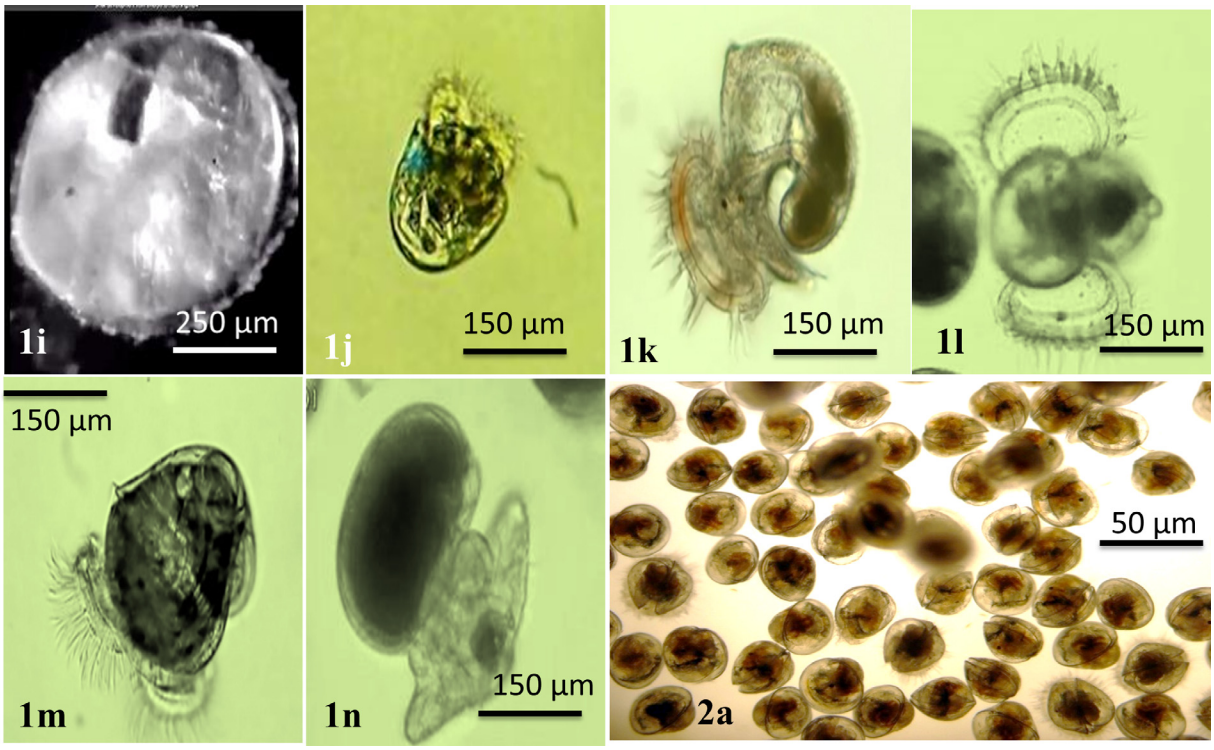

Fig. 2. a veliger larva of *Dendropoma platypus*. b.–c. impact of bioactive inducers on the larval transformation of *Dendropoma platypus*.

from veliger to juvenile stages was accelerated than larvae of control in seawater (Table 1 and Fig. 2b and 2c). Serotonin 10 μg/ml and Acetylcholine 1 mM exerted a negative effect while C8:0 0.1 or 10 μM, MF 300 μM, Asymmetric dimethylarginine (ADMA) 1 mM and 1.5 mM did not affect larval transformation. This study investigated the impact of the lipid-regulating agents Atorvastatin (5, 20 and 40 mg) and Pravastatin (50 mg and 100 mg), the antihistaminic agent Cetirizine hydrochloride (10 mg), the β-blocker Nebivolol (2.5 mg, 5 mg. and 10 mg) and Atenolol (25 mg and 100 mg) and the calcium-channel blocker Amlodipine Besylate (2.5 mg, 5.0 mg, 10 mg) on larval transformation from trochophore to veliger to early juveniles. It was found that these bioactive inducers exerted a negative impact on larvae in all stages and mortality predominated.

Discussion

The development of the embryo of *C. ramosus* from the beginning of cleavage till the early juvenile was on average 40 days post-fertilization. A more or less similar result was recorded for the same species (Mahmoud et al., 2013, Mostafa et al., 2013). This value was close to that found by (Young & Eckelbarger, 2015) for most neogastropods and in *Concholepas concholepas* (Bruguière, 1789) (Inestrosa et al., 1992). The developmental time of *C. ramosus* embryos found in this study as well as that recorded by Ruangchoy and Tantichodok (1992) for the same genus was considered short. According to Spight et al. (1974) large vitelline-rich eggs of 800 mm to 1000 mm would take a longer time to develop. According to Chou and Tan (2008) species with small eggs (125 mm) had a short time (10 to 15 days) with the hatching of planktotrophic larvae, and species with large eggs, between 600 and 1000 mm had a long developmental time (greater than 25 days) and they hatched like juveniles. This study disagreed with the previous finding since the egg of *C. ramosus* measured 120.28 ± 3.82 mm and the embryonic development till early juvenile lasted 40 days. The cleavage observed in *C. ramosus* followed the pattern presented by Gohar and Eisawy (1967). The appearance of pre-veliger was evidenced mainly by the beginning of the formation of the shell, the velum, the foot, and the differentiation of the cephalic region. According to Hyman (1967) torsion occurred in this stage and the formed shell rotated by app. 180°. The number of eggs per capsule in gastropods ranges from 1 to 200 (D'asaro, 1988; Saglam & Duzgunes, 2007; Vasconcelos et al., 2004). According to German et al. (1998) and Jagadis et al. (2013) the intracapsular protein fluid was composed of proteins and carbohydrates, which varied in different species studied. Roller and Stickle (1989) stated that intracapsular albuminous fluid uptake may begin during cleavage stages through pinocytosis. Hyman (1967) mentioned that the albuminous substance was engulfed as food by the developing prosobranch embryo. The capsule of *C. ramosus* is produced by the pallial oviduct, followed by the ventral pedal gland that shapes and adheres to the chosen substrate (Guido et al., 2007). Its formation followed the pattern presented by most species of the studied Muricidea (Thorson, 1940; D'asaro, 1991). Variations occur with regard to capsule size and preference for adhesion sites. This study showed that preference for a type of capsule adhesion substrate was quite evident in *C. ramosus*, 209 egg capsules with diameter 20.28 ± 3.82 mm were adhered to the side walls of aquaria representing 40.66%, 115 ones with diameter 19. 75 ± 7.25, on rocks representing, 137 ones 26.65% on molluscan shells with diameter 20.12 ± 6,11 and 53 ones with diameter 19.68 ± 9.16 representing 10.31% were dropped.

Authors interested to study the effects of MF (Methyl Farnesoate), 1,2-dioctanyl-*rac*-glycerol (C8), Serotonin (5-HT), and acetylcholine in signaling systems, neuronal control (neurotransmitters) or control of morphogenetic and behavioral reactions on metamorphosis of marine invertebrates (Bishop & Brandhorst, 2007; Glebov et al., 2014; Urrutia et al., 2004). 5-hydroxytryptamine (5-HT) and serotonin were proven to accelerate metamorphosis in hydroids, ascidians, barnacles and molluscs (Glebov et al., 2014). However, our data showed that serotonin has a negative effect on the transformation of *D. platypus* veliger larvae. Consistent with our study Glebov et al. (2014) showed that serotonin inhibits the development of *Helisoma trivolvis* (Say, 1817) (Mollusca) in pre-metamorphic stages. Nagaraju and Borst (2008) extended previous studies of the two-color phases of the crab *C. maenas* and provided the first evidence that MF acts as a link between environmental changes and the stimulation of crustacean reproduction. This supports our finding that MF accelerates the transformation of *D. platypus* veliger larvae. 1,2-dioctanyl-*rac*-glycerol (C8) has been shown to accelerate the rate of metamorphosis in the planula larvae of *Hydractinia echinata* (Fleming, 1823) consistent with our results in *D. platypus* (Leitz & Müller, 1987). Finally, it was found that acetylcholine accelerated the larval transformation of *Marsupenaeus japonicas* (Spence Bate, 1888) as shown in previous studies on the short-neck clam *Ruditapes philippinarum* (A. Adams and Reeve, 1850) (Urrutia et al., 2004). Regulation of metamorphosis in solitary ascidians, sea urchin and a gastropod has been shown to involve Nitric oxide (NO) signaling (Bishop & Brandhorst, 2007). ADMA can be considered as larval metamorphosis factor and is associated with development and growth. Ali et al. (2016) studied selected compounds, with known mode of action in vertebrates, on the development, metabolism and settlement of larvae of the common fouling barnacle,

Table 1
Percentage of transformation of *Dendropoma platypus* veliger to metamorphosed stages of the total number of larvae (sum of the three replicates) subjected to treatment or control.

Biochemical substance	Number of tested larvae	12 days. post-fertile.	29 days. post-fertile.	39 days hrs. post fertile.	15 days. post-hatching	25 days post-hatching
seawater	84	0%	19.0%	39.2%	69.0%	**63.3%**
MF 2.5 mM	84	0%	**50.0%**	**59.5%**	**79.7%**	**95.2%**
MF 300 μM	87	0%	4.5%	20.6%	35.7%	64.5%
C8 10 m M	82	0%	4.5%	13.4%	31.7%	68.0%
C8 1 μM	88	0%	**40.9%**	**56.8%**	**64.7%**	**89.7%**
C8 0.1 mM	82	0%	4.5%	21.9%	30.4%	66.5%
ADMA 1 mM	74	0%	3.7%	17.6%	27.2%	562.7%
ADMA 1.5 mM	78	0%	4.5%	28.2%	31.0%	69.7%
ADMA 2.5 mM	72	0%	**40.2%**	**58.3%**	**80.5%**	**95.8%**
Serotonine 0.5 mM	80	0%	**47.5%**	**62.2%**	**82.5%**	**72.2%**
Acetylcholine 1 mM	82	0%	3.7%	23.1%	32.9%	**92.6%**
Acetylcholine 0.5 mM	87	0%	**48.2%**	**64.3%**	**85.0%**	**98.8%**
Acetylcholine 30 g/ml	89	0%	**4.5%**	**31.0%**	**40.4%**	**68.8%**
cGMP 1 m M	70	0%	**4.5%**	**22.8%**	**32.8%**	**85.7%**

Amphibalanus Amphitrite (Darwin, 1854). Veliger larvae in culture were treated with Atorvastatin, a lipid-regulating compound, cetirizine hydrochloride, an anti-histamine, atenolol, a beta-blocker, and amlodipine, a calcium-channel blocker. The presence of these compounds delayed the veliger stage when compared with the control.

Conclusion

Chicoreus ramosus is a protandric hermaphrodite exhibits gonochoric and broadcast spawning. Egg, cleavage, pre-veliger, veliger and intracapsular juvenile were described. Undertreated conditions of MF 2.5 mM and 300 μM, C8:0 1 μM, ADMA 2.5 mM, and Acetylcholine 0.5 mM and cGMP 1 μM larval transformation of *D. platypus* from veliger to juvenile stages was accelerated than larvae of control in seawater. the lipid-regulating agents, Atorvastatin (5, 20 and 40 mg) and Pravastatin (50 mg and 100 mg), the antihistaminic agent Cetirizine hydrochloride (10 mg), the β-blocker Nebivolol (2.5 mg, 5 mg. and 10 mg) and Atenolol (25 mg and 100 mg) and the calcium-channel blocker Amlodipine Besylate (2.5 mg, 5.0 mg, 10 mg) exerted a negative impact on larvae in all stages and mortality predominated. Bishop and Bates (2002) concluded that HSP90 function is required for morphogenesis in ascidian and echinoid embryos. Bishop and Brandhorst (2007) commented that NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus*. Al-Aidaroos et al. (2017) tested the effect of Atorvastatin, a lipid-regulating compound, Cetirizine hydrochloride, an anti-histamine, atenolol, a β-blocker, and amlodipine, a calcium-channel locker on larvae of the fouling barnacle, *Amphibalanus amphitrite*. They found that Nauplii treated with these compounds took more days to reach the cypris stage when compared with the control. These compounds also inhibited the settlement of cyprids on Petri dishes. While exposure to these compounds led to a decrease in the metabolic activity of stage III nauplii, it increased the respiratory rate of cyprids.

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