BIOCHEMICAL COMPOSITION AND FUNCTION OF JELLY MASS OF *MARPHYSA GRAVELYI* (POLYCHAETA: EUNICIDAE) FROM PULICAT LAKE, INDIA.

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ABSTRACT

Eggs of *Marphysa gravelyi* are spawned in gelatinous masses and the developing larvae are harboured in them till they are ready to undergo settlement. In order to better understand the function of the jelly mass the morphometrics, histochemical, biochemical composition and antimicrobial analysis was performed. The observations indicate the fibrous jelly mass is composed of carbohydrate -mucopolysaccharide, protein and lipids and that the size is correlated to the number of eggs present in them. The extract from the jelly of *Marphysa gravelyi* exhibits activity against *Escherichia coli*, *Vibrio vulnificans* and *Candida albicans* but no activity was seen against other microorganisms tested. The results show that the function of the jelly mass is to nourish the developing embryos, protect against desiccation and predation from macrofauna and most importantly to prevent the dispersal of the juveniles from the desirable habitat.
Keywords: *Marphysa gravelyi*, Polychaeta, Jelly mass, Morphometrics, Biochemical Composition, Antimicrobial Property.

INTRODUCTION

Polychaetes are one of the best represented groups among marine invertebrates demonstrating a wide variety of life strategies. One reproductive strategy found in many invertebrates and a few vertebrates is the encapsulation of eggs and developing larvae. Encapsulation may be gelatinous or firm leathery egg capsule (Thorson, 1946). Although gelatinous egg masses are found in the development of many organisms, only 13.6 % of the total polychaetes displays this strategy (Wilson, 1991; Giangrande, 1997). *Marphysa gravelyi* (Southern, 1921) found in the brackish waters of Pulicat Lake, India is one such polychaete which exhibits encapsulation within jelly mass. To better understand the role of these gelatinous egg capsules, an important issue which needs to be addressed is a complete understanding of the biochemical composition of the egg mass. In fact very few studies have tried to correlate the biochemical composition of egg masses with their function. Bayne (1968) investigated the carbohydrate, lipid, protein and calcium content of egg masses or egg capsules of eight gastropod Molluscs in relation to the function of the egg system. Chapman (1965) studied the egg cocoons of the polychaete *Scoloplos armiger*, but there is very little information about the biochemical composition of the egg mass of other polychaetes. Though the larval stages and the protective function of the egg mass of *M. gravelyi* has been discussed by Aiyar (1931) and Malathi *et al*. (2011) not much work has been done on the biochemical composition of the egg mass. This study aims to identify the chemical constituents in the jelly mass of *M. gravelyi* to better understand its physical and biological role and further investigate how well the jelly mass provides a survival advantage to the developing larvae.

MATERIALS AND METHODS

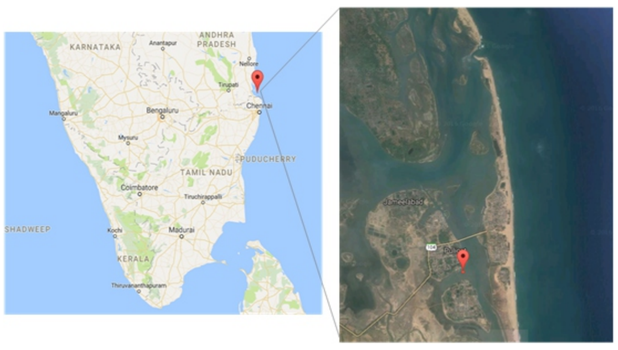

Fig. 1 - Map showing location and collection site of jelly mass of *Marphysa gravelyi* in Pulicat lake

Sample collection

The jelly masses of *M. gravelyi* are found abundantly in Pulicat Lake (Fig.1) located 60 km NE of Chennai (13.33° to 13.66° N and 80.23° to 80.25°E).The jelly masses were collected with their stalk by scooping the mud to a depth of 15cm. The stages of the larvae were assessed by microscopic examination to determine the age of the egg mass. The Jelly mass of *M. gravelyi* has four larval stages (Aiyar, 1931). For the present study jelly mass containing stage I prototrochophore and stage IV nectochaeta larva were selected.

Morphometric studies

The length and width of the jelly mass and stalk in mm was measured. The volume and number of eggs or larvae present in 1 ml of the jelly mass were counted. The relationship between volume of jelly mass and number of eggs or larvae were calculated.

Histochemical studies

The jelly masses fixed in Carnoy's fixative (Humason, 1979) were stained for histochemical analysis (Table 1).

Sample preparation for biochemical analysis

The jelly masses were teased using needles and then centrifuged at 5°C for 20 minutes at 20,000g. The pellet containing larvae and debris were discarded and the jelly mass was retained. The process was repeated until a clean, larvae free jelly mass was obtained. The jelly masses were stored in isoproponal at 4° C until further use.

Solubility of Jelly Mass

The solubility of the jelly substance was tested against solvents listed in Table 2.

Biochemical Analysis

The water content was determined by drying a known weight of jelly mass at 50–60◦C for 24 h to constant weight; Ash content was determined by burning pre-weighed dry samples in a muffle furnace at 500°C for 6 h. Total carbohydrate (Roe and Dailey, 1966); total protein (Bradford, 1976) and total lipid were extracted (Folch *et al*., 1957) and estimated (Barnes and Blackstock, 1973). The fatty acids in the jelly mass were estimated using gas chromatography and detected by flame ionization. Cholesterol levels (Allain *et al.,* 1974); Calcium content was estimated using OPCP kit and Glucose was measured by hexokinase method.

Preparation of extract for antimicrobial activity

To analyze whether the jelly mass had any antimicrobial properties, stage I and IV egg masses were washed with 0.2 µm filtered sea water to remove debris. Samples were homogenized and extracted with 10 volumes (w/v) of 70% methanol in a shaker at 37ºC for 24 hours. The extracts were centrifuged at 12, 000 g for 10 minutes at 4ºC and filtered. The solvents were removed by rotary evaporation under vacuum. The dried extracts were weighed and resuspended in Dimethyl Sulfoxide (DMSO) for antimicrobial studies.

Antibacterial Activity of Jelly Mass Extract

The bacterial pathogens listed in Table 4 were used for antibacterial analysis by disc diffusion method. Exponentially growing bacteria were spread plated and discs with 20 µl of extract (at final concentration of 1000 µg, 500 µg, 250 µg, 125 µg and 62.5 µg); 20 µl DMSO (negative control) and 10 µl (10 µg streptomycin - positive control) were placed on Mueller- Hinton agar plates containing the different bacterial cultures and incubated for 24 hours at 37ºC. The plates were examined for the zones of inhibition and the diameters measured in millimeters.

Antifungal Activity of Jelly Mass Extract

The fungal pathogens as listed in Table 5 were used for antifungal analysis by d isc diffusion method. Exponentially growing fungi were spread plated and discs with 20 µl of extract (at final concentration of 1000 µg, 500 µg, 250 µg, 125 µg and 62.5 µg); 20 µl DMSO (negative control) and 20 µl (20 µg ketoconazole - positive control) were placed on Potato Dextrose Agar plates containing the different cultures and incubated for 24 hours at 37ºC. The plates were examined for the zones of inhibition and the diameters measured in millimeters.

RESULTS

Jelly mass characteristics

The jelly masses of *M. gravelyi* were semi-transparent, gelatinous balloons (Fig.2). The newly laid jelly masses were covered by a thin, soft, sticky and transparent layer, a characteristic that gets gradually lost with the adherence of mud and algal growth. The body of the jelly mass has a thin watery central core surrounded by a thick outer gelatinous region containing individual fertilized eggs visible as diffusely distributed black specks. The development of the embryos within the gelatinous masses were highly synchronized as each egg mass represented a single stage of development with absence of unfertilized eggs in the jelly mass.

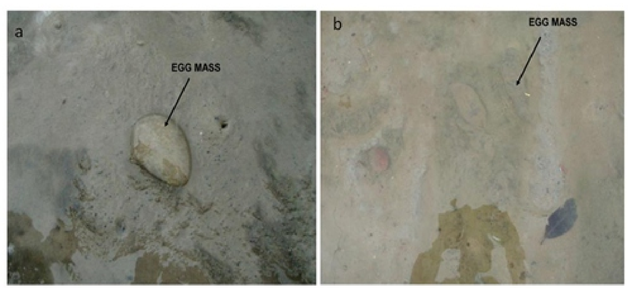

Fig. 2 - Egg mass *in situ* (a) stage I jelly mass, (b) stage IV jelly mass.

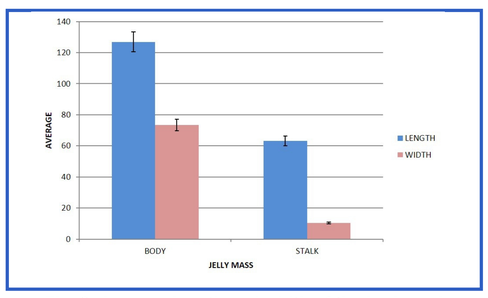

Fig. 3- Average length and width of the body and stalk of jelly mass of *Marphysa gravelyi*

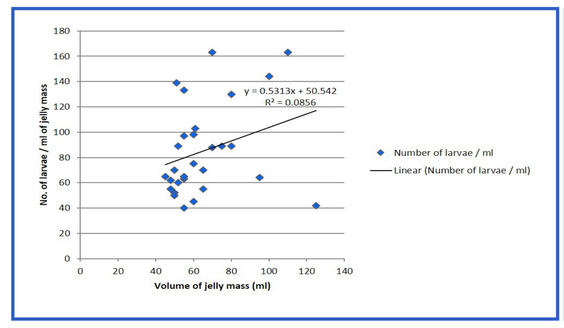

Fig. 4 - Relationship between volume of the jelly mass and number of larvae

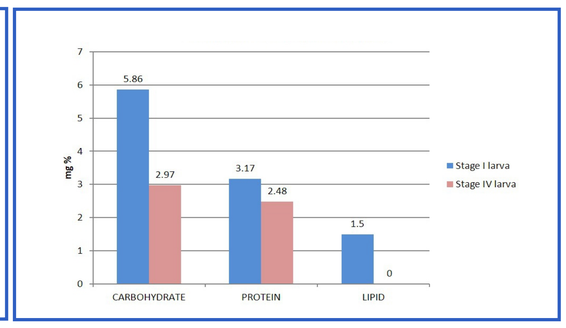

Fig. 5 - Comparison of carbohydrate, protein and lipid content in the jelly mass harbouring Stage I and IV larva

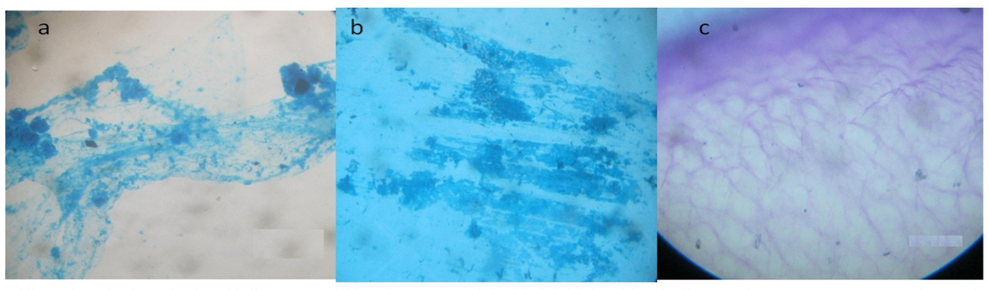

Fig. 6 - Histochemical analysis of jelly mass of *Marphysa gravelyi* with (a) Alcian blue indicates the presence mucopolysaccharide; (b) Fast green staining indicates the presence of basic amino acids; (c) Toludine blue staining indicates metachromacy.

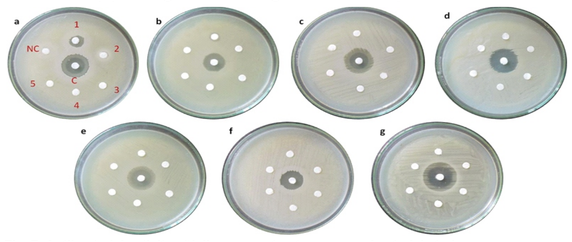

Fig. 7- Antibacterial activity of jelly mass extracts on seven bacterial strains (a-g) *Escherichia coli, Vibrio vulnificus, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio harveyi* and *Vibrio alginolyticus*. (C- Positive control (Streptomycin); NC – Negative control (DMSO); 1 - 5 concentration range of 1000 µg, 500 µg, 250 µg, 125 µg, 62.5 µg respectively)

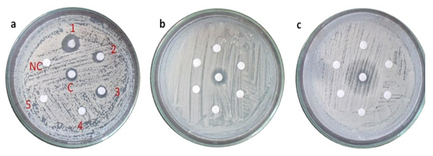

Fig. 8 - Antifungal activity of jelly mass extracts on three strains of *Candida sp* on Potato Dextrose Agar plates; (a) *Candida albicans* (b) *Candida glabrata* (c) *Candida tropicalis*. C- Positive control (Ketokonazole); NC – Negative control (DMSO); 1-5 concentration range of 1000 µg, 500 µg, 250 µg, 125 µg, 62.5 µg respectively

Table 1. Histochemical analysis of the jelly mass of *Marphysa gravelyi*

Stain	Specificity	Jelly mass of *Marphysa gravelyi*
Alcian blue	Acid mucopolysaccharide	Positive
Mucicarmine stain	Mucous	Positive
Fast green	Basic amino acid	Positive
Sudan black	Lipid	Positive
PAS stain	Carbohydrate	Positive
Toludine blue	Metachromasia	Positive

Table 2. Solubility of jelly mass of *Marphysa gravelyi* in different solvents

Solvent	Solubility
Dilute HCl	Insoluble
Dilute H_2SO_4	Insoluble
Dilute NaOH	Soluble
Acetone	Mildly soluble
Ethanol	Insoluble

Table 3. Biochemical Analysis of jelly mass of *Marphysa gravelyi*

Components	Jelly mass with stage I larvae	Jelly mass with stage IV larvae
Total Carbohydrate	5.86 % w/w	2.97 % w/w
Glucose-Hexokinase	1-2 mg/dL	1mg/dL
Total Protein	3.17 %w/w	2.42 %w/w
Total Lipid	1-2 mg%	-
Triglycerides	0.1-1 mg %	-
HDL Cholesterol	0.2-1 mg%	-
LDL Cholesterol	-	-
Fatty acid	1.1 %w/w	-
Calcium	-	-
Moisture	88.5-93.35%	93-95%

Table 4. Antibacterial activity of the methanol extracts of stage I: Prototrochophore jelly mass at different concentrations.

Microorganisms	Stage I jelly mass extracts at different concentrations Zone of Inhibition (mm)				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg
Escherichia coli	11	6	6	-	-
Staphylococcus aureus	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Vibrio paraheamolyticus	-	-	-	-	-
Vibrio alginolyticus	-	-	-	-	-
Vibrio vulnificus	9	-	-	-	-
Vibrio harveyi	-	-	-	-	-

- Indicates no microbial activity. Antimicrobial activity is depicted by the diameter of zone of inhibition (mm)

Table 5. Antifungal activity of the methanol extracts of stage I: Prototrochophore jelly mass at different concentrations.

Microorganisms	Stage I jelly mass extracts at different concentrations Zone of Inhibition (mm)				
	1000 µg	500 µg	250 µg	125 µg	62.5 µg
Candida albicans	15	11	10	-	-
Candida tropicalis	-	-	-	-	-
Candida glabrata	-	-	-	-	-

- Indicates no microbial activity. Antimicrobial activity is depicted by the diameter of zone of inhibition (mm)

DISCUSSION

Microscopic examination of the jelly mass showed it to be fibrous. The early stages of the egg mass contained only fertilized eggs fully laden with yolk and developing embryos, but slow accumulation of different phytoplanktons and meiofaunal organisms like copepods, nematodes and protozoans were found harboured along with the nectochaeta stage of the larvae in the core of the jelly mass. The size of the jelly mass and the length of the stalk were found to be variable. There is a significant relationship between the volume of each jelly mass and the number of larvae. Staining property with Alcian blue stain and metachromacy with toludine blue, revealed the general chemical nature of the jelly to be a mucoprotein having the carbohydrate moiety composed of glucosamine. The property of glucosamine to bind large amounts of water to a very small amount of organic matter and the absence of calcium contributes to the gelatinous nature of the jelly mass of *M. gravelyi.* The composition of the jelly mass reveals that it contains about 88.5-93.35% of water with very little organic matter in it. It is interesting to note the jelly mass of *M. gravelyi* showed the presence of about 1- 2 mg % lipid in addition to proteins and carbohydrates. The jelly masses of *M. gravelyi* contained 1.1 % w/w fatty acids, therefore less buoyant and did not float on water when detached but rolled freely on the muddy shores due to wave action. This along with the insoluble nature of jelly mass to various solvent treatments is advantageous to the survival of the species as the larvae in the jelly masses. The organic content present in the jelly mass of nectochaeta larvae as compared to the early stage of larval development is reduced, which may be due to grazing of the meiofaunal organisms seen in the inside and on the outer surface of the egg mass. Marine invertebrates are constantly exposed to high microbial load in seawater and sediments, many of which may be pathogenic. The survival of these organisms depends on efficient antimicrobial mechanisms to protect themselves against microbial infections. Studies on the immune response of polychaetes have shown that they have the potential for cellular and humoral immune response (Cuvillier-Hot *et al,* 2014). Antimicrobial study of the jelly mass indicates that they have mild antibacterial and antifungal property. The antimicrobial property observed in polychaete could be due to the natural population of microorganisms living in the egg mass (Benkendorff *et al,* 2001) or parental transfer of antimicrobial secretions into the jelly mass. The decreased antimicrobial activity noted could be due to loss of nutrients in the jelly mass or decreased activity of the antimicrobial secretions as the jelly mass ages. Further study is needed to better understand this observation. It is interesting to note that spawning of eggs in gelatinous masses is exhibited only by *M. gravelyi* in the intertidal waters of Pulicat Lake. This strategy presents several advantages including protection against physical stress, predator, bacterial attack (Pechenik, 1979; 1983) and offers nutritional benefits. The jelly material of *M. gravelyi* performs the important task of protection, aggregation, preventing desiccation and dispersion of larvae.

REFERENCES

Aiyar, R. G., 1931. An account of the development and breeding-habits of a brackish-water polychaete worm of the genus *Marphysa*, J. Linn. Soc., 37; 387-403.
Allain CC, Poon LS, Chan CS, Richmond W, Fu PC., 1974. Enzymatic determination of total serum cholesterol. Clin Chem. 20(4):470-5.
Barnes, H., Blackstock, J. 1973. Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanilun method for total lipids. J Exp Mar Biol Ecol 12: 103-118.
Bayne CJ. 1968. A study of the desiccation of egg capsules of eight gastropod species. J. Zool. 155(4): 401-411.
Benkendorff, K., A. R. Davis, and J. Bremner. 2001. Chemical defense in the egg masses of benthic invertebrates: an assessment of antibacterial activity in 33 mollusks and 4 polychaetes. J. invertebr. Pathol. 78:109-118.
Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry 72, 248-254
Chapman, G., 1965. The egg cocoons of *Scoloplos armiger* O.F. Müller. Biol. Bull. (Woods Hole, Mass. 128: 189-197
Cuvillier-Hot, V., Boidin-Wichlacz, C., and Tasiemski, A. (2014) .Polychaetes as annelid models to study ecoimmunology of marine organisms. Journal of Marine Science and Technology, Vol. 22, No. 1, pp. 9-14.
Folch, J, M. Lees, G.H. Sloane Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226 (1): 497-509.
Giangrande A. (1997) Polychaete reproductive patterns, lifecycles and life histories: an overview. Oceanography and Marine Biology an Annual Review. 35, 323–386.
Gretchen L. Humason., 1979. Animal Tissue Techniques. San Francisco, W.H. Freeman publications.
Malathi E., Sunder Raj S. K. and Mercy Bai P., 2011. Larval development of *Marphysa gravelyi* (Polychaeta: Eunicidae) from Pulicat Lake, India. Italian Journal of Zoology Volume 78, Supplement 1, 249-254.
Pechenik, J.A.1978. Adaptations to intertidal development: Studies on *Nassarius obsoletus*. Biol. Bull. 154: 282-291.
Pechenik, J. A. 1983. Egg capsules of *Nucella lapillus*(L.) protect against low-salinity stress. J. Exp.Mar. Biol. Ecol. 71:165-179.
Roe, J. H., Dailey, R. E., 1966. Determination of glycogen with the anthrone reagent. Analytical Biochemistry Volume 15(2) - 245-250.
Sato,M., Nishihira, M., Tsuchiya, M., 1984. Dispersal and recruitment of juveniles of the Polychaete *Lumbrinereis latreili* (AudouinetMilne-Edwards).Bull.Mar.Biol.Stn.Asamushi Tohoku University 17,191-203.
Southern, R. (1921). Polychaeta of the Chilka Lake and also of fresh and brackish waters in other parts of India. Memoirs of the Indian Museum. 5: 563-659.
Sullivan, H. Charles and Timothy, K. Maugel. 1984. Formation, organization and composition of the egg capsule of the marine gastropod, Ilyanssa obsoleta. Biol. Bull. 167: 378-389.
Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates. Medd.Dan. Fisk. Havunders. 4: 1-523
Wilson Jr. W.H. 1991. Sexual reproductive modes in polychaetes: classification and diversity. Bulletin of Marine Science, 48, 500 -516.
Woodcock, S.H., and K. Benkendorff. 2008. The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca). Aquaculture 276: 162-170.