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REVIEW OF RESEARCH

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# ESTIMATION OF PROTEIN CONTENT FROM DIFFERENT TISSUES OF MANGROVE CLAM *GELOINA PROXIMA* (PRIME, 1864) OF DAPOLI COAST, RATNAGIRI- MAHARASHTRA (INDIA).

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**ABSTRACT:**

*G.proxima is one of the indigenous mangrove clam of Dapoli coast and is used as food by the costal people. The attempt have been made to find out protein concentration quantitatively and is confirmed qualitatively.Quantitative estimation of proteins done by Lowry et.al. (1951)Method andits qualitative confirmation done by Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis: (SDS-PAGE). This,protein analysis ofG.proxima, showed variations in protein content in different tissues like foot, testes,ovary and gills in different monthsexcept hepato pancreas. Distinctive stained bands, in case of foot muscle tissue confirmed that it is a tissue that has the high concentration of proteinsfollowed by ovary and gills. Evaluation of the intensity and size of stripes and spots in SDS-PAGE allowed the relative evaluation of protein concentration in the sample.*

**KEY WORDS:***Mangrove, Clam, Geloina, Protein.*

## INTRODUCTION:

*G.proxima*is one of the indigenous mangrove clam of Ratnagiri district and is important aquatic animal due its economic and ecological effects.Bivalves forms most preferable food item for the coastal people of Ratnagiridistrict.The study of proteins have been extensively increased in recent years. Multiple forms and different properties of proteins indicate the ultimate biochemical make up and relationships among different organisms.Synthesis of proteins and their accumulation in some bivalve species, have been regarded as the main denominator of true growth by several investigators [1].

Biochemical composition certain bivalves shows high protein content *C. gryphoides*[2], *K. mormorata* [3], *P. plicata*[4],*E. radiata*[5]throughout the year and the low levels of protein content coincides with the spawning season. The protein content in different animals may vary with a number of factors such as nutritional state, reproduction, moulting and parasitism.The Bivalve *Meretrix meretrix* shows low protein values during active spawning[11] [12].

Electrophoresis is one of effective method used to describe specific proteins from different tissues of molluscs[6]. Finer separation of proteins has been achieved using the polyacrylamide disc gel electrophoretic technique[7][8].Gel electrophoresis, using sodium dodecyl sulphateis widely used that gives proteins separation basedon their molecular weight. The electrophoretic data of proteins is species specific in molluscs and these results may be useful in the analysis of systematic relations in the class Bivalvia.[09] [10]

Here an attempt have been made to find out the protein content of economically valuable clam.

## Materials and Methods:

### Extraction and Quantitative estimation of proteins done by Lowry et.al. (1951)[13]Method:

**Preparation of 40% extract:** Desired tissues such as gills, foot, Gonads, Hepatopancreas Clams were collect by dissecting clams and rinsed in 0.28% saline. Blotted 4 gms. of the each tissue and homogenized with 10 ml of 80% alcohol and centrifuged at 4000/ r.p.m. for 15 min.Supernatent mixed with 1 ml of Tris glycine buffer- pH 7 to remove non required organic and inorganic component andagain centrifuged at 4000 r.p.m. for 5 -10 min.The supernatant collected used for estimation of proteins.The entire procedure preferably carried out at $4^0$ C.

**Preparation of standard graph:**Standard protein stock solution is prepared by dissolving 100 µg of BSA in 1ml of water and used for preparation of standard curve.

**Estimation of protein content:**5 ml Lowry 'A' and 0.1 ml Lowry 'B' solutions were added to test tubesand allowed to stand for 10 minutes. Then added 0.4ml of distilled water and 0.1 ml.supernatant of individual test sample to it. All test tubes incubated 37 ° C for 30 minutes and then added 0.5ml. (1:1 diluted with D/W) Folin's Reagent and measured Optical density at 620 nm wavelength on digital colorimeter (Equiptronics model).

### Qualitative studies of protein by Sodium Dodecyl Sulphate- Polyacrylamide GelElectrophoresis: (SDS-PAGE): [14]

**Preparation of Reagents:**

1. Protein molecular weight standards as mentioned in **fig.1.2**
2. 30% Acrylamide – 0.8% Bis- acrylamide.
3. Sample buffer: Mix 4 ml of 10 % SDS, 2 ml of glycerol, 1ml of β – Mercaptoethanol, 2.5 ml of 0.5 M Tris-HCL (pH 6.8), and 0.03 gm Bromophenol blue. Bring the volume to10 ml with distilled water prior to filter with Whatman No. 1 filter paper. Divide in to 1 ml aliquots and store at $-20^0$C.
4. 10% (w/v) Ammonium per sulfate.
5. 10% (w/v) SDS.
6. TEMED ( N,N,N$^/$,N$^/$,N$^/$ - tetramethyl ethylene diamine )
7. 0.5 M Tris-HCL, pH 6.8
8. 1,5 M Tris-HCl, pH 8.8
9. **Electrode buffer:** Dissolve 3gm of Tris, 14.4gm of glycine and 1gm of SDS in distilled water. Adjust to pH 8.3. Make up volume to 1L with distilled water.
10. **Staining solution:** Dissolve 0.05 gm of Coomassie blue R-250 in 15 ml of methanol. Add 5 ml of glacial acetic acid and 80 ml of distilled water.
11. **Destaining solution I:** Mix 200 ml methanol, 30 ml acetic acid and 170ml. of distilled water.
12. **Destaining solution II:** Mix 50ml of methanol, 75 ml of acetic acid and 875 ml of distilled water.

**Pouring the running gel:**The minigel apparatus assembled according to the manufacture's detailed instructions after thoroughly cleaning and drying. Resolving gel and stacking gel solutions were prepared as defined in the table 1.1. 3.5ml of resolving gel solution is transferred to the centre of sandwich of the spacers.The top of the gel covered with a layer of distilled water. The resolving gel allowed to polymerize fully (usually 30- 60 min)

**Pouring of the stacking Gel:**The layer of distilled water was poured off completely.4 % stacking gel solution prepared as defined in the table 1.1.Resolving gel solution is transferred to the centre of sandwich of the spacers along an edge of one of the spacers.The Comb inserted in to the layer of stacking gel solution by placing one corner of the comb in to the gel and slowly lowering the other corner in. The stacking gel solution was allowed to polymerize for 45 minutes at room temperature.

**Table-1.1 Experimental set up for separating gel and stacking gel.**

| Reagents | Separating gel | | Stacking gel |
|---|---|---|---|
| | 7.5% | 10 % | 4 % |
| 30 % Acrylamide- bis | 2.500 mL | 3.333 ml | 0.667mL |
| 1.5M Tris-HCL buffer, pH 8.8. | 2.500 mL | 2.500 mL | - |
| 0.5M Tris-HCL buffer, pH 6.8. | - | - | 1.250 mL |
| Distilled water | 4.845mL | 4.012 mL | 3.005mL |
| 10% SDS | 100 µL | 100 µL | 50 µL |
| 10% Ammonium per sulfate | 50 µL | 50 µL | 25 µL |
| TEMED | 5 µL | 5 µL | 3 µL |
| Total | 10 ml | 10 ml | 5 ml |

**Loading the gel:**Protein sample was diluted to 1:1(v/v) with buffer and boiled for 3 minutes at 100 $^0$ C.The wells were filled with electrode buffer and thenwith the help of a 10-25 µL syringe, protein sample loaded as a thin layer at the bottom of the well.Then chambers filled with buffer.

**Running the Gel:**Power supply connected to the anode and cathode of the gel apparatus and allowed to run at a constant current of 15 mA/gel.After the bromophenol blue tracking dye reached the bottom of the separating gel, the power supply was disconnected.The sandwich removed and the gel oriented so that the order of the sample wells is known. It was then carefully removed from the sandwich

**Staining the gel:**Gel placed in a small plastic box and covered with the staining solution. It is agitated slowly for 3 hrs. or more on a rotary rocker.The staining solution poured off and then gel covered with a solution of destaining solution-I and again agitated slowly for about 15 minutes.Destaining solution- I poured off and the gel was destained with destaining solution-II until the gel gets cleared except for the protein bands.

**Details of Protein Molecular Weight Marker (Broad) used are as follows:**

1. **Code No.** **3452.**
2. **Size:** **for 200 lanes.**
3. Make: Takara Biotechnology (Dalian ) Co., Ltd.
4. Supplied Reagent: 5 × Loading Buffer 1 ml
   1M DTT (Dithiothreitol) 100 µl
5. Concentration: 18 µg /µl
6. Volume: 50 µl
7. Form : 50 mM Tris-HCl, pH 6.8
   1 mM EDTA
   200 mM NaCl
   50% Glycerol
8. Component Proteins of the Marker are given in the **Table 1.2.**

**Table 1.2 Component Proteins present inMolecular Weight Marker**

| Protein | Source | MW (Da) |
|---|---|---|
| Myosin | Pig | 200,000 |
| β-galactosidase | E. coli | 116,000 |
| Phosphorylase B | Rabbit muscle | 97,200 |
| Serum Albumin | Bovine | 66,409 |

| | | |
|---|---|---|
| Ovalbumin | Hen egg White | 44,287 |
| Carbonic anhydrase | Bovine | 29,000 |
| Trypsin inhibitor | Soybean | 20,100 |
| Lysozyme | Hen egg white | 14,300 |
| Aprotinin | Bovine pancreas | 6,500 |

9. Storage : Protein Molecular Weight Marker, 1M DTT : -20°C
   5×Loading Buffer: Stored at Room Temperature after used.
10. 5 × Loading Buffer (Stored at RT after used) :
    200 mM Tris-HCl, pH 6.8
    10% SDS
    0.05% BPB
    50% Glycerol

11. **Application Example :**

i. Combined the following reagents in a tube.
   1M DTT: 2 µl
   5×Loading Buffer: 20 µl

ii. Prepared 20-fold diluted marker by adding the following components to the solution prepared in- i.
   Protein MW Marker (Broad) : 5 µl
   Sterilized distilled Water : 73 µl
   20-fold diluted marker is stable for 2 - 3 months for -20°C. It is recommended to store in aliquots for several uses, not to repeat freeze-thaw cycles.

iii. Mix 20-fold diluted marker well, and heat at 100°C for 5 minutes. Load 5 µl per lane of SDS-PAGE minigel. Run 5 - 20% gradient SDS-polyacrylamide gel electrophoresis.

iv. Perform staining with Coomassie Brilliant Blue R-250.

12. **Electrophoresis results of Protein Molecular Weight Marker (5 - 20% gradient SDS-PAGE) is given in the Fig. 1.3.**

**Results:**

Obtained results arereported in the tabular format as well as photographs:

**Quantitative Analysis:**

**Table.1.3.Monthly variations in the concentrations of Proteins by Lowry Method in (µg/ml):**

| Month and Year | Foot | Gill | H.P. | Ovary | Testis |
|---|---|---|---|---|---|
| July 2013 | 52 | 38 | 16.5 | 43 | 52 |
| Aug. 2013 | 80 | 52 | 18 | 62.3 | 54 |
| Sept. 2013 | 70 | 58 | 27.5 | 70 | 80 |
| Oct. 2013 | 62.5 | 46.6 | 18 | 70 | 70 |
| Nov. 2013 | 54 | 43 | 23.5 | 52 | 58 |
| Dec. 2013 | 43 | 38 | 13 | 46.5 | 35 |
| Jan. 2014 | 38 | 35 | 16.5 | 46.5 | 38 |
| Feb. 2014 | 43 | 38 | 18 | 23.5 | 38 |
| Mar.2014 | 38 | 31.5 | 13 | 27.5 | 43 |
| April 2014 | 31.5 | 27.5 | 16.5 | 27.5 | 38 |
| May 2014 | 27.5 | 27.5 | 16.5 | 31.5 | 35 |

| | | | | | |
|---|---|---|---|---|---|
| June 2014 | 46.5 | 23.5 | 18 | 23.5 | 27.5 |

**Table. 1.4. Monthly variations in protein content in different tissues by Lowry Method (mg/ml)**

| Month and Year | Foot | Gill | H.P. | Ovary | Testis |
|---|---|---|---|---|---|
| July 2013 | 0.052 | 0.038 | 0.017 | 0.043 | 0.052 |
| August 2013 | 0.080 | 0.052 | 0.018 | 0.063 | 0.054 |
| Sept. 2013 | 0.070 | 0.058 | 0.028 | 0.070 | 0.080 |
| Oct. 2013 | 0.063 | 0.047 | 0.018 | 0.070 | 0.070 |
| Nov. 2013 | 0.054 | 0.043 | 0.024 | 0.052 | 0.058 |
| Dec. 2013 | 0.043 | 0.038 | 0.013 | 0.047 | 0.035 |
| Jan. 2014 | 0.038 | 0.035 | 0.017 | 0.047 | 0.038 |
| Feb. 2014 | 0.043 | 0.038 | 0.018 | 0.024 | 0.038 |
| Mar.2014 | 0.038 | 0.032 | 0.013 | 0.028 | 0.043 |
| April 2014 | 0.033 | 0.028 | 0.017 | 0.028 | 0.038 |
| May 2014 | 0.028 | 0.028 | 0.017 | 0.032 | 0.035 |
| June 2014 | 0.047 | 0.024 | 0.018 | 0.024 | 0.028 |

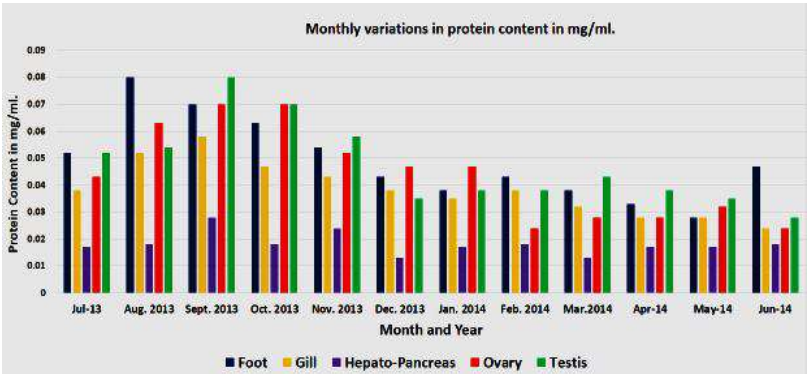


**Fig.1.1. Monthly variations in protein content in different tissues by Lowry Method.**

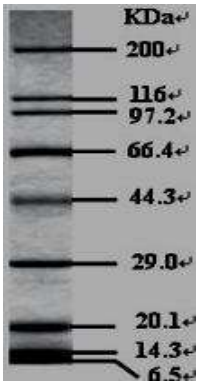


**Fig.1.2: Protein Molecular Weight Marker.**

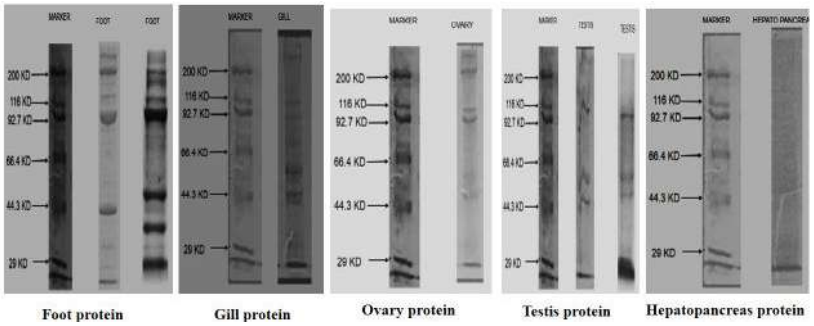


**Fig.1.3: Seperated protein bands from different tissues of *G.proxima.***

## DISCUSSION:

Bivalves are usually preferred as low cost food by the coastal community andare inexpensive source of protein, essential minerals and vitamins. Biochemical study of any edible organism is of great significance because it is an indicative of nutritive value of that organism and consequently helps to stabilize the price levels [15].

Present quantitative analysis of protein of *G.proxima*, showed highest range of proteins in foot tissue followed by testes, ovary and gills.Low protein values during the March, April and May coincide with the spawning peaks. gradual increase in the protein content values in all the tissues from the month of May to October, that matches to the pre-spawning periodindicating protein accumulation for the development of gonads. Biochemical study of bivalve *P. viridis*reported the increased levels of proteins during the maturation of gonads and decreased values during the spawning activity[16].Similar findings were reported by *V. cyprinoides*[17], on *K. mormorata*[3], *P. plicata*[4] and on *E. radiata*[5] .

A distinctive number of stained bands by SDS PAGE, in the case of foot tissue confirmed that, it is a tissue that has high proteins content in a wide range from 29 to 200 KD and above. They may be carbonic anhydrase whose molecular weight is 44.3 KD, may be Ovalbumin 44.3KD, may be phosphorylase- B 97.2 KD and may be myosin with 200 KD.Gill tissue showed the darkest band located between 44.3 and 116 KD. It can/ may be Ovalbumin, Serum Albumin and β-galactosidase.

Ovary tissue sample shows a wide range of proteins between 44.3 to 200 KDa. The proteins of ovary may be Ovalbumin, Phosphorylase B and β-galactosidase.While testis tissue sample showed the proteins with molecular weight of 44.3 to 97.2 KDa that can be ovalbumin and phosphorylase B respectively.The highest range of proteins with darker bands was observed in foot tissue followed by ovary and gills in *G.proxima.* The bands of separated molecules can be cut from the gel, digested and subjected to further structural analysis. Such quantitative and qualitative analyses may support histological and histochemical studies.

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