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Research Article

BIOCHEMICAL AND ENZYMATIC FLUCTUATION DURING OVARIAN DEVELOPMENT OF EDIBLE MARINE CRAB *PORTUNUS PELAGICUS* [LINNAEUS, 1758]

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ABSTRACT

Objectives: Variation in biochemical constituents such as protein, glucose, cholesterol, acid phosphatase and alkaline phosphatase present in the haemolymph of *Portunus pelagicus* [Blue Swimming Crab] at different reproductive stages was studied.

Methods: Haemolymphs were collected from different reproductive groups by cutting the walking legs using hypodermic syringe with No. 22 needle after rinsing with modified citrate EDTA buffer and estimated standard methodology.

Results: The protein content in the haemolymph it gradually increased from immature to ripe stage [3.4±0.49-5.5±0.74 gm/DL] with minimum at immature stage and maximum in ripe stage. Glucose content was maximum in immature stage [49.5±1.28 mg/DL] and minimum in ripe stage [35.4±1.23 mg/DL]. The cholesterol was showed that maximum value in ripe stage [98.0±1.81 mg/DL] and minimum in immature stage [79.0±1.54 mg/DL]. The synthesized enzyme, acid phosphatase was minimum in spent [2.5±0.24 IU/L] and maximum in maturing stage [3.1±0.23 IU/L]. Meanwhile, alkaline phosphatase ranged from 91.0±1.62 to 138±1.79 IU/L with minimum in spent and maximum in immature stage. It indicates that the biochemical components and enzymes are actively participated in gametogenesis.

Conclusion: This study inferred that breeding of *P. pelagicus* mainly depends on nutritive condition and reserved organic constituents of the blood.

Keywords: *Portunus pelagicus*, Haemolymph, Biochemical, Enzymes, Ovarian development.

INTRODUCTION

The fish and fishery products are main sources of animal protein to mankind [1]. Crustaceans especially shrimps, lobsters and crabs are important diet to man with great nutritive value. Marine blue swimmer crab *Portunus pelagicus* [Linnaeus, 1758] is one of the candidate species for culture due to its high growth rate, attractive colour and export value. In order to popularise its culture in the country and also to aid conservation, attempts are being made to standardize the hatchery technology for this species [2]. In crustaceans, a great amount of energy gets channeled to the gonads during reproduction, which is reflected in the deposition or depletion of nutrients with the advent or departure of the reproductive period [3, 4]. A large amount of this reserved food materials are mobilized from hepatopancreas to gonad during breeding period [5].

In the protein content decreased in hepatopancreas with its increment in the ovary during maturation in *Parapenaeopsis hardwickii* [6]. A significant increase in protein and lipid content of the ovary in the late maturing and mature stages at the expense of carbohydrate has been observed in *Uca tangeri* [7]. Lipids stored in hepatopancreas were transported to ovaries during vitellogenesis in *Fal1 farfantepenaeus aztecus* and *Litopenaeus setiferus* [8]. Adiyodi [9] estimated lipids are carried by lipoproteins to the ovary during vitellogenesis in crustaceans.

Although some aspects of the biochemical composition of certain haemolymph and tissues of *P. pelagicus* have been investigated [10,11,12], a comprehensive study about the major constituents in various tissues and haemolymph at all the maturity stages is yet to be carried out. Hence, the present study was aimed to understand the variation in biochemical constituents such as protein, glucose, cholesterol, acid phosphatase and alkaline phosphatase in the haemolymph of *P. pelagicus* at different reproductive stages.

MATERIALS AND METHODS

Alive crabs [*P. pelagicus*] were collected from the offshore region of Kottaipattinam [Lat: 10° 05' N and Long: 79° 12' E], Southeast coast of India, during 2008 by gill net through random sampling. The female crabs were dissected out and stages of sexual maturity were observed based on gonad colour. Based on the gonad colour, crabs were grouped into five maturity stages [Immature, maturing, matured, ripe and spent]. Haemolymphs were collected from different reproductive groups by cutting the walking legs using hypodermic syringe with No. 22 needle after rinsing with modified citrate EDTA buffer [13] and were stored at -20°C until analysis.

The biochemical components such as protein, glucose and cholesterol level in the haemolymph was determined. The protein content in the haemolymph was estimated by Biuret method [14], glucose was determined by enzymatic–colorimetric [15], cholesterol estimated by Enzy-colorimetric method [16]. The amount of acid and alkaline phosphatase was estimated by King [17].

RESULTS AND DISCUSSION

In general, the ovary of the crab *P. pelagicus* is a paired organ found just below the carapace in the cephalothorax region which is distinctly connecting by general bridge of ovarian tissue assuming the shape of the letter 'H' and the diameter of the ovarian arms increasing in the size as ovarian maturity advanced [18, 19, 20]. The biochemical constituents such as protein, glucose and cholesterol of *P. pelagicus* at different stages of gonadal maturity were analyzed and given in TABLE1. The protein levels in immature, maturing, matured, ripe and spent gonads were about 44.22 ± 0.51, 52.41 ± 0.84, 82.74 ± 0.62, 85.14 ± 0.70 and 37.61 ± 0.68 [mg/ml] respectively. The minimum level of protein was found to be recorded in immature and spent whereas maximum in mature and ripe gonads [p<0.0l] [Figure 1].

Proteins are essential constituents of living cells which contains carbon, hydrogen, oxygen, nitrogen and sulphur. The results of present study revealed that protein content in the haemolymph of marine crab *P. pelagicus* was significantly varied at different stages of sexual maturity and comparatively higher than other constituents. Since the proteins are important nitrogenous substances derived from food sources and biosynthesis in the serves which was gradually increases from immature to ripe stage of ovarian development. Similarly, protein content of *Uca annulipes* and *Portunus pelagicus* were found to be higher during the peak periods and lower during non-reproductive periods. The depletion of

protein in the hepatopancreas associated with ripening gonads may due to the transfer of organic materials from hepatopancreas to gonads [10]. However, the results of the present study were agreed with previous reports [21, 22, 23].

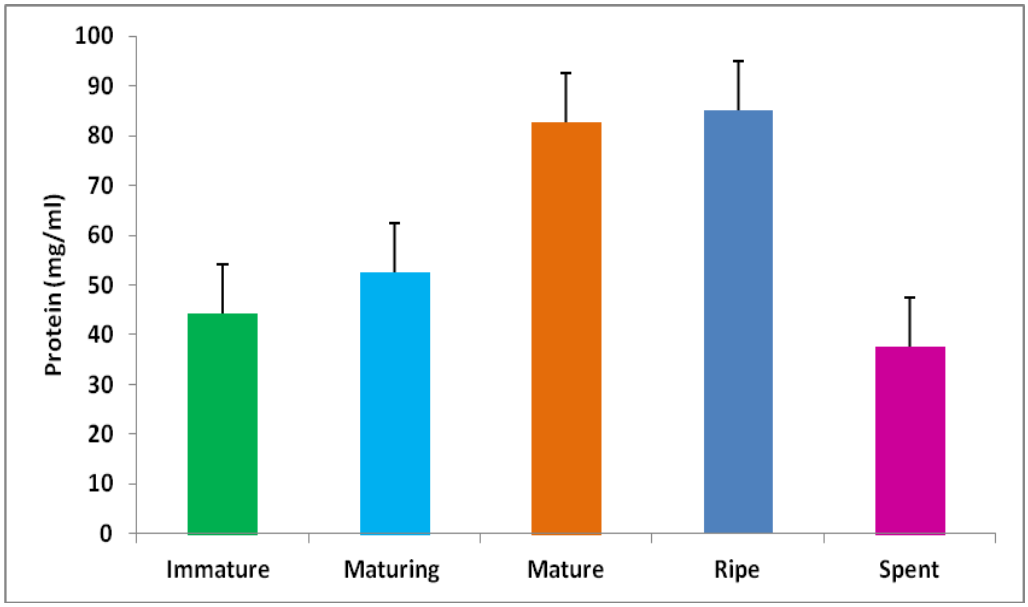

Fig. 1: The protein content in the haemolymph of *P. pelagicus* at different gonadal maturation stages

The glucose level in gonads of *P. pelagicus* in immature, maturing, matured [35.4 ± 1.23 mg/ml], ripe and spent stage were about 35.4 ± 1.23, 35.4 ± 1.23, 35.4 ± 1.23 and 35.4 ± 1.23 [mg/ml] respectively. The results revealed that higher level of glucose was recorded in ripe and lower in immature stage [Figure 2]. The glucose content of the haemolymph were found to be differ significantly [p<0.0l] with steady increase during maturation.

The glucose is an energy yielding component and precursors for the synthesis of organic components which metabolized into CO_2 and water to produce energy rich compounds in the form of ATP. The glucose level in haemolymph of marine crab *P. pelagicus* was significantly varied at different stages of sexual maturity. It was found to be higher in early stages of ovarian development and gradually decrease as gonadal maturity. Health and Barnes [24] were also reported that large amount of glycogen were found in a spent stage which decreased considerably during development on marine crab *Carcinus maenas*. The study of carbohydrate content in the ovary of *P. pelagicus* revealed that there was no significant change in glucose level in ovary during development whereas it decreased when the gonadal maturity advanced in testes. The maximum level of carbohydrate was recorded at early stages of ovary and testes and decreased as developed advanced. The results were similar to the earlier observations made in *F. indicus* [2, 22, 23, 25, 26].

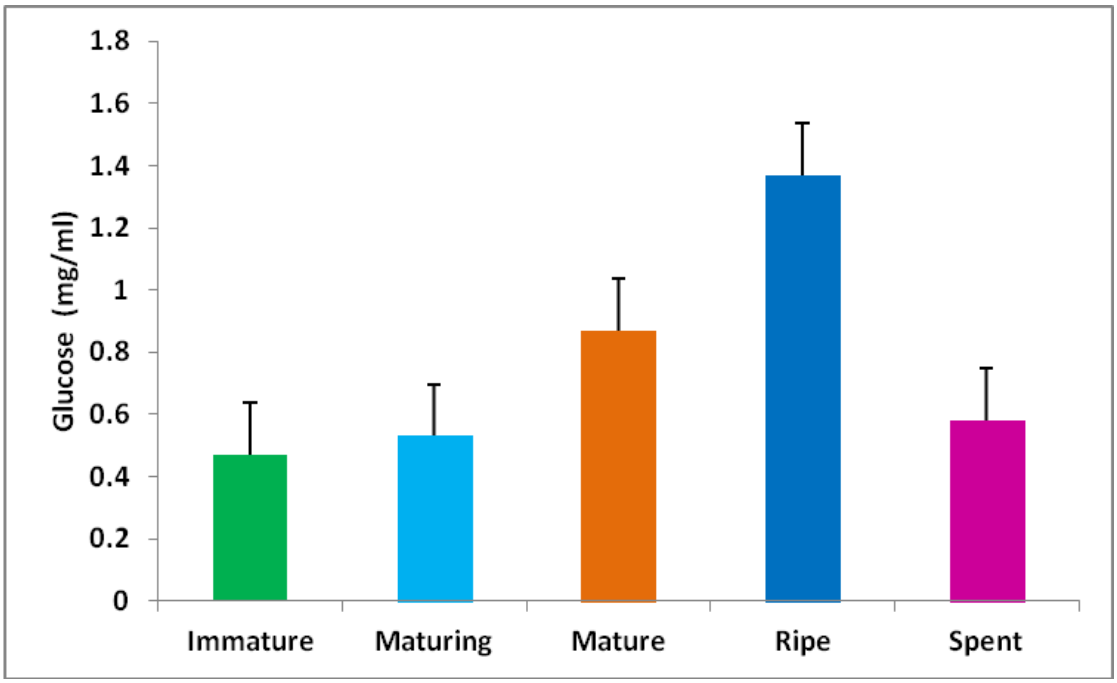

Fig. 2: The glucose content in the haemolymph of *P. pelagicus* at various gonadal maturation stages

Similarly, the cholesterol level in the haemolymphs were about 10.24 ± 0.38, 13.55 ± 0.76, 20.17 ± 0.81, 19.72 ± 0.54 and 12.51 ± 0.89 [mg/ml] in immature, maturing, matured, ripe and spent respectively. The minimum cholesterol level was recorded in immature and spent gonad whereas maximum in mature and ripe [Figure 3]. Cholesterol is an important energy yielding component which found in free state or fatty ester. In the present study, amount of cholesterol content in the haemolymph showed significant fluctuation at different stages of sexual maturity. The cholesterol content gradually increased in immature to ripe stage and decreased in spent stage was reported by [27, 28, 29, 30, 31]. The same trend was observed in our results which was similar to the earlier reports.

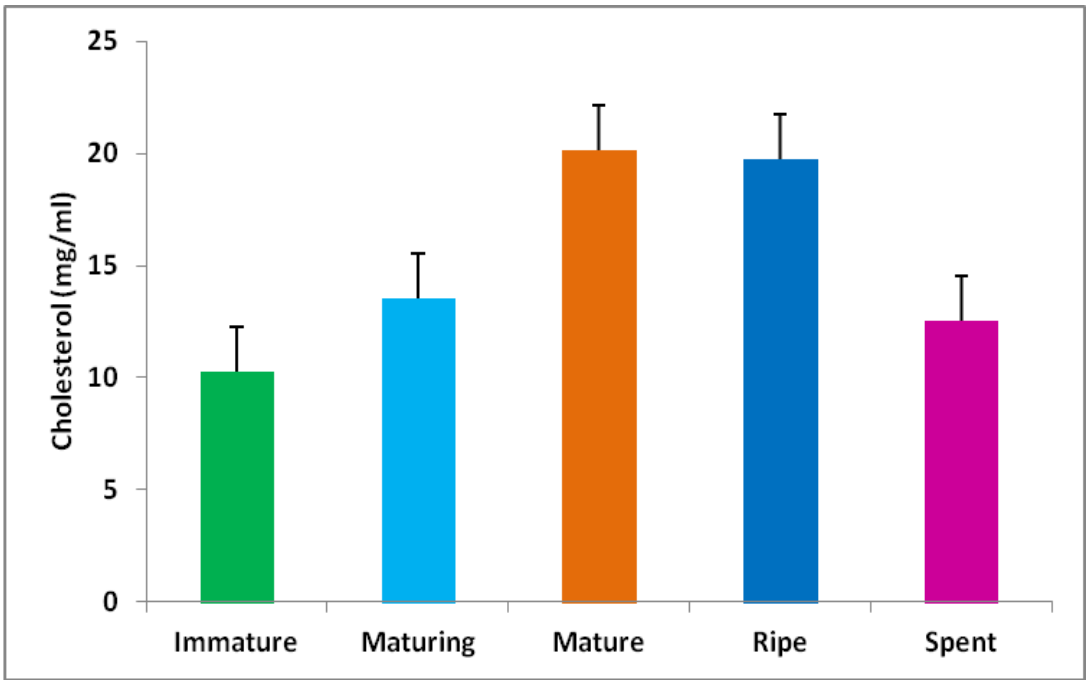

Fig. 3: The cholesterol level in the haemolymph of *P. pelagicus* at different gonadal maturation stages

The level of enzyme acid phosphatase in *P. pelagicus* was investigated at various gonadal maturation stages in *P. pelagicus*. The results revealed that higher amount of acid phosphatase was found in spent gonads [2.6 ± 0.23] followed by matured [2.9 ± 0.27] and ripe [3 ± 0.35] whereas lower in maturing gonad [3.1±0.32] [Figure 4].

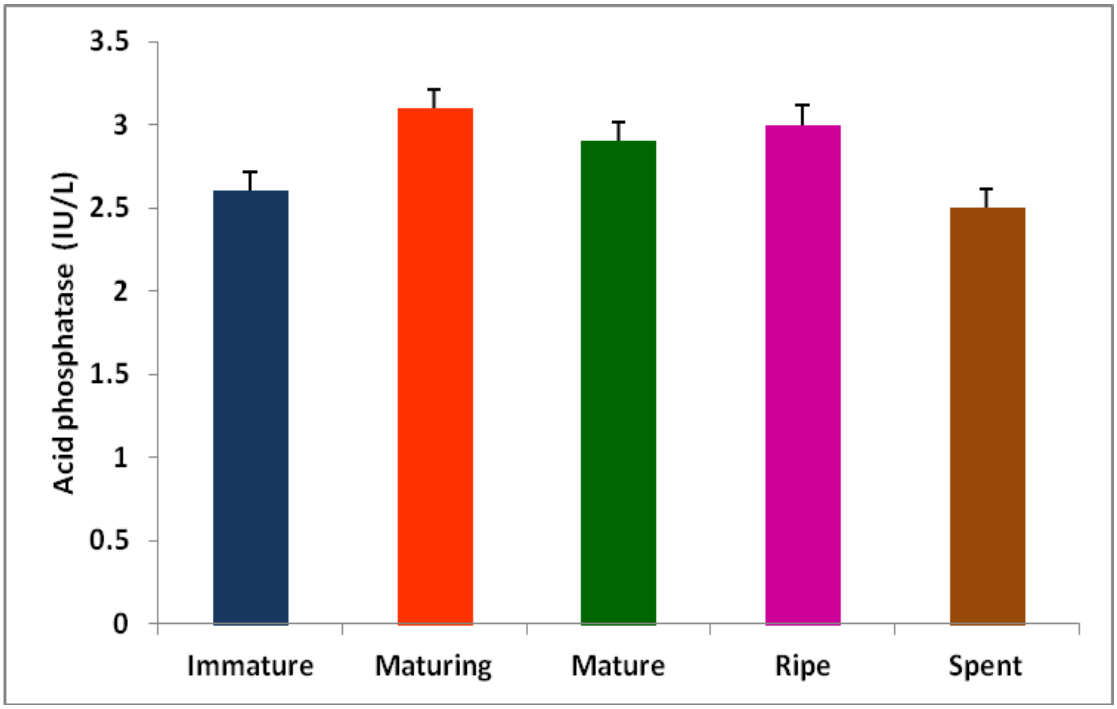

Fig. 4: Variation in acid phosphatase content in the haemolymph during gonadal maturation in *P. pelagicus*

Similarly, the alkaline phosphatase level in immature, maturing, mature, ripe and spent was about 107.21±1.89, 110.12 ± 1.94, 138.07 ± 1.94, 123.24 ± 1.76 and 91.13 ± 0.62 [IU/L] respectively. The minimum amount of alkaline phosphatase was found in immature and spent gonads and maximum in mature and ripe gonads [Figure 5].

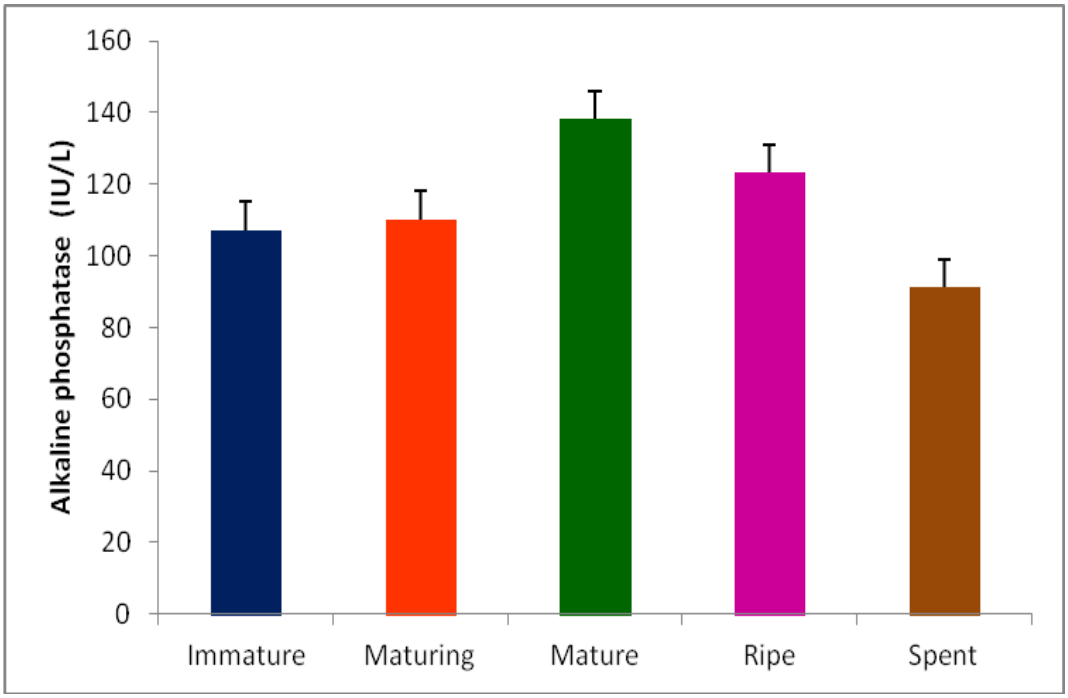

Fig. 5: Variation in alkaline phosphatase content in the haemolymph during gonadal maturation in *P. pelagicus*

The results from the study on overall biochemical content was clearly indicated that mature and ripe gonads were rich in protein, cholesterol and glucose and lower in immature and spent gonads. The acid phosphatase is an enzyme secreted by prostate cells which is active at pH 4 - 6. Bedford [28] studied the level of enzyme concentration in immature to mature crab to show the significant variation. In the present study, acid phosphatase level was slight varied in ovaries of different stages of sexual maturity. Similarly, alkaline phosphatase showed significant fluctuation in different stages of sexual maturity of *P. pelagicus*. These results were comparable with the earlier reports [25, 32].

Table 1: Variation in biochemical constituents in haemolymph during gonad maturation in *P. pelagicus*

Biochemical components	Immature	Maturing	Mature	Ripe	Spent
Protein [mg/ml]	44.22±0.51	52.41±0.84	82.74±0.73	85.14±0.70	37.61±0.68
Glucose [mg/ml]	0.47±0.22	0.53±0.23	0.87±0.20	1.37±0.31	0.58±0.28
Cholesterol [mg/ml]	10.24±0.38	13.55±0.76	20.17±0.81	19.72±0.54	12.51±0.89
Acid phosphatase [IU/L]	2.6±0.23	3.1±0.32	2.9±0.27	3.0±0.35	2.5±0.20
Alkaline phosphatase [IU/L]	107.21±1.89	110.12±1.94	138.07±1.97	123.24±1.76	91.13±1.62

CONCLUSION

As per the observed, biochemical components in haemolymph of marine crab *P. pelagicus*, protein was found to be higher than cholesterol and glucose. Moreover, the results of this study evidenced that the protein and cholesterol content were found to be varied whereas glucose and acid and alkaline phosphatase enzymes were slightly varied during ovarian development.

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