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Research Article

Biochemical composition of gonads during reproductive cycle in the Sea Urchin *Echinometra mathaei* (Echinodermata: Echinoidea)

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Maturation can also be assessed based on biochemical changes in the gonads of sea urchins. The biochemical composition (proteins, carbohydrates, and lipids) of gonads of *Echinometra mathaei* was assessed during the ovarian reproductive stages between April 2011 and November 2012. Protein constituted the major storage part of gonads in both sexes in all months (males: 47.0±22.2 mg g^{-1}, females: 73.8±25.8 mg g^{-1}) followed by lipids (males: 43.4±12.8 mg g^{-1}, females: 62.1±14.5 mg g^{-1}), and carbohydrates (males: 32.2±20.2 mg g^{-1}, females: 46.9±23.8 mg g^{-1}). The results revealed that the protein, carbohydrate, and lipid contents of gonads exhibited significant shifts from highest to lowest concentrations. This indicates that the proximate composition of the gonads of *E. mathaei* is intrinsically linked to the species' annual reproductive cycle, representing a critical consideration for assessing its potential for nutritional and commercial utilisation. The greatest quality in biochemical content was observed from February to May on the coast of Pakistan, when individuals with Stage I and II possess significantly higher concentrations of proteins and lipids, upheld by the nutritive phagocytes. Although, in Pakistan, there is no custom to eat sea urchin roe, and no sea urchin fishery exists; however, in the future, when this species becomes exploitable, such studies will be very important.

[**Keywords**: Biochemical composition, *Echinometra mathaei*, Gonad index, Pakistan, Sea urchin]

Introduction

Proteins, lipids, and carbohydrates are the chief elements of living organisms, and information about their concentrations provides insights into their physiological and nutritional importance[1,2]. When examining the economic value of sea urchins, especially their gonads, also called "roe", constituting to around 10 % of the total weight[3], have more nutritional properties than any other seafood with organic (proteins, lipids, carbohydrates, carotenoids, vitamins) and inorganic (minerals, some carbonates) constituents[4,5], hence are an extremely valued delicacy in some parts of Asia, Mediterranean and the Caribbean countries[6]. In different parts of the world, the "roe" of sea urchins has been utilised as a human food source since prehistoric times and is considered a good protein source with nutritive values like casein[7]. Other uses of "roe'' have also been witnessed, like combining with custard or ice cream[8]. According to the Nestlé[9] report, sea urchins were included among the top ten food trends due to their exclusive flavours. Worldwide harvest of sea urchins was reported to be approximately 63 thousand tons in 2018, with Japan as the largest consumer, accounting for 80 to 90 % of the current global supply[10].

Echinometra mathaei, considered the most abundant echinoid in the world[11], is distributed in tropical and subtropical countries[12,13]. Due to the smaller sizes of the species of *Echinometra*, they are not included in commercially exploited species but have now been incorporated in the landings of sea urchins, because of over-exploitation of higher-prized species[14,15]. However, basic information on *E. mathaei*'s biochemical composition and the nutritional value of its gonads is required, along with other aspects for its commercial exploitation. Somehow, the local consumption of *E. mathaei* gonads has been observed in different parts of the world. Saravanan *et al.*[15] reported that the gonads of *Echinometra mathaei* are traditionally consumed by fishermen in the Gulf of Mannar, India. Ab-Rahim & Nurhasan[16] reported the local intake of *E. mathaei* by the people of Sabah, Malaysia.

Maturation can also be assessed by biochemical changes in the gonads of sea urchins, which are the exclusive site of synthesis of biochemical components in the process of gametogenesis. The factors that affect sea urchin gonad composition can be endogenous, exogenous or both, operating in concert. Endogenous factors are genetically regulated and are associated with the animal life cycle. When gonads increase in

size, the proteins and lipids are mobilised from the muscle and transferred to the gonads[17]. In exogenous factors, it has been reported that the quality and quantity of food modify the biochemical composition of gonads[18-20]. However, it is difficult to relate the change in the biochemical component of gonads to any specific environmental factor, such as food or temperature, because of their simultaneous variation in the environment[21]. Biochemical composition and quality were found to vary between male and female sea urchins[22-25]. Similarly, studies across various species of echinoids have estimated that the biochemical components, particularly proteins of the gonads and gut, vary seasonally. In contrast, the tissue or test comprised mainly of inorganic compounds, which do not vary seasonally[21,26-29].

The studies on the biochemical components of the gonads and the reproductive cycle have been demonstrated previously for many Echinoderm species like starfishes, *Allostichaster capensis*[30], *Asterias rubens*[31], *Asterias vulgaris*[32], sea cucumber species, *Holothuria spinifera*[33], *H. scabra*[34-35] and sea urchin species, *Paracentrotus lividus*[29,36-37], *Loxechinus albus*[38], *Pseudocentrotus depressus*[39] in different parts of the world. Unfortunately, no studies on biochemical changes in gonads of sea urchin *Echinometra mathaei* are available including Pakistan to highlight important market-related traits of this valued species and its potential for commercialisation. The most recent studies on sea urchin *Echinometra mathaei* focused on reproduction[40] and population dynamics[41-42] reported from Pakistan. Accordingly, the present study is an attempt to report on the variation in the biochemical composition (that is, proteins, lipids, and carbohydrates) in gonads at different stages of gonadal maturation of sea urchin, *Echinometra mathaei* to understand the dietary requirement of adult sea urchin, which may beneficiary to the preparation of a balanced diet for the culture of this economically important species together with find out the suitable time for human consumption as food. Although, in Pakistan, there is no custom to eat sea urchin roe, and no sea urchin fishery exists. But, in the future when this species *E. mathaei* becomes exploited, as this species is found plentifully on the rocky coast of Pakistan[43], such studies will be very important.

Material and Methods

Sample collection

The sea urchin, *Echinometra mathaei*, was collected in the low tidal zone at the lowest tidal mark on the rocky shore of Buleji, Pakistan. Collections were made from April 2011 to November 2012; however, sampling was not possible during June to August (monsoon season) due to rough sea conditions in both years. For each sampling month, approximately 30 – 35 individuals of *E. mathaei* were handpicked and transported alive to the laboratory in well-aerated seawater. Individual records of test diameter and total wet weight were completed in the laboratory. In the laboratory, after taking various measurements, the sea urchins were sacrificed to obtain the five lobed gonads, which were carefully removed and weighed wet on an electric balance (Libror AEU-210, Shimadzu) to the nearest ±0.01 g for individual Gonadosomatic Index (GSI) determination, estimated as [*(gonad wet weight/ total wet weight) × 100*]. The gonads were then temporarily frozen and analysed shortly thereafter for biochemical composition, ensuring that they were not stored for extended periods. Sexes were determined based on gonad colour, which was confirmed later by histological study[40]. Monthly seawater temperature (°C) and salinity (ppt) were measured at the sampling site using a thermometer and a handheld refractometer (Atago, S/Mill-E), respectively, and photoperiod data was obtained from the web page https://www.timeanddate.com/.

For the estimation of total protein, carbohydrate, and lipid in the sea urchin's gonads, individual samples were pooled monthly by sex: three pools of female gonads (N = 3), and three pools of male gonads (N = 3) were established. Individuals of the same sex were pooled, forming groups of similar size and maturation stage, as confirmed by histological analysis. The number of individuals in a pool was variable and dependent on the sex ratio and maturation stage of the gonads of that month; however, the three pools each of male and female gonads were ensured each month.

The stages of oogenesis and spermatogenesis were identified according to Bryne[44] and Spirlet *et al*.[45] based on oocyte size (female) and thickness of the peripheral spermatocyte layer (male): Immature/ resting (stage I), with few primary gametes (oocytes and spermatogonia) and nutritive phagocytes; pre-mature (stage II) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage III) with mature gametes and few nutritive phagocytes; partially spawned (stage IV) with loosely packed mature gametes; and

spent (stage V) with empty gonads having some nutritive phagocytes[40]. For biochemical analysis, the first two stages (Stage I & II) were combined into one category, *i.e*., 'maturing stage', and were analysed along with the "mature stage (Stage III)''and "spent stage'', *i.e*., combined partially spawned and spent stage (Stage IV & V).

Biochemical characterisation

Protein analysis

Following the Folin–Ciocalteu method, as described by Lowry *et al*.[46], the total proteins were estimated using Bovine Serum Albumin (BSA) as the standard. 1 gm of wet pooled gonad was homogenised in a homogeniser (Model Polytron PT-MR 2100, Kinematic, Switzerland) with 10 ml of 0.1 M phosphate buffer of pH 7.0. Then, 1 ml of the gonad tissue homogenate and 1 ml of 0.1 N NaOH were taken and kept for at least 15 min at room temperature. Later, 8 ml of distilled water was added to make up a volume of 10 ml, and the mixture was centrifuged (Model 80-2, Seico, Pakistan) at a speed of 4000 rpm for 30 min. Later, only 0.1 ml of supernatant was taken, and to make up a volume of 1 ml, 0.9 ml of distilled water was added. Subsequently, 5 ml of alkaline reagent (2 g $Na_2 CO_3$ in 0.1 N NaOH, 4 % Na-K tartrate, and 2 % CuSO4, 200:1:1) was added, and the mixture was incubated for 30 min at room temperature. The absorbance of the sample was determined on a spectrophotometer (Model 6306, Jenway, United Kingdom) at the wavelength of 750 nm against a reagent blank.

Carbohydrate analysis

The obtained supernatant was used to estimate total carbohydrates by the Phenol-Sulphuric acid method[47] with glucose as the standard. To 1 ml of supernatant added 5 ml of concentrated sulfuric acid (H_2SO_4) and 1 ml of Phenol reagent in a test tube. The test tube mixture was heated in a water bath for 2 to 3 min, then cooled at room temperature for 30 min in the dark. The colour intensity of the sample was read using the spectrophotometer at 490 nm.

Lipid analysis

Total lipids were estimated by the Sulpho-phosphovanillin method[48] using cholesterol as the standard. Aliquots of 1 g of pooled gonads were homogenised in 10 ml of a 2:1 (v:v) chloroform-methanol mixture and centrifuged at 4000 rpm for 20 min. Subsequently, 0.5 ml of supernatant was taken in a test tube and evaporated in a hot water bath until the supernatant became dry, then cooled at room temperature for 3 min. Then 2 ml of concentrated sulfuric acid was added to these test tubes, covered with aluminium foil, and placed in boiling water for 10 min; afterwards, kept in cold water for 3 to 5 mins. 0.1 ml of the mixture was taken from the cooled test tube in another test tube, 5 ml of phospho-vanillin reagent (prepared by mixing 1.2 g of vanillin dissolved in 200 ml of distilled water, and 800 ml of orthophosphoric acid) was added, and the mixture was incubated for 15 min at 37 °C in the oven. The absorbance was read at 540 nm.

Statistical analysis

Data are displayed as means and standard deviation. Tests for normality and homoscedasticity were performed by Kolmogorov-Smirnov and Levene's tests, respectively. Data were processed by one-way-analysis of variance (ANOVA) to determine differences for each biochemical component of the gonads of both male and female sea urchins, followed by Tukey tests. The Pearson correlation coefficients were employed to compare the GSI with the different biochemical components of the male and female gonads of *E. mathaei* separately, and to compare different biochemical components of the male and female gonads with different environmental parameters (temperature, salinity, and photoperiod). In all cases, significant differences were considered at $P < 0.05$. Statistical analyses were performed using IBM SPSS 20.0 software.

Results

Environmental conditions

Monthly variation in seawater temperature during the study period ranged from 18.0 °C (the lowest record in January), which gradually increased up to 29.0 °C (the maximum record in October 2012). The salinity did not fluctuate noticeably throughout the year, ranging from 36 ppt to 41 ppt. On the other hand, the photoperiod documented minimum day lengths of 10.4 h in December, progressively increasing to a maximum day length of 13.2 h in May 2011 and 2012 (Fig. 1).

Test diameter, total weight, GSI, and gametogenic stages

Biometric variables mean ± standard deviation of the female *E. mathaei* test diameter, total weight, gonad weight, and GSI in maturing (Stage I & II), mature (Stage III), and spent (Stage V) stages of its

annual cycle presented in Table 1, were reasonably alike those of male's test diameter, total weight, gonad weight, and GSI (Table 1). Of the four variables, females showed significant differences ($P < 0.05$) in only two variables (GW and GSI) when comparisons were made among different stages, as did males ($P < 0.05$) (Table 1). Observed monthly changes in GSI of *E. mathaei* female and male gonads are summarised in Figure 1. GSI was significantly varied ($P = 0.05$) in males and females throughout the 14-month study period. In males, the maximum GSI value recorded in April 2012 was 12.2±2.1 %. However, GSI in females reached a maximum value in November 2012 (9.9±2.9 %). The minimum GSI values for both sexes were recorded during the same month (October 2011), with 2.3±1.0 % and 2.7±1.0 % for males and females, respectively. Variations in the GSI displayed gonad maturation stages (Fig. 2). During April – May 2011, more than 50 % of both males and females showed gonads at stages I and II (maturing), representing the onset of gametogenesis. Individuals at these stages (I and II) were present throughout the sampling period, except for males in November 2012. Both mature males and females (Stage III) were consistently present in high

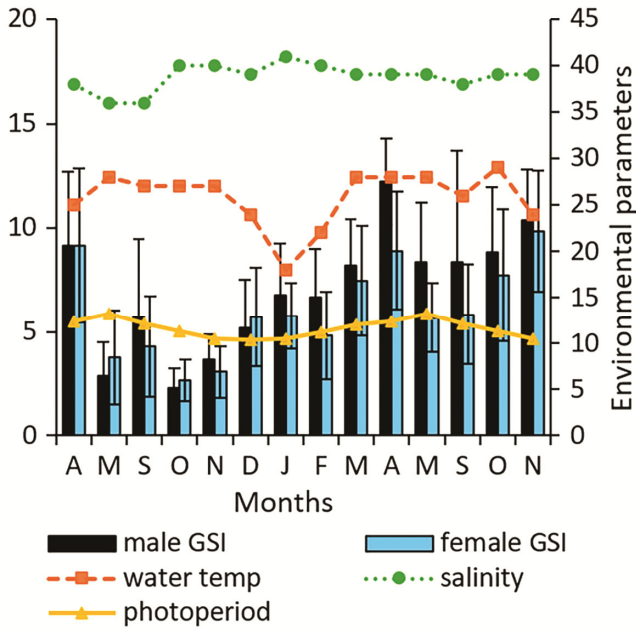

Fig. 1 — Environmental parameters (temperature (°C); salinity (ppt); photoperiod (hours)) and gonadosomatic index (GSI) of *Echinometra mathaei* males and females during April 2011 – November 2012. Values represented as columns are means ± SD of 10 – 15 individuals (on average) pooled by sex per month

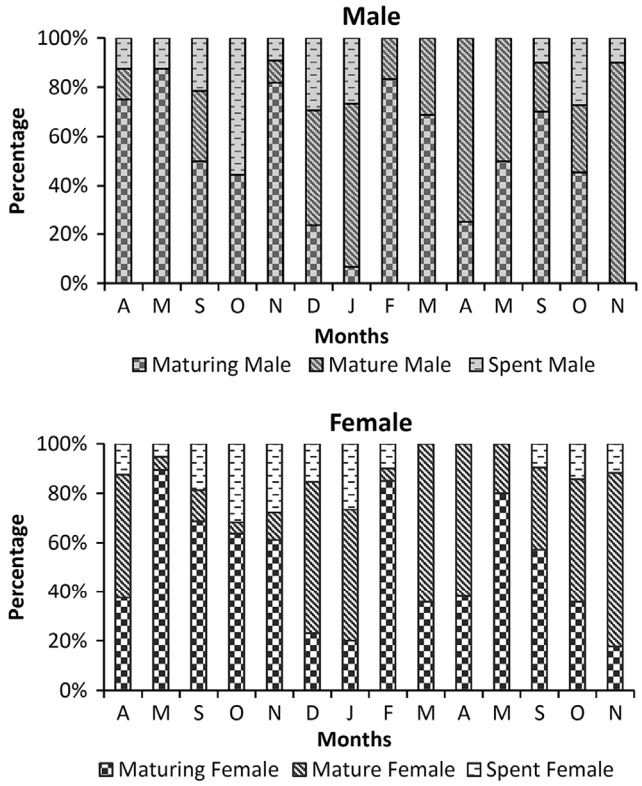

Fig. 2 — Monthly variation of Gametogenic stages of testis and ovary of *Echinometra mathaei* through histological examination from April 2011 to November 2012 at Buleji, Pakistan

Table 1 — Biometric mean ± standard deviation for females and males *Echinometra mathaei* at different stages of maturation. TD = Test Diameter; TW = Total Weight: GW = Gonad Weight; GSI = Gonadosomatic Index. Values in a row with different superscripts (a, b, c) are significantly different ($P < 0.05$). Values without a superscript are not significantly different

	Biometric data	**Maturing (Stage I & II) (n = 116)**	**Mature (Stage III) (n = 76)**	**Spent (Stage IV & V) (n = 31)**
Female	TD (mm)	46.9±6.7	48.1±5.4	45.8±5.8
	TW (g)	50.4±18.0	53.5±17.5	47.4±16.2
	GW (g)	2.2±1.3[a]	4.6±1.5[b]	1.7±0.9[c]
	GSI	4.3±2.0[a]	9.0±2.6[b]	3.8±2.3[c]
Male	**Biometric data**	**Maturing (Stage I & II) (n = 86)**	**Mature (Stage III) (n = 56)**	**Spent (Stage IV & V) (n = 26)**
	TD (mm)	45.0±7.0	46.6±6.2	47.2±5.7
	TW (g)	46.9±20.0	50.5±18.8	51.3±17.6
	GW (g)	2.4±1.5[a]	5.0±2.5[b]	2.3±1.6[c]
	GSI	5.4±2.6[a]	9.9±3.4[b]	4.4±2.8[c]

Table 2 — Monthly variation in the concentration of proteins, carbohydrates, and lipids (mg g^{-1}) (with mean ± standard deviation) in the male and female gonads during the gonadal maturation stages of *Echinometra mathaei* at Buleji, Pakistan from April 2011 to November 2012

Months	Males			Females		
	Proteins	Carbohydrates	Lipids	Proteins	Carbohydrates	Lipids
Apr'11	52.±13.1	18.7±13.2	46.1±8.6	103.0±1.4	63.5±3.5	74.4±1.1
May	66.0±5.7	34.5±3.5	43.2±4.5	83.0±7.1	44.0±5.7	78.0±1.7
Sep	24.7±6.4	12.7±3.5	27.2±2.1	33.3±6.4	19.0±12.4	48.0±8.3
Oct	48.5±9.9	25.5±4.9	61.2±7.4	66.7±5.0	34.0±16.6	63.5±8.4
Nov	25.0±7.1	23.0±5.7	47.2±6.8	52.7±16.2	37.3±4.7	52.3±3.6
Dec	23.0±11.0	23.0±10.9	47.6±7.1	47.3±17.9	35.3±12.7	65.6±4.2
Jan'12	30.0±5.7	47.5±17.7	39.2±10.2	55.5±5.3	71.0±15.0	75.2±14.8
Feb	60.0±0.0	58.5±2.1	36.0±5.7	93.3±6.4	64.3±3.1	56.0±4.8
Mar	72.0±5.7	57.5±4.9	71.2±0.0	98.7±4.2	76.3±21.7	82.8±14.9
Apr	74.0±19.8	58.0±8.5	38.4±1.1	102.0±2.8	83.0±11.3	54.0±2.8
May	82.0±5.7	53.0±21.2	56.0±6.8	124.0±0.0	64.0±0.0	88.0±0.0
Sep	34.0±7.2	9.7±8.3	27.5±5.3	67.0±11.4	14.5±9.3	44.0±5.3
Oct	39.0±18.4	14.5±2.1	33.6±1.1	78.7±8.1	33.7±7.1	52.5±6.8
Nov	73.0±12.7	51.0±26.9	44.0±6.8	105.3±3.1	56.3±3.5	65.6±2.1

proportions across all months, indicating that the population exhibits a prolonged reproductive phase. Spawning females (Stage IV & V) were abundant from September 2011 to January 2012, after which they were primarily in stages I, II, and III. Spawning females were again observed during September 2012, until November, the last month of sampling (Fig. 2).

Proximate composition of the gonads of sea urchin

Monthly variation in the biochemical composition of the gonads

The mean concentration of proteins in the male gonads of *E. mathaei* varied monthly from 23.0±11.0 to 82.0±5.7 mg g^{-1}, that of carbohydrates from 9.7±8.3 to 58.5±2.1 mg g^{-1}, and lipids from 27.2±2.1 to 71.2±0.0 mg g^{-1} (Table 2, Fig. 3). In female gonads monthly mean proteins varied from 33.3±6.4 to 124.0±0.0 mg g^{-1}, carbohydrates from 14.5±9.3 to 83.0±11.3 mg g^{-1}, and lipids from 44.0±5.3 to 88.0±0.0 mg g^{-1} (Table 2, Fig. 3). Proteins were the main component in the gonad of both male and female *E. mathaei.*

The average protein concentration in the gonad of males was 47.0±22.2 mg g^{-1} and in females was 73.8±25.8 mg g^{-1}. The highest mean concentration of proteins in the gonads of *E. mathaei* was observed in May 2012 (Summer), both in males (82.0±5.7 mg g^{-1}) and females (124.0±0.0mg g^{-1}). The lowest mean concentration of proteins was found in December (winter) in males (23.0±11.0 mg g^{-1}) and in September 2011 (late summer) in females (33.3±6.4 mg g^{-1}) (Table 2, Fig. 3).

The average carbohydrate concentration in the gonads of males was 32.2±20.2 mg g^{-1} and in females was 46.9±23.8 mg g^{-1}. The highest mean concentration of carbohydrates in the gonads of males was found in February (winter) (58.5± 2.1 mg g^{-1}) and April 2012 (Spring) (58.0±8.5 mg g^{-1}). In females, the highest mean carbohydrate concentration was observed in April 2012 (83.0±11.3 mg g^{-1}). The lowest mean concentrations were found in September 2012 (late summer), both in males (9.7±8.3 mg g^{-1}) and in females (14.5±9.3 mg g^{-1}) (Table 2, Fig. 3).

The average lipid concentration in the gonads of males was 43.4±12.8 mg g^{-1} and in females was 62.1±14.5 mg g^{-1}. The highest mean concentration of lipids in the gonads of males was found in March 2012 (Spring) (71.2±0.0 mg g^{-1}), while in females, it was in May 2012 (Summer) (88.0±0.0 mg g^{-1}). The lowest mean concentrations were found in September (late summer), both in males (27.2±2.1 mg g^{-1}) and in females (44.0±5.3 mg g^{-1}). In contrast to proteins and carbohydrates, lipids were relatively less variable in most of the months (Table 2, Fig. 3).

In male gonads, there were significant differences in the concentrations of proteins (F = 5.92, df = 13, $P < 0.05$), carbohydrates (F = 4.64, df = 13, $P < 0.05$), and lipids (F = 8.67, df = 13, $P < 0.05$) between different months. Similarly, in female gonads, proteins (F = 14.81, df = 13, $P < 0.05$), carbohydrates (F = 8.08, df = 13, $P < 0.05$), and lipids (F = 8.16, df = 13, $P < 0.05$) also showed significant differences among months (Fig. 3). Also, the concentrations of total proteins, carbohydrates, and lipids in the gonads of males and females *E. mathaei* showed significant differences between them (Proteins: F = 20.44, df = 1, $P < 0.05$; Carbohydrates: F = 7.91, df = 1, $P < 0.05$; Lipids: F = 33.30, df = 1, $P < 0.05$) (Fig. 3).

Biochemical composition of the gonads during gonadal maturation

The concentration of proteins, carbohydrates, and lipids in the gonads of female *E. mathaei* varied significantly ($P < 0.05$) with maturation, being highest in maturing stage (101.9±8.4 mg g^{-1} proteins, 67.7±13.3 mg g^{-1} carbohydrates, and 68.4±13.8 mg g^{-1} lipids) and lowest in spent stage (51.5±18.4 mg g^{-1} proteins, 21.2±9.6 mg g^{-1} carbohydrates, and 50.6±9.3 mg g^{-1} lipids) (Table 3, Fig. 4).

Similarly, the concentration of proteins, carbohydrates, and lipids in the gonads of male *E. mathaei* was found significantly different ($P < 0.05$) as maturation forward, showing the lowest concentration in spent male sea urchin (23.3±7.7 mg g^{-1} proteins, 16.1±9.3 mg g^{-1} carbohydrates, and 33.4±9.3 mg g^{-1} lipids) and highest in maturing male sea urchin (70.0±14.1 mg g^{-1} proteins, 52.2±14.4 mg g^{-1} carbohydrates, and 50.5±13.2 mg g^{-1} lipids) (Table 3, Fig. 4).

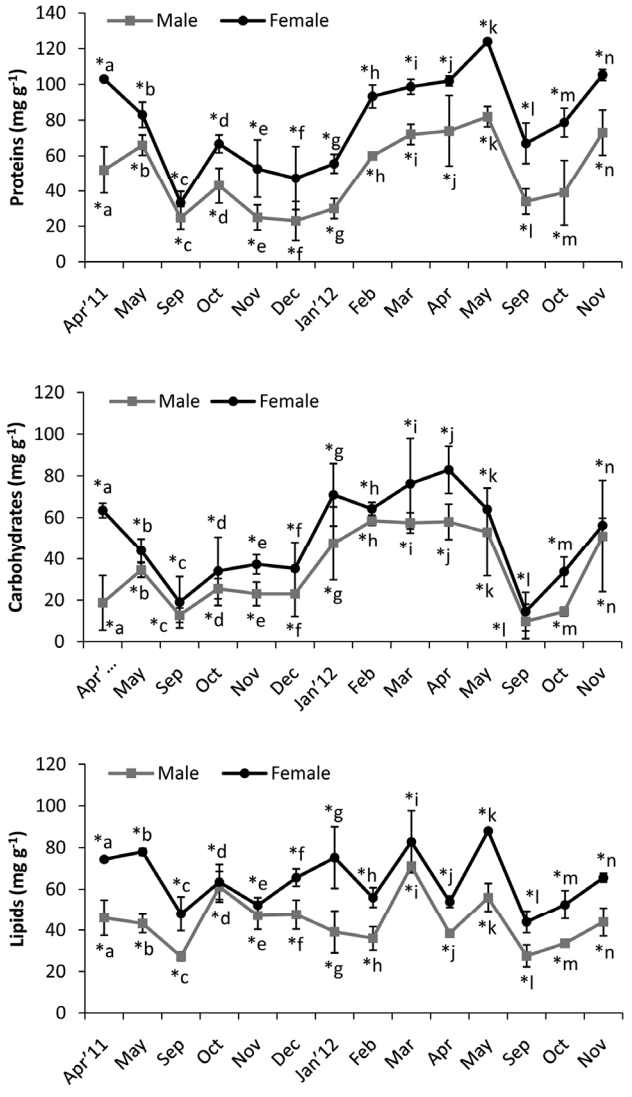

Fig. 3 — Monthly variation in proteins (mg g^{-1}), carbohydrates (mg g^{-1}) and lipids (mg g^{-1}) contents in gonads (ovary and testis) of *E. mathaei* collected at Buleji, Pakistan over 14 months from April 2011 to November 2012. Values are represented as mean ± standard deviation. Superscripts in small letters (a, b, c) indicate significant differences between months, and the asterisk (*) indicates significant differences between sexes ($P < 0.05$)

Degree of association between different parameters

No significant correlations were observed between male GSI and male gonad proteins, carbohydrates, and lipids contents (Proteins: $r = 0.469$, $P > 0.05$, Carbohydrates: $r = 0.353$, $P > 0.05$, and Lipids: $r = -0.188$, $P > 0.05$), although data revealed that the maximum level of carbohydrates and lipids occurred in February and March 2012, just before the highest male GSI (April 2012) was obtained. Male gonadal protein levels positively correlated with male carbohydrates ($r = 0.746$, $P < 0.05$), but no correlation was found with male lipids ($r = 0.401$, $P > 0.05$). No correlation was found between male carbohydrates and male lipids ($r = 0.393$, $P > 0.05$).

In female *E. mathaei*, a significant positive correlation was observed between female GSI and

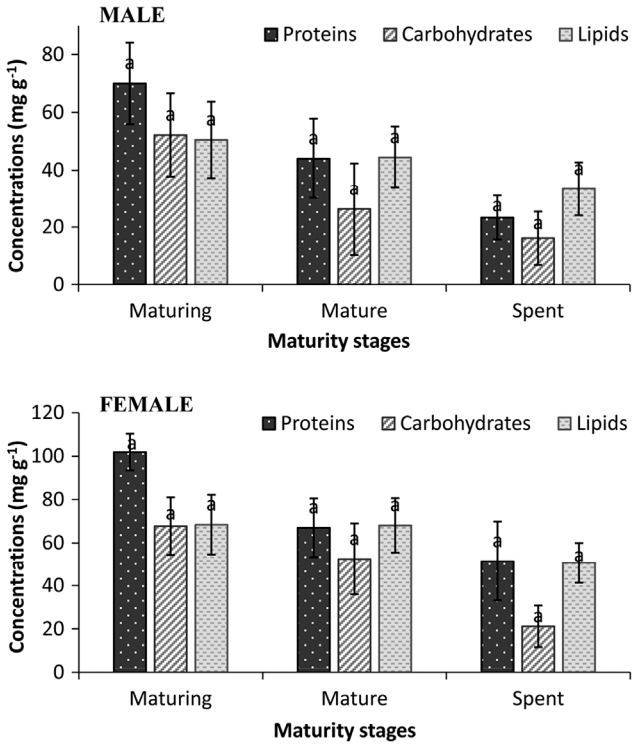

Fig. 4 — Mean (± standard deviation) of concentration of proteins, carbohydrates, and lipids in the gonads of male and female *Echinometra mathaei* at different stages of maturation. Means with different letters are significantly different at $P < 0.05$ level

Table 3 — The minimum and maximum concentration of proteins, carbohydrates, and lipids (with standard deviation) in the male and female gonads during the gonadal maturation stages of *Echinometra mathaei* at Buleji, Pakistan from April 2011 to November 2012

Mean concentrations (mg g^{-1}) in male gonads

Stage	Proteins			Carbohydrates			Lipids		
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**
Maturing	40.0	88.0	70.0±14.1	32.0	70.0	52.2±14.4	32.0	71.2	50.5±13.2
Mature	26.0	66.0	44.0±13.8	7.0	60.0	26.3±16.0	28.8	66.4	44.4±10.7
Spent	10.0	36.0	23.3±7.7	3.0	37.0	16.1±9.3	21.6	51.2	33.4±9.3

Mean concentrations (mg g^{-1}) in female gonads

Stage	Proteins			Carbohydrates			Lipids		
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Average**
Maturing	86.0	124.0	101.9±8.4	53.0	101.0	67.7±13.3	51.2	100.0	68.4±13.8
Mature	48.0	88.0	67.0±13.6	35.0	83.0	52.5±16.5	52.0	95.2	68.1±12.6
Spent	26.0	74.0	51.5±18.4	6.0	37.0	21.2±9.6	39.2	68.0	50.6±9.3

female gonadal proteins (r = 0.569, P < 0.05). In comparison, no significant correlations were observed between female GSI and female gonadal carbohydrates and lipid contents (Carbohydrates: r = 0.492, P > 0.05, Lipids: r = 0.129, P > 0.05). All biochemical components of the female gonads were found to be at their maximum before the highest female GSI was obtained (in November 2012, 9.9 %). Female gonadal protein levels significantly correlated with female carbohydrates (r = 0.669, P < 0.05), but a non-significant positive correlation was observed with the lipids (r = 0.515, P > 0.05). Seemingly, a clear, significant correlation was found between female carbohydrates and female lipids (r = 0.578, P < 0.05). There were a non-significant weak correlations between biochemical contents of both males and females gonads with temperature (Male proteins: r = 0.272, P > 0.05; Carbohydrates: r = -0.228, P > 0.05, Lipids: r = 0.207, P > 0.05; Females proteins: r = 0.211, P > 0.05; Carbohydrates: r = -0.235, P > 0.05, Lipids: r = -0.080, P > 0.05), salinity (Male proteins: r = -0.053, P > 0.05; Carbohydrates: r = 0.395, P > 0.05, Lipids: r = 0.308, P > 0.05; Female proteins: r = 0.088, P > 0.05; Carbohydrates: r = 0.402, P > 0.05, Lipids: r = 0.073, P > 0.05); and photoperiod (Male proteins: r = 0.531, P > 0.05; Carbohydrates: r = 0.053, P > 0.05, Lipids: r = 0.027, P > 0.05; Female proteins: r = 0.458, P > 0.05; Carbohydrates: r = 0.107, P > 0.05, Lipids: r = 0.278, P > 0.05).

Discussion

According to Siddique & Ayub[40], *E. mathaei* from Buleji, Pakistan, showed an annual reproductive cycle characterised by a single spawning period extending from September until January (autumn to winter). A similar pattern of reproductive cycle has been recorded in other echinoid species, such as *Echinometra* sp. by Bronstein & Loya[49], *Diadema setosum* by Bronstein *et al.*[50], *Paracentrotus lividus* by De la Uz *et al*.[51], and *Tripneustes gratilla* by Abou Elmaaty *et al*.[52].

In this study, the highest GSI was recorded in April 2012 for males and in November 2012 for females, coinciding with months when a greater proportion of individuals (male and female) were in mature stages, despite neither April nor November being the months with the highest temperatures or longest day lengths, indicating that peak gonadal development in *E. mathaei* does not directly align with these environmental maxima.

Conversely, the highest percentage of spawned individuals was observed in October 2011 in both sexes of *E. mathaei*, which corresponded with the lowest GSI values. This time of the year also marks declining temperatures and shorter day lengths, suggesting a negative correlation between spawning and photoperiod[40]. Furthermore, spawning females and males showed a weak correlation with seawater temperature and salinity[40]. This result is in agreement with the fact that the gametogenesis and breeding season in sea urchins are quite frequently affected by external factors, such as photoperiod and temperature[53-59], and salinity[60-61].

The concentrations of proteins, carbohydrates, and lipids in an animal's gonad are crucial indicators of its physiological condition and its environmental adaptability. In the current study, the biochemical composition of *E. mathaei* gonads, characterised by considerable reserves of proteins, relatively rich amounts of lipids, and lower levels of carbohydrates, is consistent with findings across numerous other echinoid species[21,29,62-64]. In echinoids, the biochemical

composition of gonads was thought to be independent of species, and its geographical address[27], and their biochemistry varies seasonally[26-28] and is directly influenced by several biotic and abiotic factors, including reproductive cycle itself[36,59,65-66]; temperature[67]; and availability, quality, and type of food, with other seasonal changes[20,49,68-73].

The strong influence of the annual reproductive cycle on the gonadal biochemical profile of *E. mathaei* is rooted in the gonad's dual function of serving as both a primary storage tissue and a reproductive organ[24,44,77-80]. Structurally, the sea urchin gonad consists of two types of primary cells, *i.e.*, germ cells (ova or sperm) and somatic cells (nutritive phagocytes)[74]. Before the start of gametogenesis, which is referred to as the growing stage, Nutritive Phagocytes (NPs) accumulate and store nutrients that can be mobilised to develop germ cells or used to carry out physiological processes during starvation[67,75-77]. Once gametogenesis begins, changes in the ratio of somatic to germ cells determine the overall biochemical profile of the gonads. These cellular dynamics explain the seasonal fluctuations in protein, lipids, and carbohydrate content observed in *E. mathaei*, linking biochemical composition directly to reproductive activity and energy allocation strategies.

During gametogenesis, lipid levels typically tend to decrease because lipids serve not only as an energy source but also as a precursor of other biochemical components[81-82] and also a critical reserve of essential fatty acids for proper embryo development[59]. On the other hand, Fernandez[28] also reported that, under natural conditions, lipid and carbohydrate levels decrease as sea urchins progress toward reproductive maturity. This trend is consistent with the present study in which *E. mathaei* exhibited higher levels of lipids, carbohydrates, and protein in immature gonadal stages than in spent stages (Table 3). Lipid content also showed relatively less periodic fluctuation compared to other biochemical components[21].

In both sexes of *E. mathaei*, the gonadal carbohydrate concentrations were found to be very low. Similar findings have been reported in other sea urchin species, such as *Stomopneustes variolaris*[64] and *Paracentrotus lividus*[5] as well as in holothurians[83-85]. Low carbohydrate reserves in echinoderms could be related to the limited contribution of glycogen, which is generally considered the main storage carbohydrate in animal tissues[86]. However, Chen *et al.*[72] further suggested that carbohydrate concentration may vary with gonadal developmental stages.

Protein content in the gonads of *E. mathaei* showed a strong association with the reproductive cycle, displaying peak levels prior to spawning, consistent with previous studies[28-29,65]. In both sexes, proteins represented the dominant biochemical component[21,29,64,87]. However, female gonads consistently exhibited higher concentrations of proteins, lipids, and carbohydrates than male gonads, in line with observations in other echinoid species[32,39,88-91]. This elevated biochemical content in females likely reflects the greater reproductive investment required to produce nutrient-rich eggs compared to the lower energetic demands of male gametes.

The biochemical composition of the gonads of both sexes of *Echinometra mathaei* showed weak correlations with temperature, salinity, and photoperiod in this study, which is consistent with other studies that reported the effects of different environmental parameters on the biochemistry of sea urchin gonads[63,92].

Prior investigations have shown that the best time to consume nutritionally rich gonads with good market value is during the early growing stages, generally before the onset of gametogenesis[93], because in these stages, nutritive phagocytes are abundant and accumulate a significant amounts of proteins, lipids, and carbohydrates[36,94]. In the current study, the gonads of *E. mathaei* displayed the highest protein, carbohydrate, and lipid contents in Stage I (Immature stage) and maintained this high nutritional value until Stage II (Pre-mature stage). These findings suggest that stage I-II is the optimal period for utilising nutrient-rich gonads. Later, when gonads mature (stage III), their biochemical composition, particularly lipids and proteins, begin to decrease, supporting earlier reports that raw gonad quality deteriorates with the onset of gametogenesis[59,95].

Alteration in the biochemical composition of gonads can spoil their taste and texture, as it was observed that Major Yolk Proteins (MYP), stored in Nutritive Phagocytes (NPs) of immature gonads and responsible for the synthesis of gametes[39], likely contribute to the shift in flavour and texture as gametogenesis progresses, thereby reducing their economic value[36]. Therefore, gonads should be utilised preferably before the beginning of gamete development, when protein and lipid reserves are at their maximum[36].

Furthermore, apart from these explanations, no correlation was observed between the biochemical

components of the gonad and the GSI (indicating reproductive stages), except for proteins in females; this outcome is consistent with the previous study reported by Monteiro-Torreiro & Garcia-Martinez[29] for *Paracentrotus lividus*.

Conclusion

From the present study, it might be concluded that the concentration of proteins, carbohydrates, and lipids in the gonads of both female and male *E. mathaei* varied significantly ($P < 0.05$) with maturation, peaking at the maturing stage, and reaching their lowest levels at the spent stage. If the gonads of *Echinometra mathaei* were to be utilised, they have the best quality in biochemical contents throughout the year (as Stage I and Stage II individuals are present in every month), but from February to May (when individuals with Stage I and II individuals are found more abundantly), significantly higher concentrations of proteins and lipids are upheld by the NPs. In Pakistan, there is no traditional sea urchin fishery exists and no local consumption is reported. This sea urchin species has possible potential that be exploited commercially[42], but there is a need to put in place a sustainable harvesting strategy before commercial exploitation begins to protect and regulate this valuable resource and to make sure that a significant percentage of the sea urchin population reproduce, attain maturity, and successfully sustain unaffected population sizes.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

The authors followed all applicable international, national, and institutional guidelines for animal testing, animal care, and animal use.

Author Contributions

SS: Measurements and data collection, statistics, analysis of the results, and writing and editing of the manuscript. ZA: Conceptualization and supervision.

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