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Nutritional susceptibility to morphological, chemical and microbial variability: An investigation on mud crab, *Scylla serrata* in Bangladesh

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Abstract
Nutritional susceptibility of *Scylla serrata* to chemical and microbial variability with varying morphological capacity was the thematic area of the present investigation. In detail, morphological capacity considering carapace length, carapace width, total length, chelate leg length and crawl leg length; the nutritional composition considering protein, lipid, ash, moisture; chemical assessment considering TVN, TMA; and microbial assessment considering TBC, TMC were measured for both male and female mud crab. Morphological observation revealed that the weight of male mud crab was changed by 65% with changing per unit of length (b=0.65) and in female it was by 42%. Moreover, for males the b values of weight-condition factor and length-condition factor ranges from above 2 to above 3; whereas in females the range of values were less than that found in males. TBC and TMC found in muscle of male mud crab were higher than that of female. The beta co-efficient of multivariate regression analysis showed (TVN= 9.2+0.48 TMA+0.02 TBC+ 1.7 TMC) that the TVN is changed by 0.48 unit/TMA, 0.02 unit/TBC and 1.7 unit/TMC changes indicating that the TVN value is highly changed with changing TMC followed by TMA and TBC. In case of TMA, the beta co-efficient of multivariate regression analysis (TMA=52.859+ 0.032TBC+3.624TMC) showed that the TMA is changed by 0.032 unit/TBC and 3.624 unit/TMC changes indicating that the TMA value is highly changed with changing TMC rather than that of TBC. The protein content found to negatively relate with the moisture content by 0.661 co-efficient values and with lipid with 0.04 values. The correlation co-efficient of proximity composition considering primarily protein and lipid with chemical and microbial variability showed that protein is negatively sensitive to TVN, TBC and TMC values. It can then be inferred that the nutritious proximity of mud crab is significantly susceptible to sex with varying morphological capacity, chemical and microbial variability.

Keywords: Mud crab, Nutritional susceptibility, Morphological variability, Proximate composition

1. Introduction
The *Scylla serrata* being the most important and high demand coastal aquatic species in the international market, due to its nutritional richness, becomes in consideration of holistic management approach of maintaining the microbial and chemical quality with varying condition factor to ensure its nutritional proximity in all level of its supply chain [1, 2]. Crab meat contains essential amino acids, proteins, unsaturated fatty acids and also an excellent source of minerals [3, 4]. A novel protein was isolated from the seminal plasma of the mud crab, *Scylla serrate* [5]. The nutritional quality of the crab proteins were compare very favorable than that of muscle meat of mutton, chicken, duck and fish [6, 7, 8]. At present major export markets of mud crabs from Bangladesh are China (about to 92%), Malaysia, Japan, Hong kong, Thailand, Taiwan and Singapore [9].
Hence the major challenge ensuring nutritional status or value of mud crab is supposed to be microbial risk because it is directly associated with food safety for human consumption as well as importer's highly concern. This is because, mud crabs are sold primarily in the live state and during the process of preparing mud crabs for the market, various procedures may induce stress responses as reflected by some biochemical changes that may lead to reduced nutritional quality of the crabs [10]. Published literature on all-inclusive dissertation of the *Scylla serrata* of Bangladesh is very scanty [11, 12, 13]. Only a few insulated study such as biological investigation was reported by [11] without incorporating proximate, chemical and microbial

analysis. Similarly, [12] and [13] have conducted study on biochemical composition of *Scylla serrata* but they have not mentioned about chemical, microbial and morphological condition. In addition [14, 15, 16, 17], have executed study on marketing system and socio-economic status of crab collectors of *Scylla serrata*, but they have not remarked about biochemical, chemical, microbial and morphological status. Microbial analysis is too few to be mentioned [18].

Hence the present investigation is the first of its kind of holistic approach which covers microbial analysis, chemical analysis, proximate composition and morphological capacity both in male and female mud crab of Bangladesh. Correspondingly this paper explores a comprehensive explanation of nutritional susceptibility against microbial and chemical variability in mud crab in order to provide adequate management measures.

2. Materials and methods

The experimental specimen (both male and female of *Scylla serrata*) were collected at rate 100 Taka/kg from Farmgate Bazar Fish Market, Dhaka. The collected samples were washed in chlorinated water and preserved in a deep freezer (-20 ºC); afterwards those were taken out for performing analysis. Each type of sample was divided into four lots. One used for proximate composition, one for chemical assessment, one for microbial quality and another lot was for determination of morphological characteristics.

2.1. Morphological analysis

The collected mud crabs were analysed for recording the morphological capacity considering carapace length, carapace width, total length, chelate leg length and crawl leg length. Individual carapace width (CW) between tips of the longest lateral spines across the middle line between the frontal notch and posterior margin were measured using a Vernier caliper (0.1mm accuracy) and individual weight was taken in Docbel (BRAUN) weighing balance (Accuracy of 2g) after removing all adhering water from the body using a blotting paper.

2.2. Biochemical composition

Moisture, lipid, protein and ash contents were determined by Association of official Analytical Chemist (AOAC) official method [19]. Protein was estimated by kjeldahl's method [20]. Fat was determined by Soxhlet's method [21]. The percentage of ash content was estimated by burning the materials in a Muffle Furnace at 550-600˚C for 4-6 h and moisture was determined by drying the samples at 100-105˚C in an oven [21].

2.3. Chemical assessment

Total Volatile Nitrogen (TVN) and Trimethylamine (TMA) were investigated using AOAC official method to observe chemical changes [19]. Conway Micro Diffusion technique was employed for the estimation of TVN and also TMA to observe chemical changes [22].

2.4. Microbial assessment

The microbiological changes in samples *such as* total bacterial count (TBC) and total mould count (TMC) were determined by decimal dilution technique followed by pour plating [23, 24].

2.5. Analysis

The length-weight relationships were determined separately for males and females in *Scylla serrata* by the linear equation $Y = a + bX$ where X= independent variable, Y = dependent variable and 'a' and 'b' are constants. For this purpose, the observed values of individual crabs were transferred into logarithmic values and regression analysis was carried out to calculate the 'a' and 'b' values. The variation in male and female *S. serrata* was calculated using ANOVA (Analysis of covariance). Descriptive statistics was used to analyze and represent the data. Statistical analysis was executed with the SPSS software package (verson11.5, SAS Institute Inc, USA) and with Microsoft Excel.

3. Results

3.1. Length-Weight measurements and Condition factor

The length of male mud crab was around 4 cm while in female it was near about 3 cm (Table 1). Weight of male mud crab was nearly 250 g while nearly 150 g was found in female mud crab. A scatter diagram in respect to *Scylla serrata* was obtained by plotting the Length (total length, crawl leg length and chelate leg length) against weight and carapace width against weight of individual crabs (Figure 1). From the closeness of the scatter and from the parabolic nature of the plot, it is clear that there exist a good relationship between length and weight as well as between carapace width and weight. The coefficient of correlation (r) obtained for the total length-weight, crawl leg length-weight, chelate leg length-weight and carapace width-weight were nearly equal to 1 (0.985, 0.963, 0.973 and 0.992 respectively) indicating that the values were significant and hence, a high degree of positive correlation existed between total length-weight, crawl leg length-weight, chelate leg length-weight and carapace width-weight in the crabs.

Table 1: Morphological characteristics of male and female mud crab *Scylla serrata*

Sex	Length (cm)	Carapace Width (cm)	Chelate Leg Length (cm)	Crawl Length (cm)	Weight (gm)	Condition Factor
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Male	4.27 ± 0.15	2.70 ± 0.12	17.00 ± 0.58	4.17 ± 0.15	248.33 ± 21.43	2.45 ± 0.12
Female	2.83 ± 0.15	1.63 ± 0.15	11.67 ± 0.33	2.70 ± 0.06	147.67 ± 15.65	1.44 ± 0.13
Combine	3.55 ± 1.02	2.165 ± 0.76	14.335 ± 3.77	3.435 ± 1.04	198 ± 71.18	1.95 ± 0.56

Table 2: Length-Weight and Width-Weight relationship in male and female

Sex	Length vs Weight						Width vs Weight					
	a	b	R^2	SE	t	P	a	b	R^2	SE	t	p
Male	1.47	0.65	0.99	0.0003	2368	0.000	1.71	0.35	0.99	0.0001	4261	0.000
Female	1.59	0.42	0.99	0.0002	2050	0.000	1.69	0.11	0.99	0.001	82	0.000

The b values of width-condition factor and length-condition factor are 2.586 and 3.273 for male whereas 1.360 and 2.368 for female.

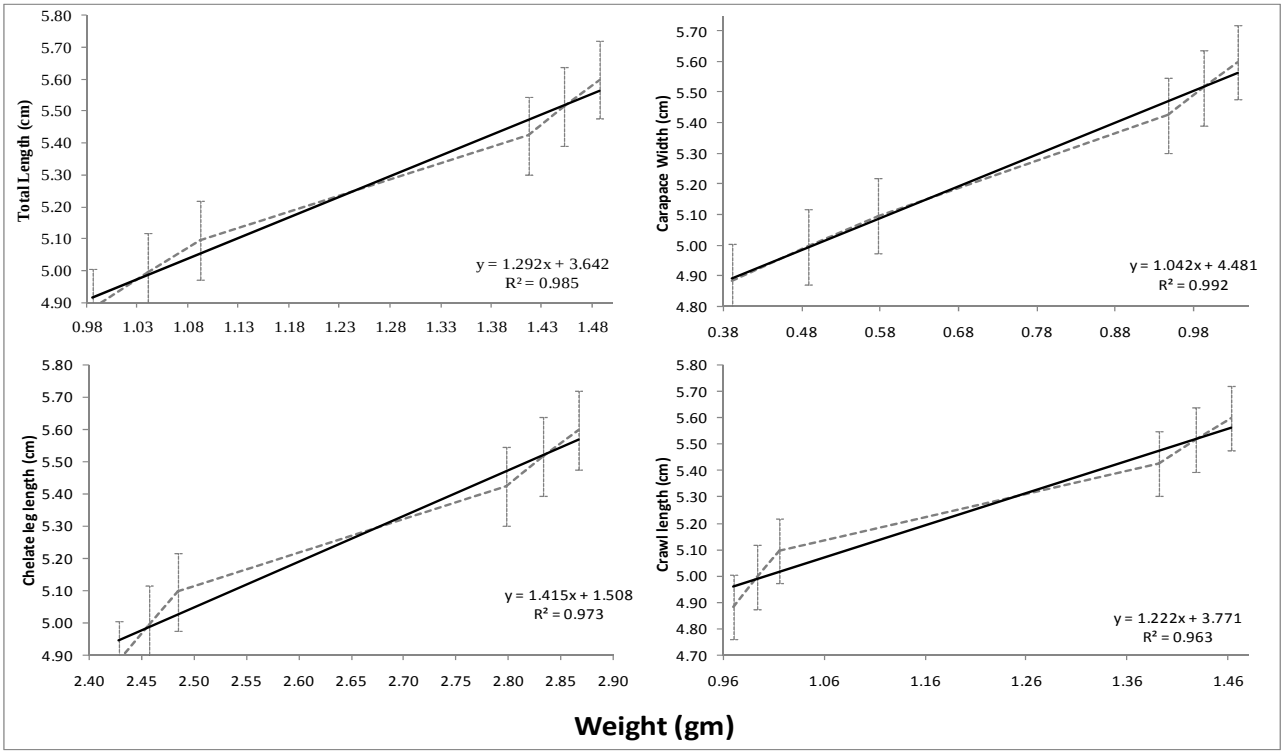

Fig 1: Relationship matrix of total weight with total length, chelate leg length and crawl leg length and carapace width of Mud Crab (*Scylla serrata*)

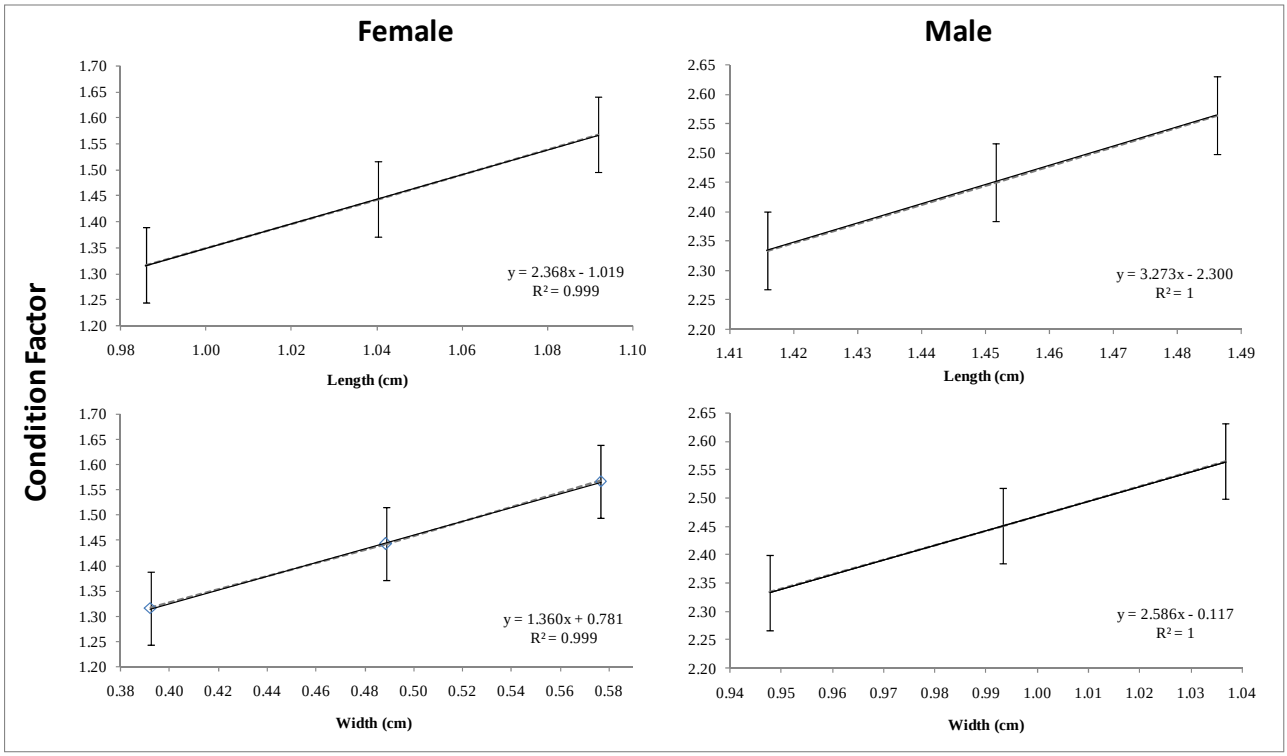

Fig 2: Proximity of condition factor to total length and carapace width of Mud Crab (*Scylla serrata*)

3.2. Proximate composition

Near about 11% protein was measured in both male and female mud crab (Figure 5). Nearly 84% moisture and 2.3% ash was measured in male mud crab while about 85% moisture and 1.6% ash was observed in female crab. The present study has found nearly 2.4% lipids in male while about 2.3% in female mud crab.

3.3. Chemical quality

Near about 20 mg N/100g of TVN was measured in both male and female mud crab (Figure 3). Moreover, about 50 mg TMA/100g of TMA was measured in male mud crab while 25 mg TMA/100g sample was observed in female crab.

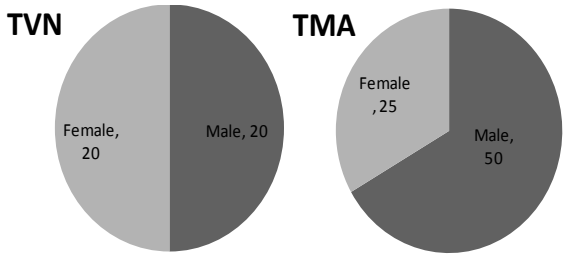

Fig 3: Chemical quality (TVN and TMA) in male and female mud crab, *Scylla serrata*.

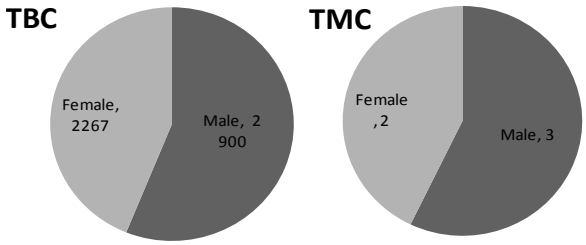

Fig 4: Total bacterial count (cfug^{-1}) and total mould count (cfug^{-1}) in male and female mud crab, *Scylla serrata*.

3.4. Microbiological quality

Total bacterial count observed in male mud crab was 2.90 ± 0.15×10^3 cfug^{-1} while in female was 2.27 ± 0.12×10^3 cfug^{-1} (Figure 4).

Moreover, in case of male mud crab total mould count was 4.67 ± 0.67 cfug^{-1} while in female was 4.33 ± 0.33 cfug^{-1}.

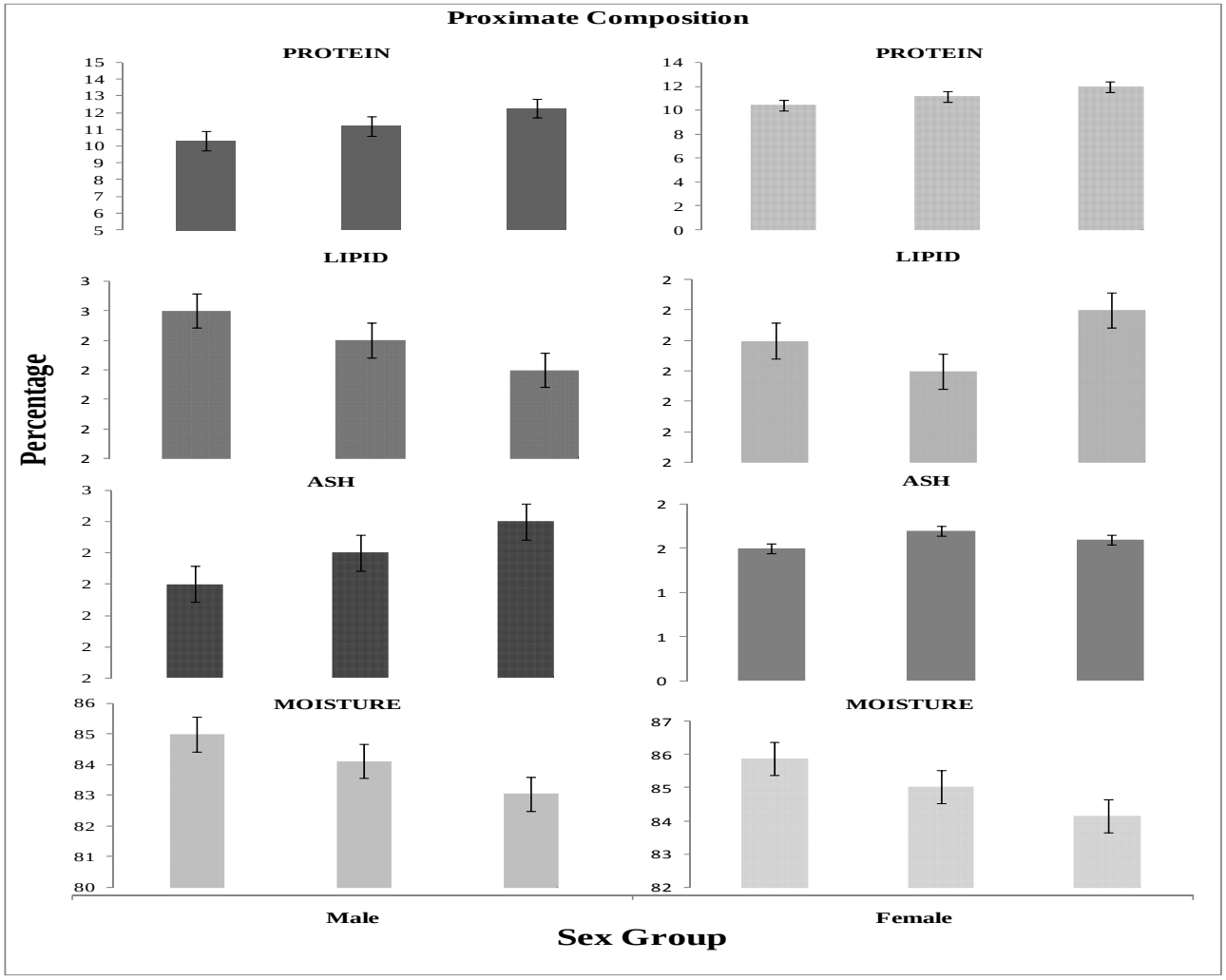

Fig 5: Proximate composition in male and female mud crab (*S. serrata*).

4. Discussion

4.1. Length–weight measurement and condition factor

Knowledge of carapace width-weight relationships of a species is necessary to provide adequate management of its fisheries and aquaculture [25]. In the present study, the beta coefficient indicates that the weight of male mud crab is changed by 65 % with changing per unit of length (b = 0.65) and in female it is by 42% (Table 2). It indicates that mud crab shows a sexual dimorphism and male mud crab has tendency to be heavier when compare to the female as found in three species of *S. serrata* by [26]. This result has consistency with the previous observation in case of *S. serrata* [27] and *S. tranquebarica* [26]. Moreover, for males the b values of weight-condition factor and length-condition factor ranges from above 2 to above 3 whereas in females the range of values were less than that found in males indicating that male mud crab is haviar than female mud crab [26]. Stated with showing 'b' values *that* the males are heavier than females at a given width and length against weight in *S. tranquebarica* which is similar with the present study.

4.2. Proximate analysis

The nutritive substances existed in a dissolved condition. All the important chemical reaction in cells also occurred in aqueous medium [12]. Except lipid content all the studied nutritional values show more or less similar pattern in composition for male and female. [12] Reported that the protein values of *Scylla serrata* male were 17.69% and 19.39% for females. [28] Investigated 11.60% protein in body meat of male and 19.92% protein in female body meat of mud crab which is in consistency with the present study. However [12], reported that, the highest moisture content found in male mud crab were 83.50 % and 79.50% for females which is a little different as compared to the present study. [12] also reported that in male mud crab highest ash content was measured 2.22% in December and 1.20% in May; In female highest ash content was measured during April 2011, 2.34% and lowest in August was 1.62%. In the present study the protein content relates negatively with the moisture content by 0.661 co-efficient values and with lipid with 0.04 values (Table 3).

The result of the present study (2.4% lipids in male while about 2.3% in female mud crab) is more or less has got similarities with the findings works done earlier [27]. Noticed that the lipid content in in *Scylla serrata* from body meat was 1.65% and claw meat was 2.01 %. However [29], stated that biochemical composition of fish differs due to types of food, age, size, stage of maturity, place of collection etc.

Table 3: Relationship matrix among the nutritious values in the mud crab

Sex	Protein	Lipid	Ash	Moisture
Protein	1	-0.019	0.069	-0.661
Lipid			0.04	-0.04
Ash		1	1	0
Moisture				1

The correlation co-efficient of proximity composition considering primarily protein and lipid with chemical and microbial variability shows that protein is negatively sensitive to TVN, TBC and TMC values. According to [30], due to the presence of TMC (pro-biotic micro-organism) protein is able to produce some organic acids such as lactic acid, acetic acid etc having anti-microbial specifications and is able to prevent the bacterial. It has also been supposed that an increases in the amount of TVN may be due to the effect of production of free fatty acids on the denaturizing of protein [30, 31]. As they stated, these factors causes the prevention of proteolysis, which subsequently increases TVN.

Table 4: Susceptibility of nutritious values to chemical and microbial variability in the mud crab

	PROTEIN	LIPID	TVN	TMA	TBC	TMC
PROTEIN	1.00	0.00	-0.33	-0.09	-0.60	-0.31
LIPID	0.00	1.00	0.85	0.21	0.04	0.77
TVN	0.33	0.85	1.00	-0.22	0.53	0.63
TMA	-0.09	0.21	-0.22	1.00	0.80	0.21
TBC	0.60	0.04	0.53	-0.80	1.00	-0.16
TMC	-0.31	0.77	0.63	0.21	-0.16	1.00

4.3. Chemical quality

Volatile nitrogen (TVN mg-N/100g of fish sample) is the main indicator of spoilage. It has been reported that the acceptance range of average TVN value in case of fin fishes ranges from 4.05 to 46.75 mg/100g (large size), 5.15 to 47.36 mg/100g (medium size) and 3.35 to 47.97 mg/100g (small size) over 18 days of iced storage [32, 33]. A similar range of TVN values has been measured in the present investigation. Moreover, in the current work TVN value showed no variance with the sex as similar as reported by [34]. He has also stated that the upper limit of acceptable limit of TVN value is 30 mg-N/100g. However, TVN value significantly varies with the variation of TBC at 95% significant level in the present study. It has also been found that TVN value positively correlated with the TMA, TBC and even with TMC. The beta co-efficient of multivariate regression analysis showed (TVN = 9.2 + 0.48 TMA + 0.02 TBC + 1.7 TMC) that the TVN is changed by 0.48 unit/TMA, 0.02 unit/TBC and 1.7 unit/TMC changes indicating that the TVN value is highly changed with changing TMC followed by TMA and TBC.

Trimethylamine (TMA) is produced by many spoilage micro-organisms from a compound known as trimethylamine oxide (TMAO). Some research suggested the TMA value ranged from 10-15 mg N/100 g of fish muscle as the upper limit of acceptability while in case of marine fish [35, 36]. But others referred the acceptable limit of TMA is 30 g N/100 g of fish [34]. In the present investigation, higher TMA value has been found in male mud crab than the accepted TMA value of other findings, but TMA value in case of female belonged to the acceptable range. This indicates that the TMA value varies with sex dimorphism of mud crab which is similar to the findings of [34]. Moreover, it has also been found that the TMA value significantly varies with TBC at 95% confidence level. The beta co-efficient of multivariate regression analysis (TMA = 52.859 + 0.032 TBC + 3.624 TMC) showed that the TMA is changed by 0.032 unit/TBC and 3.624 unit/TMC changes indicating that the TMA value is highly changed with changing TMC rather than that of TBC.

4.4. Microbial Quality

Several aspects of aquaculture of mud curb species have been the basis of intensive research during the last two decades [24, 37, 38, 39, 40]. However, disease in crab populations received little attention [41]. Therefore, microbial infections (i.e., bacterial and fungal) have been the major concern of mud crab researchers. Furthermore, infected crabs may potentially transmit pathogens to various rearing facilities, adjoining farms, and even to natural environment, although much data are not available [41].

In this present study, therefore, total bacteria and total mould have been counted for investigating the microbial risk of nutritious values of mud crab. It has been found that both the TBC and TMC found in muscle of male mud crab was higher than that of female indicating that the microbial quality varies with sex in case of mud crab.

5. Conclusions

Mud crab shows a sexual dimorphism in its microbial, chemical and nutritional proximity and male mud crab has tendency to be heavier when compare to the female. Spoilage of both male and female mud crab is increased with increasing TMA, TBC and even with TMC values. It has also found that the nutritional value changes with changing spoilage indicator, and even chemical and microbial quality. It is, thus, concluded that the nutritious proximity of mud crab is significantly susceptible to sex with varying morphological capacity, chemical and microbial variability.

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