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Nutritional Value of the Egyptian Freshwater Bivalve *Spathopsis rubens arcuata* Under the Effect of Depuration

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Abstract

On the view of finding a new source of protein, *Spathopsis rubens arcuata* is one of fresh water bivalves inhabiting Nile River, Egypt. The quality of this clam as a human food was investigated. The herein results showed that *S. rubens arcuata* is a good source of protein, carbohydrate and with a negligible source of fat when compared with edible fishes. Myristic acid was the most abundant saturated fatty acid (SFA) while Myristoleic acid and Linoleic acid were the two most abundant unsaturated fatty acids. The nonessential amino acid Glutamine is the most dominant amino acid followed by Aspartic, Alanine and Leucine. Studying the safety of consuming the investigated clam was based on: 1) Studying the heavy metal content in relation to the environment and after a depuration period, 2) Studying the associated parasites. The results showed that the initial heavy metals (Cu, Cd and Pb) concentrations detected in soft tissues of the clam were high and exceeded the legal values. But the 8 days of depuration was an excellent strategy to reduce Cu, Cd and Pb to an acceptable level for human consumption. Seasonal study of the clam associated parasite showed that *S. rubens arcuata* harbors nonpathogenic ciliated protozoa *Conchophthirus sp.* and an annelid *Batracobdelloides tricarinata* as commensal organisms and a trematode *Aspidogaster conchicola* as a parasite. The present work concludes that *S. rubens arcuata* can be a good source of food for human on condition that arises in the market after a period of depuration reached to 8 days.

Keywords: Bivalves; Depuration; Fatty acids; Amino acids; Parasites

Introduction

Mollusks, especially bivalves clams and mussels are an important food source for humans. It is well known that clams are a good source of some important nutrients such as proteins, carbohydrates, steroids, minerals, especially iron, zinc, and copper, and vitamins such as vitamin B-12. Also, these shellfish can be considered as a reliable source of fats especially saturated one and have a high content of the omega-3 fatty acids [1]. The shellfish have cholesterol concentrations of less than 80 milligrams per 100 grams (edible portion) therefore, can be consumed by people trying to limit their dietary cholesterol intake [2]. They provide a high-quality protein with almost all the dietary essential amino acids [3]. They also generally store carbohydrates in large amounts during their growing season and use them over the rest of the year [4]. Although proteins may be an energy reserve in some bivalve species [5,6].

Different toxins might be accumulated in these clams under certain conditions [7] This risk is increased due to eating these clams raw or lightly cooked [8]. To minimize this risk, the source of the shellfish should be investigated, and better quality would be attained by appropriate treatment following the harvest. The best strategy that has been developed for bivalve risk management is the depuration [9]. The depuration helps bivalves to expel and isolate contaminants from their gills and intestinal tract over a period. The most abundant heavy metals such as copper (Cu), lead (Pb) and cadmium (Cd) were found in the Egyptian irrigation system [10].

In Egypt, *S. rubens arcuata* is among the benthic invertebrates in an irrigation canal that in direct contact with heavy metals both of natural and anthropogenic origin. The quality and economic importance of this species are still unknown. The present work aimed to investigate the effect of the depuration on the nutritional value of *S. rubens arcuata* clams, assessment of the most abundant heavy metals in their tissues and the prevalence of the infection rates of different parasites which could be hosted in the clams tissues.

Materials and Methods

Samples collection

Spathopsis ruben arcuata were collected from Al-Mahmoudia irrigation canal at Damanhour, El-Beheira Governorate, Egypt. Dead or damaged specimens were eliminated and a standardized shell size, only shell sizes ranged from 110 to 130 mm in length and 23 to 41 mm in width were used.

Depuration experiment

The depuration experiment was commenced within 4h of shellfish collection. Depuration was studied for three and eight days. Ten clams were placed in aquaria contained 10 litters dechlorinated tap water in three replicates under laboratory conditions and with continuous aeration. Water was changed and replaced with new dechlorinated tap water and the aquaria were cleaned every day to avoid refiltration of depurate contaminants.

Heavy metal analysis

Heavy metal (Cu, Cd, and Pb) concentrations in soft tissues of *S. rubens arcuata* were analyzed according to Pearson's chemical analysis of foods [11] by using an atomic absorption spectrophotometer Shimadzu model (AA-6650). The results of metal concentrations are expressed as µg/g of dry weight of soft tissues.

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Determination of the chemical composition

Moisture content was determined according to AOAC in 2000 [12]. Ash was determined according to AOAC in 1986 [13]. Total carbohydrate was determined colorimetrically according to the study of Duboies et al. [14]. Total lipid was analyzed using Soxtherm; Gerhardt, laboratory instrument while total protein was analyzed by Kjeldatherm and Vapadest 50s; Gerhardt, laboratory instrument [15].

Amino acid analysis

The dried ground sample (1 g) was put in diethyl ether for 24 hours to remove lipid from it and then dried. 0.3 grams of sample were hydrolyzed with 6 N HCl)5 mm^3) in a sealed tube at 110°C for 24 hours, then cooled for 10 min. The sample was filtered with the aid of 10 mm^3 of distilled water. Thereafter, the sample was put in a water bath for 5 hours till dryness. At the end of this step, the digestion process was finished, and distilled water and HCL were evaporated. The residue of the sample was dissolved in 2 ml of sample dilution (diluting buffer) (0.2 M, pH 2.2) to complete the sample dissolving. Amino acid quantities were determined by using an automatic amino acid analyzer [16].

Fatty acid analysis

In a tube, 50 mg of lipid was weighed, then 5 ml of methanol sulphuric acid (1 ml conc. sulphuric acid and 100 ml methanol) and 2 ml of benzene were added. The tube was closed well and placed in a water bath at 90°C for an hour and a half. Then, it was cooled, and 8 ml of water and 5 ml of petroleum ether were added. The tube was shaken strongly, and the ethereal layer was separated out in a dry tube. Evaporation was done until dryness occurred. All solution was placed in a tube and put in the oven at 90°C for an hour and a half. The resulting methyl esters were analyzed using an Agilent Gas Chromatography system [17].

Examination methods for parasites

The samples were examined for parasitic infections using shedding method [18]. The thin hemolymph film was prepared for any protozoan parasites according to Abd El-Rahman [19] collected Helminth and annelid samples were identified according to Brooks and Welsch, Hendrix and Overstreet & Abd El-Rahman [20-22] Scanning electron microscopy (SEM) for helminths and annelids occurs according to Hassan and Saeed [23].

Statistical analysis

Data on metal contents and biochemical measurements (protein, carbohydrate, lipid, amino, and fatty acids) of *S. rubens arcuata* were statistically analyzed. One-way analysis of variance (ANOVA) and post-hoc multiple-comparison tests (Tukey) was used. The level of significance was presented at $p< 0.05$. All statistical analyses were performed using the statistical software package SPSS 20.0.

Results

The heavy metal content of *S. rubens arcuata*

The results showed that the concentrations of heavy metals Cu, Cd, and Pb (µg/g of dry tissue weight) were gradually decreased in all soft tissues of *S. rubens arcuata*a the depuration period was increased. On the 3rd day of depuration, the levels of heavy metals in the foot, mantle, and adductor muscle tended to decrease in the order Cd>Pb>Cu but tended to decrease in the order Pb>Cd>Cu in the gills. While after 8 days of depuration, the levels of heavy metals in the previous tissues tended to decrease in the order of Cd>Pb>Cu. the highest reduction rate of Cu and Pb was on the 3rd day of depuration in the gills, while on the foot, the highest reduction rate for the concentration of Cd. On the other hand, at the 8th day of depuration, the reduction rate of Cu was higher in the gills, but in mantle for the Pb. After 8 days of depuration, Cu and Pb showed the lowest percentage in the foot tissues. By day 8 of depuration, all soft tissues were eliminated form Cd as shown in Table 1.

Biochemical composition of depurated *S. rubens arcuata*

The biochemical compositions of the soft parts of *S. rubens arcuata* were assessed after 3- and 8-days post depuration. The moisture percentages were statistically significant increased through the depuration periods ($p=0.000$). Lipid percentage was not statistically significant changed ($p>0.05$) after the depuration for 3 days but significantly decreased after 8 days of depuration ($p<0.05$). On the other hand, the total protein and carbohydrate contents in *S. rubens arcuata* after 3 days of depuration significantly decreased ($p=0.003$), but after 8 days of depuration decreased significantly ($p=0.000$ and $p=0.008$, respectively) when compared with the initial content as shown in Table 2.

Variables		Foot	Gill	Mantle	Adductor muscle
Cu	Before depuration	8.21 ± 0.34^{c}	11.20 ± 1.94^{c}	9.47 ± 0.54^{c}	15.96 ± 0.75^{b}
	3rd days- post depuration	7.32 ± 0.33 ^{b*}	7.52 ± 0.30 ^{b*}	7.37 ± 0.37 ^{b**}	15.96 ± 0.60^{b}
	8th days- post depuration	3.45 ± 0.12 a***	0.73 ± 0.02 ^{a***}	2.20 ± 0.27 ^{a***}	1.67 ± 0.10 ^{a***}
Cd	Before depuration	1.06 ± 0.14^{c}	0.49 ± 0.03^{c}	0.27 ± 0.05^{c}	0.78 ± 0.08^{c}
	3rd days- post depuration	0.34 ± 0.04 ^{b***}	0.31 ± 0.02 ^{b***}	0.16 ± 0.01 ^{b*}	0.26 ± 0.01 ^{b***}
	8th days- post depuration	N.D ^{a***}	N.D ^{a***}	N.D ^{a***}	N.D ^{a***}
Pb	Before depuration	14.35 ± 1.86^{b}	10.33 ± 1.12 ^{c}	4.69 ± 0.59 ^{c}	14.96 ± 0.87^{c}
	3rd days- post depuration	12.09 ± 0.06^{b}	6.07 ± 0.10^{b***}	2.83 ± 0.30 ^{b**}	10.76 ± 1.89 ^{b*}
	8th days- post depuration	0.85 ± 0.04 ^{a***}	0.21 ± 0.02 ^{a***}	0.03 ± 0.003 ^{a***}	0.02 ^{a***}

ND: Non-detected
*Significant at p-value ≤ 0.05
** Significant at p-value ≤ 0.0
***Significant at p-value ≤ 0.00
Values are mean ± SD.
Different letters $^{(a, b, and c)}$ in each row for each metal are significant, $p<0.05$ (One-way ANOVA).

Table 1: The heavy metal concentrations in foot, gill, mantle, and adductor muscle of *Spathopsis rubens arcuata*, during the first day of collection, third, and eighth day of depuration.

Biochemical composition (%)	Depuration Time		
	Before depuration	Day 3 post depuration	Day 8 post depuration
Moisture	20.27 ± 0.31^{a**}	23.78 ± 0.43^{b}	54.69 ± 0.42^{c***}
Ash	8.31 ± 0.18 ^{c}	6.29 ± 0.01^{b***}	5.35 ± 0.07^{a***}
Proteins	39.17 ± 0.02^{c**}	36.19 ± 0.41^{b}	10.35 ± 0.21^{a***}
Carbohydrates	32.56 ± 0.15^{c**}	30.30 ± 0.14^{b}	28.61 ± 0.28^{a**}
Lipids	1.70 ± 0.14^{b}	1.41 ± 0.01^{b}	1 ± 0.01^{a*}

SD: Standard Deviation, F ratio: Frequency, p-value: Probability.

Table 2: Biochemical composition in the total soft tissues of *Spathopsis rubens arcuata* collected from Al-Mahmoudia canal, Zawyet Ghazal, at the first day of collection, third, and eighth days of depuration. Different letters $^{(a, b, and c)}$ in each row are significant, $p<0.05$ (one-way ANOVA).

Analysis of fatty acid composition after depuration periods

The contents of fatty acids were detected through Gas Chromatography analysis from soft parts of *S. rubens arcuata* after depuration for 3 days. On the first day of collection and after three days of depuration,13 different fatty acids in *S. rubens arcuata* were analyzed including 7 saturated fatty acids (SFA), 4 monounsaturated fatty acids (MUFAs), and 2 polyunsaturated fatty acids (PUFAs). The initial content of the tissue showed that Myristic acid (C14:0) was the most abundant SFA in *S. rubens arcuata*. The major acid was detected among the MUFAs is Myristoleic acid (C14:1), while Linoleic acid (C18: 2c) was in PUFAs. At the first day of collection, there are one MUFA omega 9 (Oleic acid C18:1c), one PUFA omega 3 (Eicosatrienoic acid C20:3w3), and one PUFA omega 6 Linoleic acid C18:2c), while after 3 days of depuration, 20 different fatty acids were detected in *S. rubens arcuata* tissues including 9 saturated fatty acids (SFA), 5 monounsaturated fatty acids (MUFAs), and 6 polyunsaturated fatty acids (PUFAs). The Palmitic acid (C16:0) became the most abundant SFA. While, among the MUFAs, Oleic acid (C18:1c) was the most abundant acid and Linoleic acid (C18:2c) among the PUFAs. Also, in addition to Oleic acid C18:1c (MUFA omega 9), Eicosatrienoic acid C20:3w3 (PUFA omega 3), and Linoleic acid C18:2c (PUFA omega 6) that found in the initial content, there are 3 PUFAs omega 3 (Alpha Linolenic acid C18:3, Eicosapentaenoic acid C20:5, and Docosahexaenoic acid C22:6) and one PUFA omega 6 (Arachidonic acid C20:4) were found after depuration of three days.

Some of the saturated fatty acids such as palmitic acid, stearic acid, margaric acid, and heneicosylic acid and all unsaturated fatty acids, except myristoleic acid and 14, pentadecanoic acid were increased significantly after the 3rd day of depuration ($p<0.01$). Furthermore, the overall content of SFAs and UFAs increased significantly after 3 days of depuration ($p<0.01$ and $p=0.000$, respectively). Moreover, the initial content of total saturated fatty acids significantly increased when compared with total unsaturated fatty acids ($p=0.003$), but after the 3rd day of depuration total unsaturated fatty acids significantly higher

No	Fatty acids Saturated fatty acids (SFAs)	Before depuration	Day 3 post depuration
1	Caprylic acid	0.28 ± 0.01	0.23 ± 0.04
2	Lauric acid	3.53 ± 0.28	1.91 ± 0.08*
3	Tridecylic acid	16.02 ± 0.39	5.11 ±0.419***
4	Myristic acid	26.57 ± 0.41	9.117± 0.453***
5	Pentadecylic acid	11.002 ±0.16	5.182± 0.068***
6	Palmitic acid	10.547±0.65	51.234 ±0.045***
7	Margaric acid	N.D.	2.602 ±0.010***
8	Stearic acid	2.318±0.69	20.793± 0.163***
9	Heneicosylic acid	N.D.	8.378 ± 0.104***
	Total saturated fatty acids	70.281±2.60	104.573 ± 0.700**
Monounsaturated fatty acids (MUFAs)			
10	Myristoleic acid (Tetradecanoic acid)	12.36 ± 0.48	2.300 ± 0.178***
11	14, Pentadecanooic acid	9.336± 0.22	3.041 ± 0.257***
12	Palmitoleic acid (9 Hexadecenoic acid)	4.706 ± 0.16	11.266 ± 0.776**
13	Margaroleic acid (Heptadecenoic acid)	N.D.	2.005 ± 0.145**
14	Oleic acid (Elaidic acid m) Omega 9	4.630 ± 0.23	31.400 ± 0.502***
	Total	31.03	50.012***
Polyunsaturated fatty acids (PUFAs)			
15	Alpha Linolenic acid (ALA) Omega 3	N.D.	0.391±0.006***
16	Linoleic acid (LA) Omega 6	4.775 ± 0.187	31.301 ± 0.507***
17	Arachidonic acid (ARA) Omega 6	N.D	15.381 ± 0.789***
18	Eicosapentaenoic acid (EPA) Omega 3	N.D	6.610±0.226***
19	Eicosatrienoic acid (ETA)Omega 3	1.879 ± 0.112	20.683 ± 0.412***
20	Docosahexaenoic acid (DHA) Omega 3	N.D.	10.715 ± 0.401***
Total		6.654	85.081***
Total unsaturated fatty acids		37.68 ± 0.156	135.09 ± 1.045***
Total fatty acids		107.96 ± 2.764	239.66 ± 0.34***
Total SFAs/ Total FAs		65.09%	43.632%
Total UFAs/ Total FAs		34.90%	56.367%
N.D: Non-detected			

Table 3: Fatty acid analysis of the total soft tissues of *Spathopsis rubens arcuata* collected from Al-Mahmoudia canal, Zawyet Ghazal during the first day of collection and third day of depuration. Values are concentrations of fatty acids in mg/100g of sample. Values are significantly different at ($p<0.05$).

No	Amino acids	Before depuration	Day 3 post depuration
Essential amino acids (µg/g)			
1	Threonine	16.4 ± 0.11	18.90 ± 0.66*
2	Valine	10.98 ± 0.81	13.43 ± 0.47
3	Methionine	5.69 ± 0.26	6.15 ± 0.76
4	Isoleucine	7.41 ± 0.38	8.45 ± 0.47
5	Leucine	21.3 ± 0.15	26.62 ± 0.53**
6	Phenylalanine	0.41 ± 10.35	11.67 ± 0.02*
7	Histidine	0.63 ± 13.84	17.11 ± 0.77*
8	Lysine	16.27 ± 0.91	22.06 ± 0.73*
9	Arginine	20.36 ± 0.71	26.33 ± 0.53*
TEAA		122.62	**150.75
Non-essential amino acids (µg/g)			
10	Aspartic acid	36.96 ± 0.75	46.11 ± 0.28**
11	Serine	15.80 ± 0.25	20.19 ± 0.05**
12	Glutamine	45.25 ± 0.33	61.2 ± 0.10***
12	Proline	18.79 ± 0.26	19.49 ± 0.66
14	Glycine	20.67 ± 0.35	22.75 ± 0.24*
15	Alanine	21.88 ± 0.76	27.11 ± 0.03**
16	Tyrosine	7.32 ± 0.42	8.99 ± 0.38
17	Cysteine	12.58 ± 0.26	14.13 ± 0.03*
TNEAA		179.24	***219.95
TAA		301.86	**370.7
TEAA/TNEAA		0.68	0.68
TEAA/ TAA		0.41	0.41
LYS/ARG		0.79	0.83
TEAA: Total essential amino acids, TNEAA: Total nonessential amino acids, TAA: Total amino acids, LYS/ARG: Lysine Arginine ratio. Values are concentrations of amino acids in ppm (µg/g). Values are significantly different at ($p<0.05$).			

Table 4: Amino acid analysis of the total soft tissues of *Spathopsis rubens arcuata* collected from Al-Mahmoudia canal, Zawyet Ghazal during the first day of collection and third day of depuration.

Variables	*% of Spathopsis rubens arcuata* **Infection**		
Prevalence	*Conchophthirus*	*Aspidogaster conchicola*	*Batracobdelloides tricarinata*
Autumn	0%	4.44%	26.66%
Winter	79.41%	5.88%	23.52%
Spring	61.76%	20.58%	11.76%
Summer	0%	22.22%	0%

Table 5: The seasonal prevalence of parasites in *Spathopsis rubens arcuata*.

when compared with total saturated fatty acids (p=0.001) as shown in Table 3.

Determination of amino acid composition after depuration times

The amino acid contents of the investigated *S. rubens arcuata were determined* before and after 3 days of depuration. 17 different amino acids in soft tissues of *S. rubens arcuata* including 9 essential amino acids and 8 nonessential amino acids were detected. Glutamine is the most abundant amino acid in *S. rubens arcuata* (45.247 to 61.196 µg/g) which constituted 14.98 to 16.50% of the total amino acids, followed by Aspartic acid (36.959 to 46.111 µg/g), Alanine acid (21.875 to 27.105 µg/g), and Leucine (21.308 to 26.624 µg/g). Total amino acids (TAA) significantly increased after the third day of depuration ($p<0.01$). before and after 3 days of depuration, the content of essential amino acids (EAA) was lower than nonessential amino acids (NEAA), and the ratio of TEAA: TNEAA was (0.68). Lysine: Arginine ratio was low on the first day of collection and third day of depuration (0.79 and 0.83, respectively) as shown in Table 4.

Investigation of *S. rubens arcuata* for parasitic infection

A type of ciliates, *Conchophthirus sp.* (Figures 1A and 1B) was isolated from the hemolymph, mantle cavity, mantle, and gills of *S. rubens arcuata*. No trematode cercariae were shed from these *clams*. Gonads and intestine of *S. rubens arcuata* appeared free from any larval and adult stages of parasites. Adult *Aspidogaster* trematodes (Figure 2) were isolated from the mantle cavity, pericardial cavity, and gill filaments of *S. rubens arcuata*. The morphological study of this trematode using scanning electron microscope shows oral sucker and adhesive disc (Figures 3A-3E). During searching for parasites in *S. rubens arcuata*,

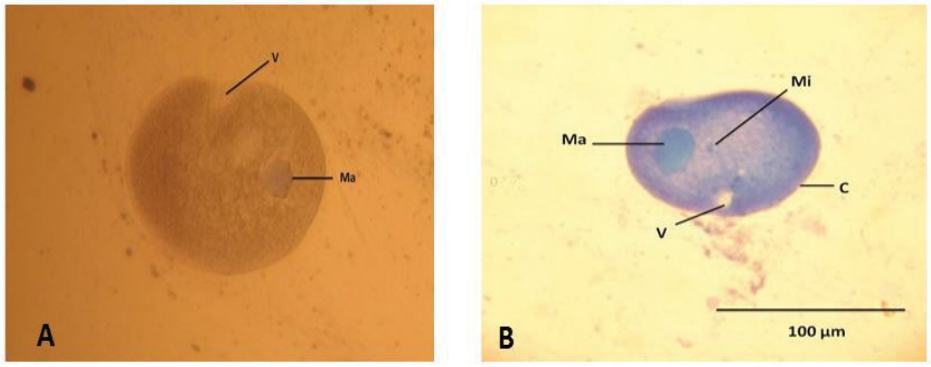

Figure 1: Light micrographs of *Conchophthirus sp.*, 1A) Unstained whole mount; 1B) Stained whole mount showing cilia.

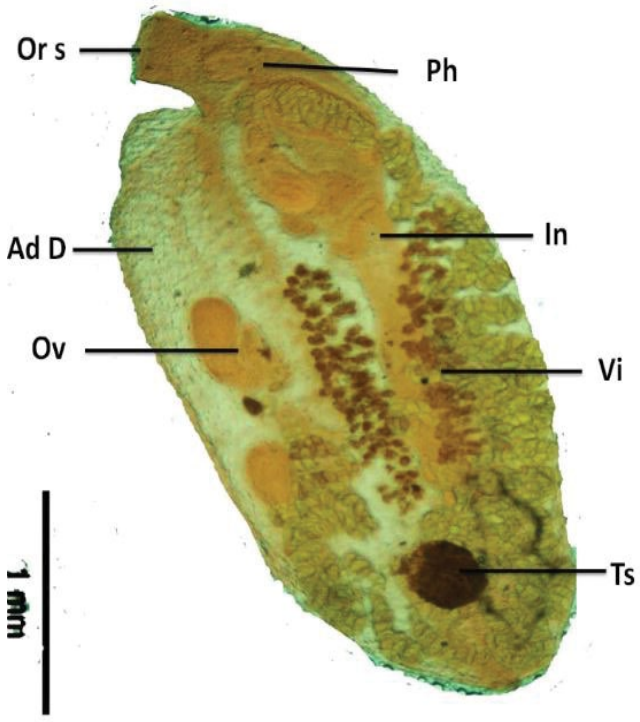

Figure 2: Light micrograph of *Aspidogaster* sp. Adhesive disc (Ad D), Intestine (In), Oral sucker (Or s), Ovary (Ov), Pharynx (Ph), Testis (Ts) and Vitellaria (Vi).

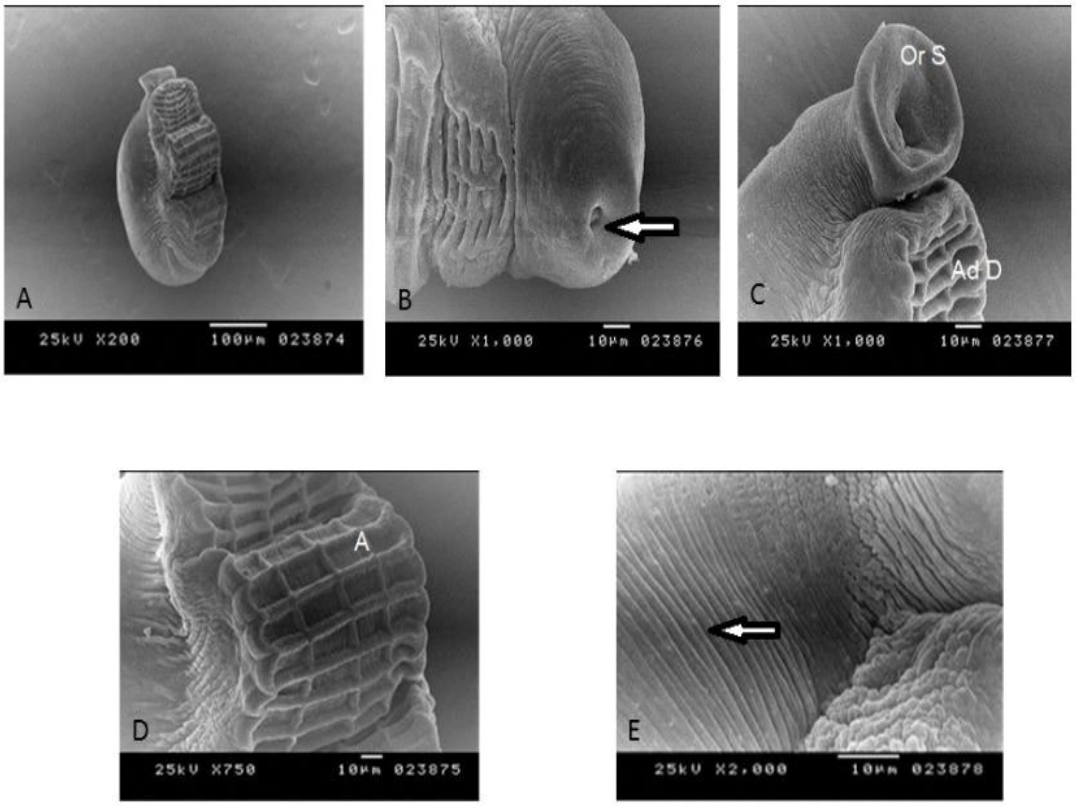

Figure 3: Scanning electron micrographs showing, 2A) Whole mount of *Aspidogaster conchicola*; 2B) Mouth opening (arrow); 2C) Oral sucker (Os); 2D) Ventral adhesive disc (Ad D) consisting of four longitudinal rows of alveoli (suckerlets) (A); 2E) Dorsal body surface elevated by transverse folds (arrow).

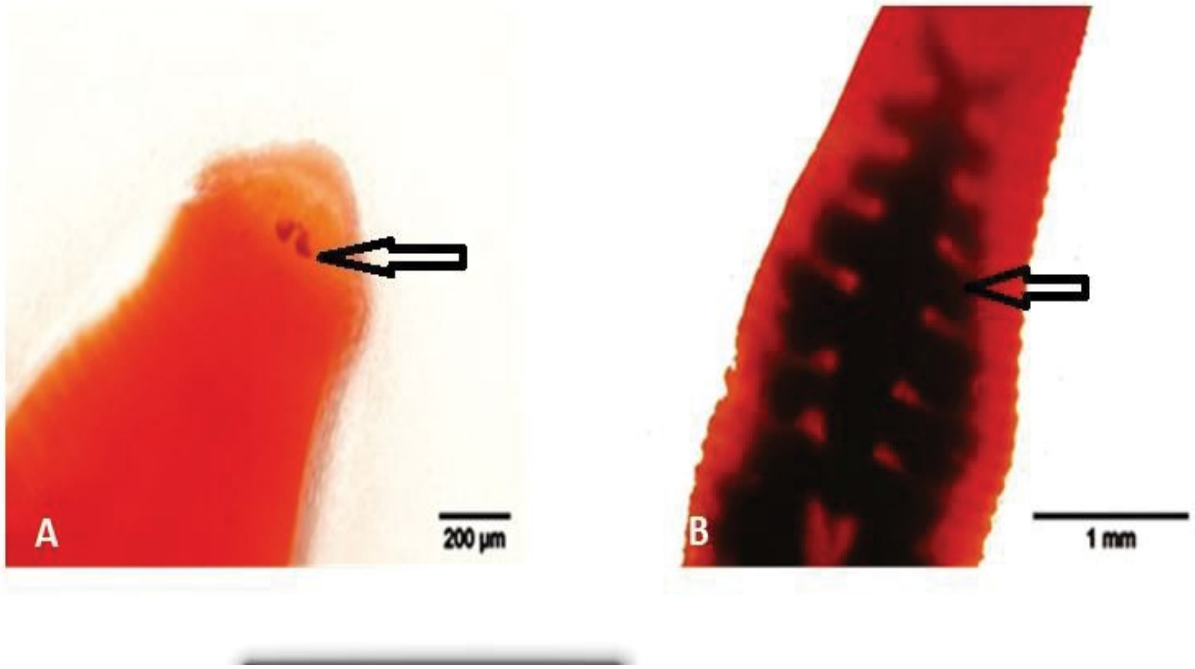

Figure 4: Light micrographs showing, 4A) The head of *Batracobdelloides tricarinata* having two pairs of eyes (arrow); 4B) Seven pairs of the lobed crop caecae (arrow).

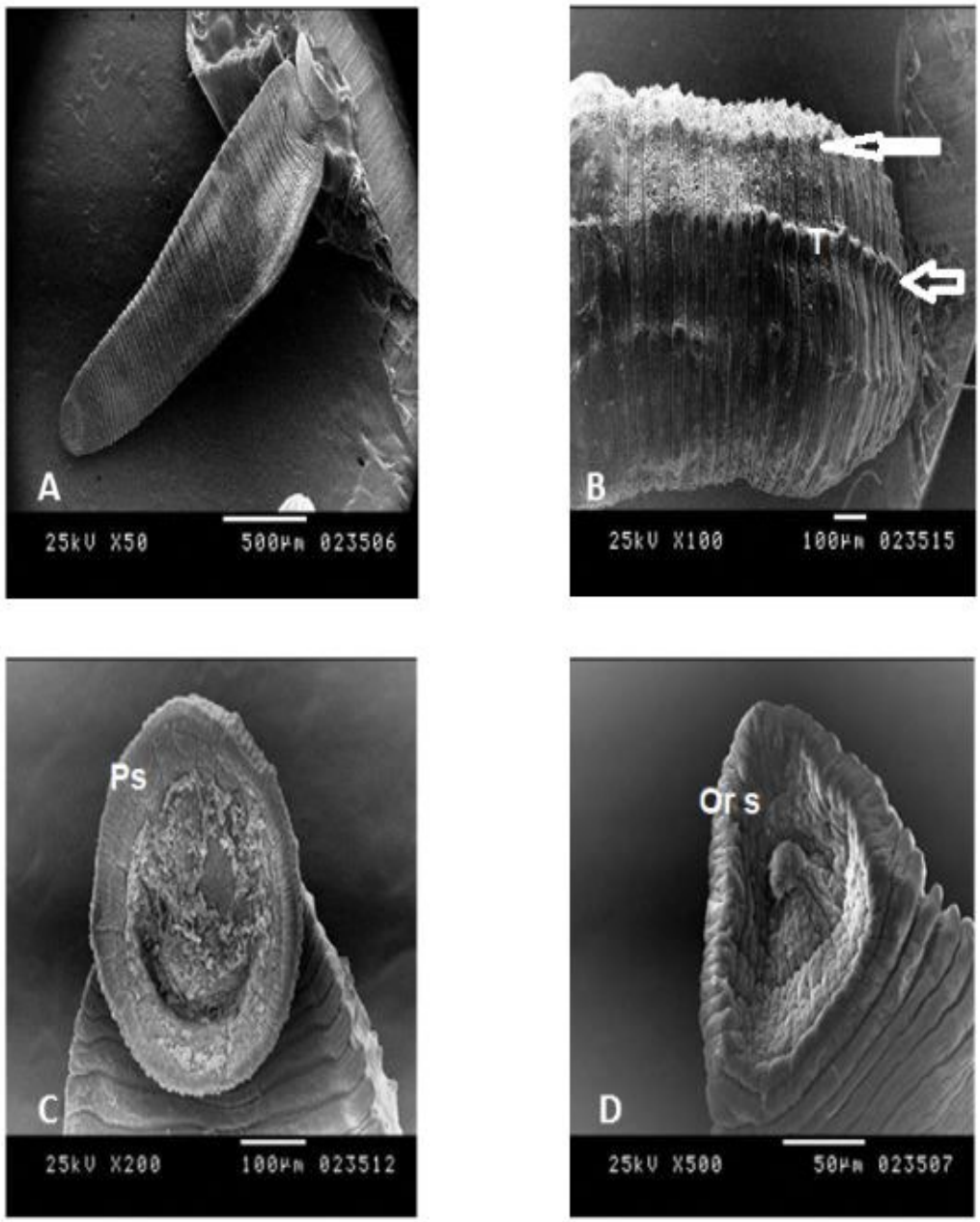

Figure 5: Scanning electron micrographs showing, 5A) Whole mount of *Batracobdelloides tricarinata*; 5B) Longitudinal rows of tubercles (T) which form ridges (arrows); 5C) Posterior sucker (Ps); 5D) Anterior sucker (Or s).

a few specimens of the African fish leech *Batracobdelloides tricarinata* that ranged from one to two specimens lay within the mantle cavity of *S. rubens arcuata.* This type of leech has two eye spots (Figure 4). The external structure studied by scanning electron microscope and shows that the tegument covered with longitudinal rows of tubercles (Figures 5A-5D).

The results showed that the prevalence of *Conchophthirus* in *S. rubens arcuata* during the winter season (79.41%) was higher than spring season (61.76%), while during autumn and summer seasons *Conchophthirus* was not detected in *S. rubens arcuata* (0%). The prevalence of ***A. conchicola*** infection in *S. rubens arcuata* was calculated, and it was higher in summer season (22.22%) than in autumn, winter and spring seasons (4.44%, 5.88%, and 20.58%, respectively). The results in Table 5 showed that the prevalence of *B. tricarinata* in the mantle cavity of *S. Rubens Arcuata* during autumn season (26.66%) was higher than winter and spring seasons (23.52% and 11.76%, respectively), while during summer *B. tricarinata* was not detected in the mantle cavity of *S. rubens arcuata* (0%).

Discussion

The real problem with eating freshwater clams is the fact that they are filter feeders, meaning that they constantly ingesting the water

around them, and accumulating a variety of substances, including pollutants and toxins, in their own tissues. The current study was conducted to investigate the nutritional value, and assessment of the heavy metal levels in the *S. rubens arcuata* before and after depuration periods. In addition, to detect the presence of parasites in this clam. The present study showed that all metals measured in the collected tissues exceeded the permissible levels and this come in accordance with Fol and Abdel-Gaber [24]. But the heavy metal contents after a period of depuration in the soft tissues of these clams. it was appeared that heavy metal concentrations (Cu, Cd, and Pb) in all tissues (foot, gills, mantle, and adductor muscles) of *S. rubens arcuata* showed a significant decrease on the 3rd and 8th days of depuration compared to their initial concentrations, but these reductions were more potent on the eighth day of depuration. This result was consistent with Cheung and Wong [25] who reported that the depuration for 7 days decreased Cd and Pb in three clam species *Circe Sinensis, Gafrarium fumidum*, and *Tapes philippinarum*. A similar result was reported by Geffard et al. [26] who found that Cd was eliminated more quickly than Cu and Zn when the kinetics of heavy metal elimination from *Crassostrea gigas* was recorded. Also, our findings were agreed with Yap et al. [27].

The obtained data showed that, the moisture percentages significantly increased after 8 days of depuration. This may be due to the increase of filtration rate. Liu et al. [28] found that the chronic toxic effects of heavy metals including (Cu, Pb, Cd) on the filtration rate of immature blood clams, *Tegillarca granosa* were significantly inhibited. However. the protein, carbohydrates, ash, and lipids contents significantly reduced after 8 days of depuration. This may be agree with Anacleto et al. [29], who showed that the high mortality in *Scrobicularia plana* and reduced glycogen content may be due to the stressful conditions (e.g. lack of feed and sediment) during the depuration process, leading to a greater utilization of biochemical reserves.

Due to the depuration, the protein and carbohydrate contents increased on the 3rd day and this could be due to the decreasing of electrolytes concentrations in *S. rubens arcuata* soft tissues after depuration period which in turn affect protein and carbohydrate synthesis. Previous data have reported that metal ions inactivate protein molecules through nonspecific binding or cross-linking of essential side chains and by promoting irreversible denaturation [30]. Moreover, depuration might lead to stress, which in turn affect proteins, carbohydrate, and lipids metabolism [31,32] Consistent with our findings, cadmium (Cd) was found to interfere with many protein and carbohydrate metabolisms by inhibiting the enzymes involved in these processes [33]. The significant reduction of protein, carbohydrate, and lipid levels after 8th days of depuration may be due to the absence of food during the depuration process and the consumption of these substances during the depuration periods.

In the present study, increasing of fatty acids number in the soft tissues of *S. rubens arcuata* after 3 days of depuration, especially the unsaturated fatty acids, which indicate that eating *S. rubens arcuata* after 3 days of depuration, could reduce the amount of cholesterol in the blood [34]. Moreover, previous studies showed that PUFA plays a vital role in alleviating cardiovascular disease, type-2 diabetes, inflammatory ailments, and autoimmune disorders [35-37], and Connor [38] reported that omega 3 fatty acids prevent cardiovascular disease. Also, Nettleton [39] found that omega 3-fatty acids are a good nutritional aspect of fats since they have a much lower incidence of heart disease and lower total plasma cholesterol. The present study reported that after depuration for 3 days, a significant increase of the EPA, DHA, ARA, Palmitic and Oleic acids, these findings were consistent with previous studies reported by Fokina and Nemova [40].

The concentrations of total essential and nonessential amino acids were significantly increased in the total soft tissues of *S. rubens arcuata* after depuration periods, and this can be backed to the decreasing of heavy metals (Cu, Cd, and Pb) concentrations in *S. rubens arcuata* tissue after depuration period. Whereas Cd toxicity can impact on biochemical constituents such as glycogen, total proteins, lipid, and free amino acids [41]. Also, Cu ions can inhibit the physiological activity, thereby decreasing the uptake of amino acids and consequently their distribution to other tissues [42].

Parasitic investigation of *S. rubens arcuata* during seasonal periods showed the presence of a type of ciliates *Conchophthiridae* sp. This parasite was found in the epithelial surfaces of the mantle, outer gill surfaces, in the gill water tubes, and supra-branchial cavities of the clam and this finding was consistent with the previous study by Grizzle and Brunner [43].

Our results indicated that the prevalence of *Conchophthirus* in the mantle cavity of *S. rubens arcuata* during the winter season was high, while in summer season was absent. The absence of *Conchophthirus* in the mantle cavity of *S. rubens arcuata* during summer and autumn seasons could hypothetically be explained by the difference in the chemical content of the mantle cavity mucus. Chuseve et al. [44] reported that the abundance of protozoans *Conchophthirus acuminatus* in zebra mussels *Dreissena* was positively associated with water temperature, whereas they were highest during summer and lower in winter.

Aspidogaster conchicola was found in the mantle cavity, pericardial cavity, and gill filaments of *S. rubens arcuata.* This consistent with Duobinis-Gray et al. [45] who found that *A. conchicola* specimens are typically found in the pericardial and renal cavities of bivalve molluscs. A high prevalence of *A. conchicola* in *S. rubens arcuata* was low and ranged from 4.44-22.22%. Similar results have been reported previously by Yuryshynets and Krasutska [46]. High prevalence of *A. conchicola* was observed in the summer season. The reproduction rate of parasites may be greater at a higher temperature and lower water quality leading to increased parasites [47,48]. This finding was explained by Khurshid and Ahmad [49] who found that the period, which starts in the spring and continued to early summer. Also, Singh and Mishra [50] found that the higher abundance of helminth parasites during summer season was probably due to a higher temperature and lower immunity during this season which can facilitate increasing the susceptibility of disease and the transmission of parasites in their hosts.

Conclusion

In this study, a few specimens of the African fish leech *Batracobdelloides tricarinata* ranged from one to two specimens were found in the mantle cavity of *S. r. arcuata* as indicated by Appleton [51] who reported *B. tricarinata* in mollusc species such as *Bulinus africanus*, *Lanistes ovum*, *Caelatura kunenensis*, *Mutela mabilli*, and *Aspatharia wahlbergi.* According to Oosthuizen [52], Who observed no indication of parasitism by *B. tricarinata* in a variety of aquatic mollusc species. The present study indicates that the relationship between *B. tricarinata* and *S. r. arcuata* may be commensal, the leech utilizing the clam's shell for protection, and the leech partially protects the clam from infection by larval trematodes whereas in this study, none of the clams harboring *B. tricarinata* was infected by larval and adult trematodes. This observation agrees with Brooks and Welsch [53]. who found that

the same commensal relationship between *Marvinmeyeria Lucida* (Annelida: Hirudinea) and *Helisoma trivolvis* (Mollusca: Gastropoda) in Nebraska. This can explain the high prevalence of *A. conchicola* in summer season (22.22%) when *B. tricarinata* was completely absent and low prevalence of *A. conchicola* (4.44%) in the autumn season when there was a high prevalence of *B. tricarinata* (26.66%). According to the given results it is suggested that *S. rubens arcuata* can be a good source of food with high protein and low-fat contents and free of parasitic diseases. But with a recommendation that marketing of this clam must be after a period of depuration ranged from 3 to 8 days.

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