COMPARISON OF PROXIMATE AND MINERAL ANALYSIS OF *PILA SCUTATA (MOUSSON, 1848)* WITH *SARDINELLA LONGICEPS* AND *RASTRELLIGER KANAGURTA*

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Abstract

In Kerala till today, nobody has become interested in snail farming even though snail capturing and its utilization as human food, poultry feed and in large scale as supplementary feed for prawn farms are lavishly going on. This over exploitation of *Pila* for prawn farming as well as reclamation of ponds and paddy field for urbanization has reached an alarming condition. This ecologically and economically important biological resource will become extinct within a very short time, if no steps are taken to establish the commercial farming of these species immediately. Considering the above facts, the present study was marked out to develop strategies on management of the adults in captive condition, natural breeding, maintenance of juveniles in the hatching pond and ranching for ensuring the sustainable natural population and stocking of this by the commercial farming of *Pila*. The survey score recommended desirable necessary actions to be taken in conservation of this species and should create an awareness on the medicinal properties of *Pila*. The proximate and mineral composition analysis show the importance of *P.scutata,* since it has higher nutritional value. The ecologically and economically important biological resource if not protected will become extinct within a very short time, if no steps are taken to establish the commercial farming of this species immediately.

Key words: Breeding, *Pila scutata*, proximate composition, snail farming, urbanization

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INTRODUCTION

In the freshwater environment, molluscs are an important group and their abundance plays pivotal role in freshwater ecosystem functioning (Vaughn *et al*., 2004). Fresh water molluscs are common in ponds, lakes, paddy fields, quiet water pools, and flowing waters like lower section of perennial rivers, irrigation canals etc. Freshwater gastropods are generally found attached to submerged vegetations, rocks, sticks, bricks etc., but bivalves live partly buried in the sand or mud. Similar to the gastropods, bivalves exhibit variations in shell features depending upon ecology of the species and may produce eco-phenotypes (Subba Rao, 1989).

Molluscs form an important component of biological monitoring in terms of rating the water quality and status of aquatic systems (Strong *et al*., 2008). Based on their occurrence, freshwater molluscs are distinguished into two - primary and secondary freshwater species. The primary freshwater molluscs are confined exclusively to freshwater habitats, whereas secondary freshwater species are distributed to both in estuarine and freshwater habitats. There are estimated 5,000 freshwater molluscs in the world for which valid descriptions exist besides the 10,000 undescribed species (Balian *et al.*, 2008).

The freshwater aquaculture plays an important role in the development of additional food resource for the increasing human population of our country. Inland aquaculture is limited to raising the fish and to some extent shellfish production. During faunistic surveys, it has been observed that there is a regular sale of shellfish in several markets of eastern and north-eastern states. Shellfishes are harvested from the natural resources by different methods today and finally brought for sale to the market. There are no regular markets in some places, but still people there use shellfish as food very frequently.

Arunachal Pradesh Shellfishes are consumed throughout, although no regular marketing was observed during the visit. Three species (one gastropod and two bivalves) were found to be consumed as food. Meghalaya Almost all freshwater molluscs that occur abundantly are used as food. Shell fishes are more popular in the Garo hills than Khasi hills. Seven species, 6 gastropods and one bivalve are, commonly used there as food. These species, are known under different names in Garo hills but a common term "Mattah" used for all molluscs in Khasi hills. Molluscs are regularly collected from the different water bodies for own consumption and also sold regularly in the market.

These molluscs are sold in packets made out of fresh banana leaves or in lot. Manipur Shellfishes regularly sold in different markets of Manipur form staple food of the local people. Mizoram Freshwater molluscs form a staple food for the people of Mizoram (Subba Rao and Dey,1986). Almost all freshwater molluscs occurring abundantly are used there as food.

Among the nine genera recognised (Hayes *et al*., 2015), species of the two genera *Pomacea* and *Pila* in particular are frequently known as ‘apple snails’ because many of them bear large, round, sometimes greenish shells. The main reason for introduction of these species is as a human food resource, a domestic aquarium snail, for biocontrol of other snails acting as vector of the parasites causing schistosomiasis, and for control of aquatic weeds. It is commonly found in freshwater ponds, pools, tanks, lakes, marshes, rice fields and sometimes even in streams and rivers even in brackish waters of low salinity. They occur abundantly in those areas where a large amount of aquatic vegetation like *Vallisneria, Pistia* is found. They are amphibious, being adapted for life in water and on land. The animal creeps very slowly by its ventral muscular foot, covering about 5 cm per minute.

Fish and shellfishes have been found as a major source of protein to both riverine and the general population at large, as they occur abundantly in the brackish and fresh waters. They have also been found to be a good source of protein, low in fat and calories, making a very healthy choice of food (Ahmed et al.,2022). The different species of these shellfishes are periwinkles (*Tympanotomus* spp), oysters, prawns, snails and clams. The fresh water snails commonly harvested by the artisanal fishers are *Lanistes* spp, *Nucella* spp, *Bulinus* and *Pila*.

Although, it has been found that shellfishes have a high content of cholesterol, it is low in saturated fat, which is not dangerous to human health. The land snail *Anchanchatina marginata* is high-quality food rich in protein, low in fat and a good source of iron. It has a protein content of 88.42%, which compares with animal protein of 82.37% for pork and 92.75% for beef (Imevbore and Ademosun, 1988).

Shellfishes are a good source of protein, low fat and macro - minerals and trace elements such as

copper, iron, zinc and manganese. Eating of shellfishes is part of a healthier lifestyle. Difference in nutritional values could be attributed to species, regions and environments, therefore snails like many other shellfishes have been observed to contain high concentration of iron, calcium, magnesium and zinc (Baby et. al., 2010).

Today, it can be found in high numbers in most reservoirs and their tributaries, and in many ponds and canals throughout Kerala. Habitat loss, environmental disasters, freezing conditions, high temperature, and drought are among the major threats faced by these terrestrial gastropods (Nicolai & Ansart, 2017). Many people might think of a typical land snail habitat as humid, cool, and shaded. Nevertheless, a number of pulmonate snail species live in arid, semiarid, and Mediterranean regions, where the climate is, at least temporarily, dominated by high ambient temperatures and low humidity (Mizrahi, Heller, Goldenberg, & Arad, 2010). Rising temperature and concomitant drought, however, rapidly bring ectotherms close to critical limits, particularly species that are not able to rapidly alter their distribution, such as land snails (Dillon, Wang, & Huey, 2010).

In Kerala, edible fresh water molluscs such as *Pila scutata*, *Pila globossa* and *Pomacea canaliculata* populations and numbers have been diminishing progressively, due to the urbanization and anthropogenic interventions.

The ecologically, economically, and nutritionally important biological resource will become extinct within a very short time if no steps are taken to establish the commercial farming of this species immediately. Considering the above facts, the present study was imputed out to develop strategies on management of the adults in captive condition, natural breeding, maintenance of juveniles in the hatching pond ensuring the sustainable natural population and stocking of this by commercial farming of *Pila*.

The main objectives of the study are to review the present status on the distribution of *Pila, t*o analyse the nutritional quality of *Pila* and compare its nutritional quality with local available two fish species (*Sardinella longiceps* and *Rastrelliger kanagurta*).

MATERIALS AND METHODS

1. The candidate species

Table 1 Systematic Position

Phylum	Molluscs
Class	Gastropoda
Subclass	Prosobranchia
Order	Megagastropoda
Genus	*Pila*
Species	*scutata*

Fig 1 The candidate species – *Pila scutata* (Dorsal and ventral view)

2. Collection of specimens and Experimental Design

Fig 2 Collected from natural pond

The specimens (Table 1 and fig 1) were collected from natural pond around Uliyakovil in Kollam during July 2019 (Fig 2). The experimental, studies on growth and production of a freshwater snail using different substrates in artificial ponds, was carried out for a period of 6 months from July to November in 2019.

Proximate analysis of Pila

The collected samples were cleaned and measured for wet weight (g), total length with shell (cm) and without shell(cm). Edible parts from the shells of each species were removed carefully using a needle and weighed to the nearest 0.1 g in a electronic weighing machine (Shimadzu) and meat content was calculated (Table 2). Flesh samples were analysed for proximate and mineral contents. Proximate and mineral contents were determined by the methods of the Association of Official Analytical Chemists (Lynch et al, 1995) given in Table 3 and Plate IV.

Statistical Analysis

The statistical analysis was performed using statistical software - SPSS (Statistical Package for Social Sciences) version-24. Analysis of variance (ANOVA) was performed to test any significant variation among the water quality parameters and morphometry in terms of substratum. Level of Significance was assigned at the probability of *0.01 to 0.05.*

Assessing water quality

Assessing water quality parameters such as temperature, pH and dissolve oxygen were measured. The surface water temperature was noted on the tank by dipping a calibrated mercury Celsius thermometer (0 - 50°C). The chemical parameters such as pH and Dissolved oxygen were also measured. Hydrogen ion concentration (pH) of the water was recorded at the pond by using a digital portable pH pen (PHILIPS, model pp 9046). Dissolved oxygen (DO) was determined manually by Winkler (1888) and modified by Strickland and Parsons (1968) based on the principles of titration methods.

Present status of Pila

Survey method is used to gathering data by direct data collection via questionnaire to people living near paddy fields and fresh water ponds to gather the required information. (Questionnaire containing 10 questions related to the environmental degradation).

RESULTS

Proximate analysis of Pila

Table 2 Length and weight of *Pila* sample collected for proximate analysis

SL.No	Length (cm)	Weight with shell (g)	Weight without shell (g)
1	4.2	15.9	5.7
2	2.8	9	2.8
3	3.8	12.5	4.4
4	3.5	8.2	4.6
5	3.6	11.4	3.8
6	4.1	15.5	4.4
7	3.6	10.8	2.9
8	3.4	13.9	4.4
9	3	8.9	2.2
10	3.3	9.3	2.6
11	4.7	32.6	15.5
12	4	26.5	12
13	3.8	23	11.4
14	3.7	17.4	10.2
15	3.7	22	11.7
16	3.5	18.5	11
17	3.6	22.1	12.7
18	4.4	29.3	13
19	3.5	17.7	10.1
20	4.4	27	11.7
21	3.5	14.6	8.5
22	3.2	12.9	7.3
23	3.5	13.7	7.8
24	3.3	13.3	6.9
25	3.6	15.2	5.7
26	3.7	17.5	6.3
27	3.5	13.5	4.6
28	3.2	13.6	4.7
29	3.3	14.8	5.1
30	3.6	12.5	5.1
31	3.8	15.4	6.9
32	3.5	12.2	4.9
33	3.7	14.3	6.4
34	3	10.4	4.7

Table 3 Proximate composition of *P. scutata*

Sl. No	PARAMETERS	PROTOCOL	MEAN±SD	UNIT
1	Moisture	AOAC 21ST EDITION 950.46	57.92 ± 0.06	mg/100g
2	Energy	AA/CH/SOP/48	436.35 ±0.17	Kcal/100g
3	Carbohydrate	IS 1656: 2007	355.37 ±2.75	mg/100g
4	Sugar	IS 6287: 1985	0.0	mg/100g
5	Fat	AOAC 21ST EDITION 960.39,2019	235.81 ±2.28	mg/100g
6	Protein(NX6.25)	AOAC 21ST EDITION 940.25,2019	209.29±1.11	Mg/100g

ND - Not detected/DL - Detection Limit

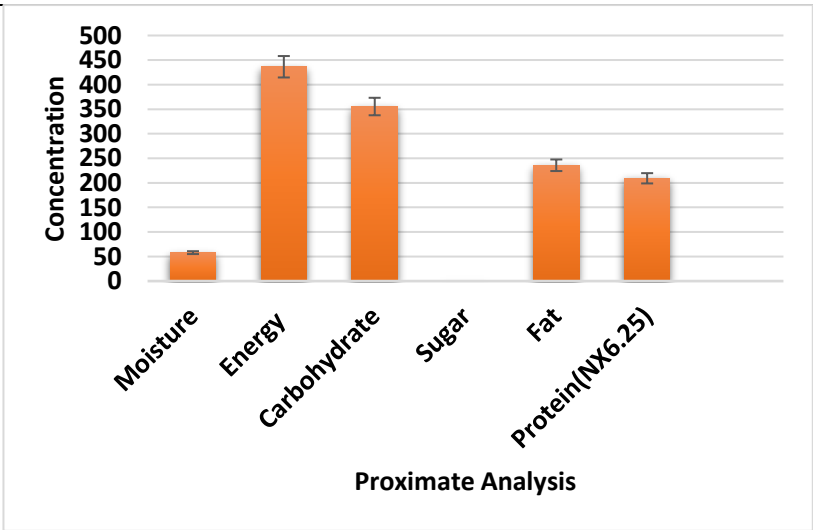


Fig 3 Proximate composition of *P. scutata*

Table 4 Mineral analysis of *P. scutata*

Sl. No	PARAMETERS	PROTOCOL	MEAN±SD	UNIT
1.	Sodium	IS 9497: 1980	70.41±1.27	mg/100g
2.	Potassium	IS 9497: 1980	13.43 ±0.12	mg/100g
3.	Iron	AOAC 21ST EDITION 991.11,2019	3.23 ±0.04	mg/100g
4.	Calcium	IS 5949-1990	39.25±0.05	mg/100g

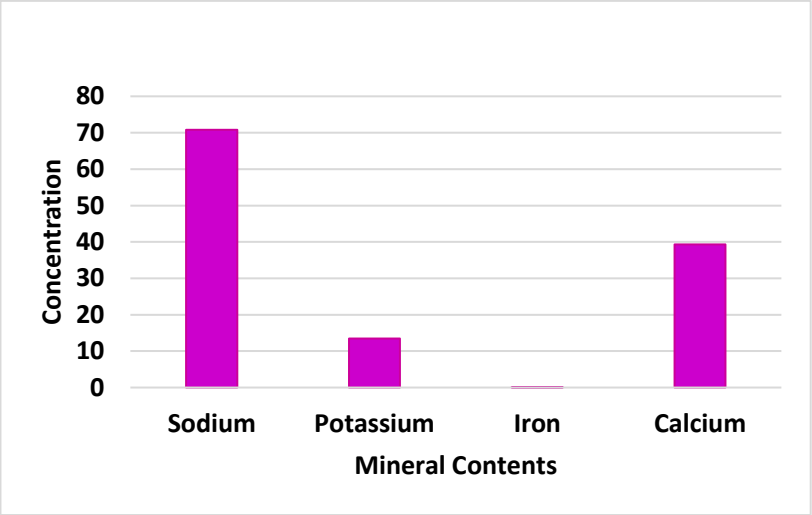


Fig 5 Mineral analysis of *P. scutata*

Table 5 Comparison of proximate and mineral analysis of *P.scutata* with *S.longiceps* and *R.kanagurta*

Sl. no	Parameters	*P. scutata*	*S.longiceps*	*R.kanagurta*
1	Moisture (mg/100g)	57.92 ± 0.06	73.79±0.30	76.08±0.58
2	Energy (Kcal/100g)	436.35 ±0.17	186±0.30	205±0.02
3	Carbohydrate (mg/100g)	355.37 ±2.75	3.58±0.004	1.12±0.003
4	Sugar (mg/100g)	ND	ND	ND
5	Fat (mg/100g)	235.81 ±2.287	60.0±0.00	24.0±0.33
6	Protein (NX6.25) (mg/100g)	209.29 ±1.11	159.4±0.10	208±0.02
7	Sodium (mg/100g)	70.41±1.27	289.53±0.12	90±0.01
8	Potassium (mg/100g)	13.43 ±0.12	166.5±0.05	314±0.03
9	Iron (mg/100g)	3.23 ±0.04	3.07±0.02	2.33±0.08
10	Calcium (mg/100g)	39.25±0.05	35.9±0.09	12±0.01

ND - Not detected/DL – Detection

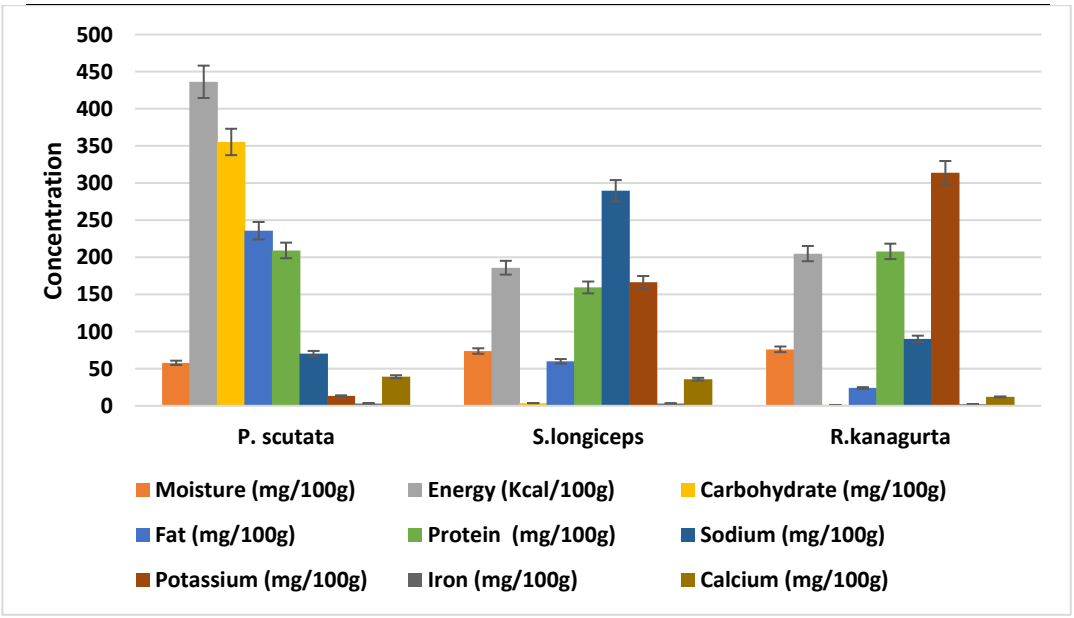


Fig 6 Comparison of proximate and mineral analysis of *P.scutata* with *S.longiceps* and *R.kanagurta*

The results of ANOVA indicated that, in water quality parameters, only DO showed significant variation ($p>0.015$) between treatment tanks; whereas in morphometry, number of egg clusters

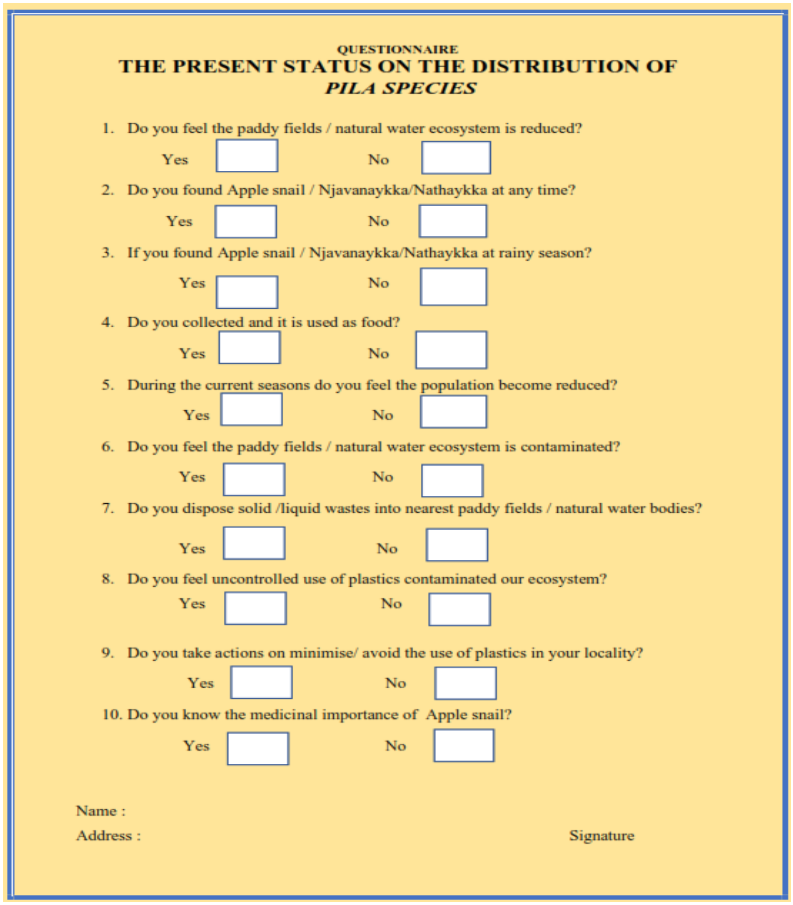
(p<0.29) and length (p<0.20) showed no significant variation. Therefore Level of Significance was assigned at the probability of 0.01 to 0.05 (Table 6).

Table 6 ANOVA of Morphometry of snail and water quality parameters in different treatment tanks during the experimental period

		Sum of Squares	df	Mean Square	F	Sig.
Temperature (0C)	Between Groups	18.491	7	2.642	21.132	.166
	Within Groups	.125	1	.125		
	Total	18.616	8			
pH	Between Groups	.082	7	.012		
	Within Groups	0.000	1	0.000		
	Total	.082	8			
DO (mg/l)	Between Groups	14.943	7	2.135	2668.349	.015
	Within Groups	.001	1	.001		
	Total	14.944	8			
No.of Cluster of Eggs	Between Groups	203.500	7	29.071	6.460	.294
	Within Groups	4.500	1	4.500		
	Total	208.000	8			
Length (mm)	Between Groups	4.177	7	.597	13.261	.208
	Within Groups	.045	1	.045		
	Total	4.222	8			
Weight (mg)	Between Groups	15.429	7	2.204		
	Within Groups	0.000	1	0.000		
	Total	15.429	8			

Present status of Pila

The survey questions were assessed and the percentage of responses calculated and represented in figure 7. The highest response was recorded in question numbers 3 and 5 (100%), whereas the least response percentage was recorded in question number 4 (20%), followed by question number 9 (50%). Other questions (1, 2, 6,7,8 and 10) showed similar percentage responses ranging from 80-95%.



QUESTIONNAIRE

THE PRESENT STATUS ON THE DISTRIBUTION OF *PILA SPECIES*

1. Do you feel the paddy fields / natural water ecosystem is reduced?
   Yes ☐ No ☐
2. Do you found Apple snail / Njavanaykka/Nathaykka at any time?
   Yes ☐ No ☐
3. If you found Apple snail / Njavanaykka/Nathaykka at rainy season?
   Yes ☐ No ☐
4. Do you collected and it is used as food?
   Yes ☐ No ☐
5. During the current seasons do you feel the population become reduced?
   Yes ☐ No ☐
6. Do you feel the paddy fields / natural water ecosystem is contaminated?
   Yes ☐ No ☐
7. Do you dispose solid /liquid wastes into nearest paddy fields / natural water bodies?
   Yes ☐ No ☐
8. Do you feel uncontrolled use of plastics contaminated our ecosystem?
   Yes ☐ No ☐
9. Do you take actions on minimise/ avoid the use of plastics in your locality?
   Yes ☐ No ☐
10. Do you know the medicinal importance of Apple snail?
    Yes ☐ No ☐

Name :
Address : Signature

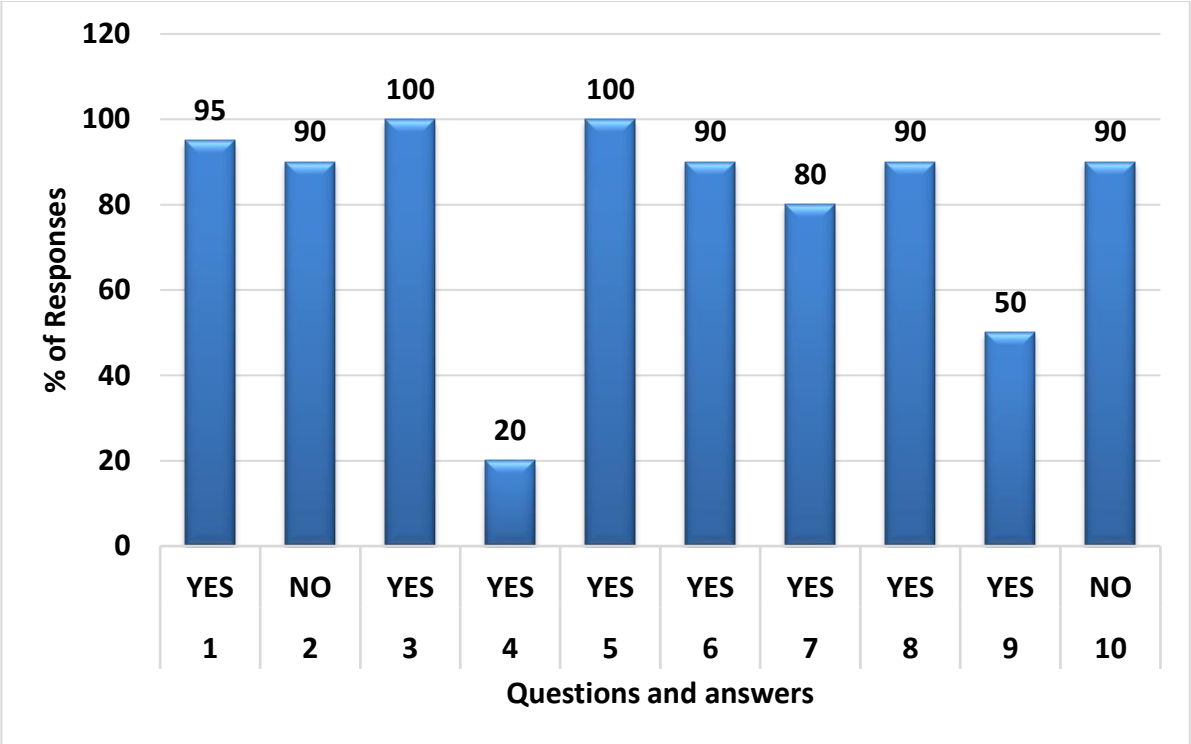
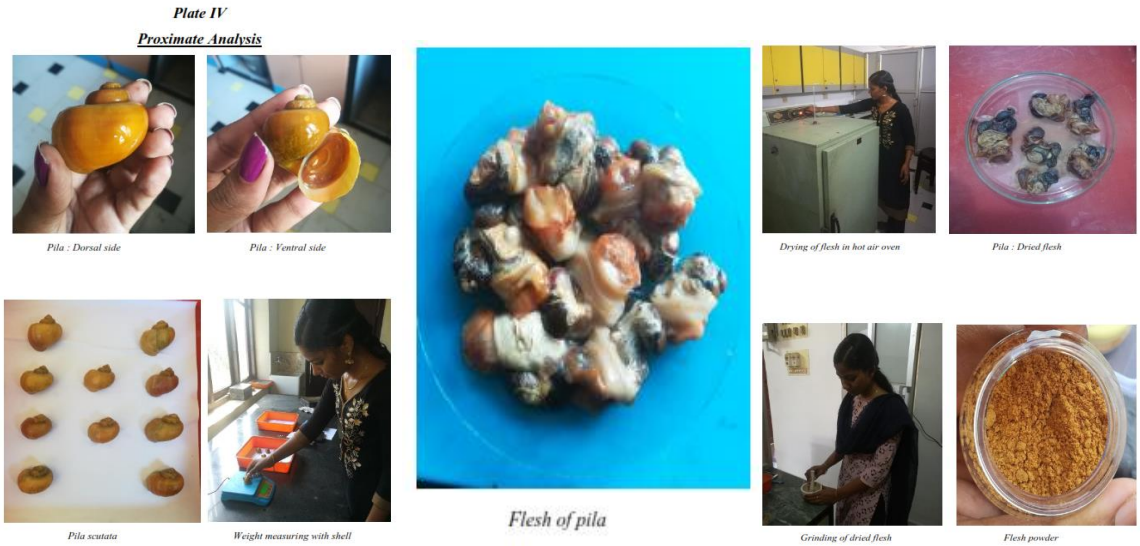


Fig 7 Percentage of responses in survey

The morphometric characteristics used in the study are given in Table 2. The weight of snails varied between 2.64 to 15.55 g, with a total length varying between 2.80 – 4.71 cm. The proximate and mineral analysis in *P.scutata* were determined and given in tables 3 & 4 and figs 5 & 6. The percentage of moisture content was recorded as (57.92 ± 0.06 mg/100g), protein (209.29 ±1.11 mg/100g), carbohydrate (355.37± 2.75 mg/100g) and fat (235.81 ±2.28 mg/100g) respectively. The mineral content analysis, the mean sodium content was obtained as (70.41 ±1.27 mg/100g), the potassium content was (13.43 ±0.12 mg/100g), the Iron content was (3.23 ±0.04 mg/100g) and the calcium content was (39.25± 0.05 mg/100g).

The proximate composition values obtained from the analysis of *P. scutata* is compared with two commonly available food fish; *Sardinella longiceps* and *Rastrelliger kanagurta* (Table 5 & fig 6) for comparing the nutritional value of *P. scutata* as a food source. The mean moisture content in the fish *S.longiceps* was (73.79± 0.30mg/100g), in *R.kanagurta* was (76.08± 0.58mg/100g). The energy content in both fishes were (186Kcal/100mg and 205 Kcal/100mg) respectively. The mean carbohydrate content in *S.longiceps* was (3.58±0.004mg/ 100g),in *R.kanagurta* was (1.12±0.003mg/100g). The mean fat content present in *S.longiceps* was (60.0±0.00mg/100g) and it is least in *R.kanagurta* with the value of (24.0±0.33mg/100g). The mean protein content in *S.longiceps* was (159.4±0.10mg/100g) and in *R.kanagurta* was (208±0.0mg/100g).

In mineral content, the mean sodium content present in *S.longiceps* was (289.53±0.12 mg/100g) and in *R.kanagurta* was (90±0.01 mg/100g), the mean potassium content was *S.longiceps* was (166.5±0.05 mg/100g) and in *R.kanagurta* was (314±0.03 mg/100g), the Iron content was *S.longiceps* (3.07±0.02 mg/100g) and in *R.kanagurta* was (2.33±0.08 mg/100g). The mean calcium content was in *S.longiceps* was (35.9±0.09 mg/100g) and in *R.kanagurta* was (12±0.01 mg/100g).



DISCUSSION

Pila is commonly known as pond snail or apple snail, a typical representative of the class Gastropods. It is one of the largest freshwater molluscs, commonly found in fresh water ponds, pools, tanks, lakes, marshes, rice fields and sometimes even in streams and rivers and brackish waters of low salinity. The body consists of head, foot, and a visceral mass. The digestive system comprises a tubular alimentary canal, a pair of salivary glands and a large digestive gland.

Pila is unique as it has double mode of respiration, aquatic by Ctenidium and aerial by Pulmonary sac, with an open circulatory system which consists of heart, arteries, veins and the sinuses. Blood of *Pila* is colourless with its excretory system, comprises of one or two kidneys and pericardial gland. Each kidney has two openings, one internally communicating to the pericardium and the other to the exterior called nephridiopore. The nervous system consists of a series of paired ganglia with commissures and connectives and their sense organs include two pairs of tentacles, osphradium, a pair of eyes, and a pair of statocyst. Sexes are separate, but sexual dimorphism is almost absent.

The animal burrows into the mud in winter and summer, an adaptation to these climatic changes. An animal out of water aestivates after a period of activity. The duration of activity depends upon relative humidity of the environment. The onset and cessation of aestivation at any temperature within favourable range depend solely on the availability of water. When an animal is forced to aestivate with a portion of the shell or operculum removed so as to expose the soft parts, the animal loses water and does not survive more than 10 days. At death, water content is between 55 and 60 per cent of the total soft body weight. The shell is resistant to the passage of water and electrolytes. Water is only at the opercular region, where junction with the shell is sealed with mucus, initiates the return to an active state.

The results of ANOVA indicated that in water quality parameters, only DO showed significant variation ($p>0.015$) between treatment tanks; whereas in morphometry, number of egg clusters ($p<0.29$) and length ($p<0.20$) showed no significant variation. The survey results indicated that population of the species, *Pila globosa* is vanishing and non – native species *P.scutata* is also decreasing day by day due to urbanization and other anthropogenic interventions which was clearly depicted in the questions? (If you found Apple snail / Njavanaykka / Nathaykka at rainy season?) and 5 (During the current seasons, do you feel the population become reduced?) have 100% score and remaining questions had 80-90% score except question number 4 (Have you collected it and used it as food?).

The proximate composition values obtained from the analysis of P. *scutata* is compared with the two commonly available food fish; *Sardinella longiceps* and *Rastrelliger kanagurta* for analysing the nutritional value of *P. scutata* as a food source. The mean moisture content in the fish *S.longiceps* was (73.79±0.30mg/100g),in *R.kanagurta* was (76.08±0.58mg/100g) (Palanikumar et al,2014). Where as in *P.scutata* mean moisture content is (57.923±0.068mg/100g), it is relatively lower than compared to the two common fishes. In fishes, the energy content in was (186Kcal/100mg) and (205±0.02Kcal/100mg) respectively (Lana Soko.2020), as compared to these values, the species *P.scutata* have more energy content with

(436.351±0.175 Kcal/100mg). The mean carbohydrate content in in *S.longiceps* was (3.58±0.004mg/100g),in *R.kanagurta* was (1.12±0.003mg/100g) comparing these values with the mean carbohydrate content in *P.scutata* (355.370±2.754mg/100g). The results revealed that the carbohydrate content is very high in *P.scutata*.

The flesh of *P.scutata* is rich in mean fat content (235.810±2.287mg/100g)compared to the fishes. The mean protein content in *S.longiceps* was (159.4±0.10mg/100g) and in *R.kanagurta* was (208±0.0mg/100g), where as in *P.scutata* (209.090±1.119mg/100g) was more or less similar protein content. In minerals Calcium content is higher in *P.scutata* (39.25±0.05mg/100g) compared to *S.longiceps (*35.9±0.09 mg/100g) and *R.kanagurta* (12±0.01 mg/100g).

The survey score recommended desirable necessary actions to be taken in conservation of native flora and fauna and should create an awareness on the medicinal properties of Pila. Consequently, a study of induced breeding of Pila species was found to be essential, that it may provide information and exploitation of this freshwater resource. Despite being densely populated, Kerala possesses vast suitable habitats for freshwater edible molluscs which can supplement animal protein for people, especially the lower income group and various types of aquacultures such as prawn farming and cat fish farming.

No information is available on the breeding and stocking of Pila. But these are the most important components to play vital role for commercial farming of *P. scutata*. The snail, *P. scutata* is an important component of the biodiversity playing very vital role for the maintenance of the aquatic ecosystem in one hand and on the other hand recently the animal is being utilized as the supplementary food for prawn culture. Snail meat has been reported to be highly nutritious because it contains the essential amino acid, rich in vitamins and minerals but low in fats and cholesterol. It is also reported that the land snail meat is very rich in protein which could be as high as 14.52% (Yusuf and Oseni, 2004). Other values like 12.87% and 12.2g/100g of protein have been reported for the garden and apple snails respectively.

However, the nutritional composition of snail might vary depending on its feeding habits, species, and method of collection, location, season and sexual conditions. Data on the nutrient composition of edible snails in Kerala state is scanty, fragmentary, and inadequate. A comprehensive data is therefore necessary. This work was therefore carried out to explore the nutritional potentiality of the species of snails. This natural renewable resource is under threat due to anthropogenic intervention. Certainly, if not sustainable uses of the snail species can be achieved through commercial farming the biological resource will be extinct due to non-scientific management and unwise habitat destruction. Development of artificial breeding ground and stocking of *Pila* are the essential serving for the commercial farming.

CONCLUSION

The proximate composition and mineral analysis of *P.scutata* shows its relevance as a food source in current scenario. Global capture fishery is presently at crossroads with over 70% of the resources exploited but the protein need increases with the increase in human population .Some under developed countries must need high level of protein to avoid malnutrition. The analysis and comparison shows the importance of *P.scutata* because it have the highest protein content than locally available common fish food like sardine and mackerel. Hence culture and consumption of *P.scutata* can act as an alternative for increased protein need and it also follows simple culture practice.

High energy content, carbohydrates and no sugar content hence it can be used by diabetic patients in association with their other nutritional need and provide a healthy diet. Fat content in *P. scutata* is also highest than comparing species. Induced breeding in captivity and sustainable management in nature as well as development of commercial production of apple snails might reduce the pressure on ecosystems and positively contributed to the continued expansion of freshwater prawn farming in Kerala. The ecologically and economically important such a biological resource will become extinct within a very short time if no steps are taken to establish the commercial farming of this species immediately.

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