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Proximate Composition and Heavy Metal Accumulation in Deep-Sea Crustaceans from Selected Stations in the Indian Exclusive Economic Zone (EEZ)

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Abstract

Proximate composition and heavy metal accumulation in the muscle of deep sea crustaceans v*iz., Acanthephyra armata, Heterocarpus gibbosus, Plesionika spinipes* and *Puerulus sewelli* were analyzed. Crustacean samples were collected from Lat. 11.026° – 11.405°N; Long. 74.508° – 75.212°E and Lat. 17.103° – 19.983°N; Long. 83.042°- 87.428°E at the depth of 200 – 1200 m off west coast and east coast of India. Moisture content was higher in *H. gibbosus* (81.84±0.11%) off west coast and *P. spinipes* (81.05±0.36%) off east coast, highest protein content (20.34±0.48%) was observed in *P. sewelli* from west coast followed by *H. gibbosus* (17.42±1.10%) off east coast. Fat content of the samples varied from 2.3-3.8%; with the highest in *A. armata* collected from west coast, whereas ash was higher in *A. armata* (2.21±0.14%) off east coast. Analysis of heavy metal content indicated higher levels of zinc in samples off west coast while iron and cobalt content was significantly higher in east coast samples. Lead was below detectable level in the samples analysed and cadmium was detected only in *H. gibbosus* off west coast. The deep sea crustaceans analysed in the present study contained significant quantity of protein and hence can find market in the food industry. Heavy metals analysed were within the prescribed limit, indicating that the selected stations of Indian EEZ are free of heavy metal pollution.

Keywords: Deep-sea, crustaceans, proximate composition, heavy metals

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Introduction

Seafood is significant in human nutrition because of its unique nutritive value related to the presence of proteins, fats, vitamins and minerals. At present, India is the second largest producer of fish in the world. As the demand for fish is continuously increasing, making the required protein available to the existing population is a challenge. With an increasing population, the fishing pressure is also increasing in the capture sector. Most of the crafts used in Indian waters are of 40 to 50 foot in length, having the capability of trawling only up to 200-300 m in coastal waters of Indian EEZ. The catch in coastal waters has already reached sustainable level beyond which there may be depletion of resource in inshore areas (Vivekanandan, et al., 2003). Hence, in the past decade, importance has been given for exploring deep sea resources (Vivekanandan, 2001; Thirumilu & Rajan 2003; Sebastine et al., 2011). Several exploratory surveys (Rajan et al., 2001; Karuppasamy et al., 2008) conducted along the deep waters of both the Indian coasts indicated that deep sea fisheries can be of much commercial and economical value.

The current level of commercial exploitation of deep-sea resources is limited only to deep-sea shrimps and sharks (Vivekanandan, 2011). However, unfamiliar appearance and rapid discoloration due to melanosis are the reasons for the medium market preference of deep-sea shrimp (Shanis et al., 2014). It is essential to assess the nutritive value of these resources as many of them are thrown back to the sea because of the non availability of information pertaining to their biochemical composition (Shanis et al., 2014). In India, studies on fishery, catch, species composition and biological aspects of deep-sea shrimps are reported mainly from Kerala, Karnataka and Tamil Nadu coast (Kumar et al., 2001; Radhika, 2004; Dineeshbabu et al., 2001; Thirumilu & Rajan 2003). Information about proximate composition of deep-sea fishes might raise their value as table food. Fernandez et al. (2014) has compared the nutritional characteristics of two myctophid fishes collected from deep-sea trawlers against common food fishes (*Sardinella longiceps, Mugil cephalus and Rastrelliger kanagurta*) and they found that the protein and lipid levels were comparable to that of the selected food fishes. However, very little information is available on the nutritive value of crustaceans from deep waters off the Indian EEZ.

Crustaceans can be employed as bio-indicators to assess the marine environment, since they can accumulate heavy metals and other pollutants (Darmono & Denton, 1990; Kress et al., 1998; Mantelatto et al., 1999). Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms like shrimp (Khansari et al., 2005). Although investigations about the trace metal behavior in the Arabian Sea and Bay of Bengal started in the early 1990s, (Couture & Kumar, 2003) they were mainly focused on metals in sediments, including their speciation, distribution and transformation. In this context, the present study was aimed to analyze the proximate composition and heavy metal accumulation in the predominant deep-sea crustaceans in the southeast and southwest coast of India.

Materials and Methods

Samples were collected from deep-sea stations located in the east and west coast of Indian EEZ (Fig. 1), during two deep-sea cruises of the research vessel *FORV Sagar Sampada*. The most prominent crustaceans caught in deeper waters are shrimps (*Acanthephyra armata* (900 – 1120 m depth), *Heterocarpus gibbosus* (280 – 380 m), *Plesionika spinipes* (250 – 400 m) and *Puerulus sewelli* (300 – 380 m) which are shown in Fig. 2. Twenty specimens of each species of the same size were kept in polyethylene bags and stored in the freezer at -20^{o}C for further analyses. Moisture, protein, fat and ash contents were determined as per standard methods (AOAC, 1990) and heavy metal analysis was done by Atomic Absorption Spectrophotometry (GBC 932AA, GBC Scientific Instruments, Australia) following AOAC method (AOAC, 1998).

Statistical analysis was carried out in Microsoft Excel. All the analyses were done in triplicate and the means were compared using Analysis of Variance (ANOVA). ANOVA was carried out for species wise and coast wise data comparison. The level of significance was fixed at 5%.

Results and Discussion

The mean values of each component of proximate parameters of *A. armata, P. spinipes, H. gibbosus* and *P. sewelli* are given in Table 1. Among the samples analysed, protein content was highest in *P. sewelli* collected from west coast followed by *H. gibbosus* off east coast. Lowest level of protein content was with *P. spinipes* off east coast. A significant difference (p<0.05) was noticed between the protein content of *H. gibbosus* collected off east and west coast. Among

Table 1. Proximate composition of deep-sea crustaceans off east and west coast of India

Species name	Moisture (%)		Protein (%)		Fat (%)		Ash (%)	
	East	West	East	West	East	West	East	West
Acanthephyra armata	77.83^{a}±0.04	78.37^{b}±0.26	16.05^{b}±0.34	15.00^{a}±0.19	3.64^{b}±0.18	3.82^{a}±0.11	2.21^{b}±0.14	1.79^{a}±0.04
Plesionika spinipes	81.05^{Bb}±0.36	78.77^{Ab}±0.43	14.92^{Aa}±0.56	16.68^{Bb}±0.10	2.30^{Aa}±0.10	3.12^{Ba}±0 .32	1.79^{a}±0.11	1.78^{a}±0.07
Heterocarpus gibbosus	77.00^{Aa}±0.66	81.84^{Bc}±0.11	17.42^{Bbc}±1.1	14.92^{Aa}±0.52	3.67^{b}±0.12	3.07^{a}±0.18	1.93^{a}±0.21	1.28^{a}±0.17
*Puerulus sewelli**	NA	76.53^{a}±0.54	NA	20.34^{c}±0.48	NA	2.37±0.22	NA	1.25^{a}±0.13

NA=Not analyzed, Values are mean ± SE (n=3), ^{a,b} and ^{c} denote significant difference (p<0.05) between row, ^{A} and ^{B} indicate significant difference (p<0.05) between column

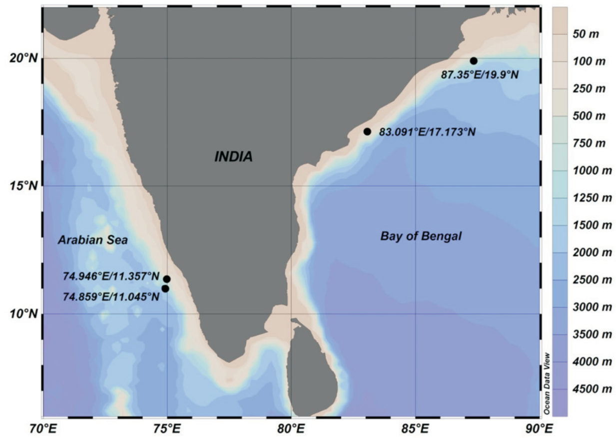

Fig. 1. Sampling stations from east coast and west coast of India

the species, significantly higher (p<0.05) fat content was observed in *A. armata,* while *H. gibbosus* collected from west coast had highest moisture content. Ash content was significantly higher in *A. armata* collected from east coast followed by *H. gibbosus* of east coast.

Protein is the most important constituent in seafood from the nutritional point of view. However, compared to finfish species, shell fish falls under lower protein category, the range being 5-14% (Balachandran, 2001). Protein content of *A. armata* and *H. gibbosus* agreed with those reported in the above study. Protein content of *P. spinipes* from east coast (14.92%) was similar to those reported for deep water red shrimp *Plesionika martia* by Oksuz et al. (2009). *P. sewelli* in our study showed higher content of protein (20.34%) compared to shrimps, indicating that it can be a good source of amino acids. *P. sewelli* comprises the major catch of deep-sea lobsters collected from depth up to 300 m in India and has a good export market potential. A study conducted by Rosa & Nunes, (2003) proved that protein content of a deep sea lobster, *Nephrops norvegicus* varied from 19.5 to 20.8% during an yearly survey off the Portuguese south coast. The same authors reported a protein content as high as 22-23% in the deep water blue and red shrimp, *Aristeus antennatus* whereas the protein content of a deep-sea lobster, *Linuparus somnisus* from Southern Java Ocean was found to be 12.29% (Suseno et al., 2014) which was lower compared to our study.

Seafood lipids are rich source of essential and polyunsaturated fatty acids. Lipid levels in shell fish are reported to be dependent on the source and proportion of lipids in the diet, moulting cycle and the season. In this study, crustaceans collected from the deep-sea had fat content varying from 2.37 to 3.32%. This indicates that fat content was higher compared to the previous reports on other crustacean species (Gopakumar & Nair, 1975). Moisture has little role in nutrition whereas it is important from technological point of view. The significant difference observed between few parameters of east coast and west coast could be due to the difference in feed availability. Proximal chemical composition in crustacean muscle were governed by many factors, including species, growth stage, feed and season (Karakoltsidis et al., 1995; Sikorski et al., 1990; Yanar et al., 2004). Depth and productivity both affect food availability and thus influence

Table 2. Heavy metal accumulation (µg g^{-1}) in deep-sea crustaceans off east and west coast of India

Species name	Cu		Zn		Fe		Co		Cd		Pb	
	East	West	East	West	East	West	East	West	East	West	East	West
*Acanthephyra armata**	22.28^{Ab} ±0.03	33.92^{Bc} ±7.38	8.15^{Aa} ±0.03	22.64^{Bb} ±2.10	39.54^{Bb} ±2.71	19.63^{Ab} ±3.85	3.03^{Bb} ±1.36	1.45^{Ac} ±0.65	BDL	BDL	BDL	BDL
Plesionika spinipes	7.15^{Ba} ±1.56	4.11^{Aa} ±0.79	13.73^{Ab} ±0.02	36.54^{Bc} ±1.06	50.06^{Bc} ±2.75	28.26^{Ac} ±0.16	1.12^{Ba} ±0.08	0.25^{Aa} ±0.20	BDL	BDL	BDL	BDL
Heterocarpus gibbosus	29.80^{Bc} ±4.30	12.65^{Ab} ±0.79	12.51^{Ab} ±0.47	15.35^{Ba} ±1.11	22.51^{a} ±1.39	20.70^{a} ±0.39	1.52^{Ba} ±0.09	0.25^{Aa} ±0.16	BDL	0.06±0.03	BDL	BDL
*Puerulus sewelli**	NA	36.02^{cd} ±3.33	NA	14.79^{a} ±0.55	NA	18.09^{a} ±0.44	NA	0.37^{ab} ±0.05	NA	BDL	NA	BDL

NA=Not analyzed, BDL=Below detectable limit, Values are mean±SE (n=3), ^{a,b} and ^{c} denote significant difference (p<0.05) between row, ^{A} and ^{B} denote significant difference (p<0.05) between column

Acanthephyra armata

Plesionika spinipes

Heterocarpus gibbosus

Puerulus sewelli

Plate 1. Deep-sea crustaceans selected for the study

chemical composition (Childress et al., 1990b). However, reports pertaining to biochemical composition of deep-sea crustaceans of Bay of Bengal and Arabian Sea are rare in literature to compare the data of the present study.

The results of heavy metal analysis indicated that heavy metal accumulation varied among the crustacean species. Shrimps collected from the west coast waters contained significantly higher Cd, Zn than those collected from east coast whereas, the concentration of Fe, Cu and Co was higher in the east coast samples (Table 2). This difference could be due to the variations in the metal concentrations in the water bodies and their absorption by the living organisms through food and water. Among all the heavy metals, iron accumulation was highest followed by Zn, Cu, Co and Cd. The concentration of all the heavy metals analyzed was lower in the lobster obtained from west coast when compared to the deep-sea shrimps.

Concentrations of Fe vary widely among marine crustaceans (Eisler, 1981). Maximum Fe concentration was observed in *P. spinipes* off the east coast (50.06 $\mu g\ g^{-1}$), and the lowest Fe concentration was observed in *P. sewelli* (18.09 $\mu g\ g^{-1}$). Shrimp collected from east coast contained significantly higher (p<0.05) Fe content than their counterparts collected from west coast. Higher concentrations of Fe in tissue samples could be due to an increased input of organic matter and anthropogenic metals from industrial pollution. Port activities such as shipping, loading of Fe-Mn ore, and unloading of Zn-Pb ore concentrates and other bulk cargo also partly contribute to the enhanced levels of some of these trace metals in seawater of both the Arabian Sea and Bay of Bengal (Rejomon et al., 2008). Moreover, large quantities of sediments impregnated with heavy metals are carried by major rivers along the east coast (Rengasamy & Jing, 2005).

Zinc and Cu are essential elements and play important roles in growth, cell metabolism and survival of most animals including crustaceans. Hence, the relatively high levels of these metals can be their metabolic requirement. Except for *A. armata* off west coast, Cu content of the crustaceans studied were within the limits prescribed by FAO, 30 $\mu g\ g^{-1}$ (FAO, 1983). Even though Cu is essential, very high intake will cause adverse health problems (Demirezen & Uruc, 2006; Satheesh kumar & Senthil kumar, 2011). Concentrations of copper in crustaceans also tend to be elevated, in comparison to other aquatic species, because they contain haemocyanin, a copper-containing protein that functions as an oxygen-transport molecule. Baboli & Velayatzadeh (2013) compared the Cu content in the tissue of Penaeid prawns, reported by various authors and it varied from 2.4 to 60.3 $\mu g\ g^{-1}$.

Zinc was found to be the second most abundant metal in the crustaceans sampled in this study. Shrimp samples off west coast showed significant difference ($p<0.05$) in Zn concentration in the order: *P. spinipes*>*A. armata*>*H. gibbosus*. Even though not significant, a little variability was also noticed in the shrimp samples collected from east coast following the same order as that of west coast. The European Union's permissible level of Zn for human consumption is 30 mg kg^{-1} wet weight (EU 2008). The analysis revealed that only *H. gibbosus* of west coast exceeded this limit. Cobalt is a natural element found throughout the environment. In the present study, cobalt accumulated in lower concentrations in samples from west coast (mean 0.65 $\mu g\ g^{-1}$) compared to east coast (mean 1.89 $\mu g\ g^{-1}$) species. Heavy metals like Pb and Cd in the muscle of deep sea crustaceans were particularly determined to analyze the seepage of these metals from polluted coastal waters to deep-sea waters if any. The sources of these exogenous metals in seafood can be industrial and agricultural discharges as well as from possible spill of lubricants/fuel during fishing operations round the year (Ololade et al., 2007). Lead is a toxic element that has no biological role and causes carcinogenic effects in marine biota and humans (Velusamy et al., 2014). The maximum limit established by European Union for crustaceans is 0.3 $\mu g\ g^{-1}$ (European Union, 2006). Zaza et al. (2015) reported that rose water pink shrimp from Eastern Central Atlantic Fishing had lead content up to 270 $\mu g\ g^{-1}$, which was the highest among the seafood collected from that area. In the present study, lead content was below detectable level in all the crustacean species, indicating safety for human consumption. The International Agency for Research on Cancer (IARC, 1993) classified cadmium as a human carcinogen. In particular, crustaceans appear to be more sensitive to cadmium than fish and mollusks (Sadiq, 1992). Similar to lead, cadmium was also below the detectable levels in all the samples, except for *H. gibbosus.* Cadmium content in *H. gibbosus* was 0.06±0.03 $\mu g\ g^{-1}$, which was well below the maximum levels (0.5$\mu g\ g^{-1}$) proposed by European Union (2008) for crustaceans. The results indicate that consumption of these deep sea crustaceans do not pose threat of Co & Pb to consumers as their levels were negligible. The nominal differences noticed in the accumulation of metals between the two coasts could be attributed to the variability of feeding habits (Romeo et al., 1999), ecological needs, metabolism (Canli & Furness, 1993), age and size of the species (Linde et al., 1998) and their habitats (Canli & Atli, 2003; Tuzen & Soylak, 2007).

Deep-water fisheries are becoming more and more important, and there is a paucity of safety monitoring of the ecosystem. The present study provides information on proximate composition and heavy metal accumulation of few deep sea crustaceans. The results indicated that the fish resources analysed contain significant protein content, and hence can be exploited commercially for meeting protein requirements. The concentrations of heavy metals analyzed were well within the regulatory limits indicating that the samples off deep waters do not pose any threat to the consumers. The findings of the study are important in the perspective of exploring deep sea resources as edible seafood. In a fast growing country like India, exploitation of deep sea resources within the judicious management practices is necessary to overcome resource crunch to ensure food and nutritional security.

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