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# Studies on the seasonal variations in the proximate composition of ascidians from the Palk Bay, Southeast coast of India

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ABSTRACT

**Objective:** To investigate the seasonal fluctuations of the proximate composition of the ascidians muscle. **Methods:** The moisture content was estimated by drying 1 g of fresh tissue at a constant temperature at 105 ℃ for 24 h.The loss of weight was taken as moisture content. The total protein was estimated using the Biuret method. The total carbohydrate in dried sample was estimated spectrophotometrically following the phenol- sulphuric acid method. The lipid in the dried sample tissue was gravimetrically estimated following the chloroform-methanol mixture method. Ash content was determined gravimetrically by incinerating 1 g dried sample in muffle furnace at about 550 ℃ for 6 h and results are expressed in percentage. **Results:** It was found very difficult to compare the monthly variations, as all the ten species, exhibited wide fluctuations in their proximate compositions. For the sake of convenience, average seasonal values were calculated by summing the monthly values. **Conclusions:** The proximate composition of the 10 commonly available ascidians showed high nutritive value and hence these groups especially solitary ascidians can be recommended for human consumption in terms of pickles, soup, curry and others after ensuring the safety of consumers.

## 1. Introduction

Ocean has potent bioactive compounds isolated from marine organisms which are currently used as food. A large proportion of natural compounds have been extracted from marine invertebrates[1]. More than 70% of our planet's surface is covered by oceans, and life on Earth has its origin in the sea. In certain marine ecosystems, such as coral reefs or the deep sea floor, experts estimate that the biological diversity is even higher than in tropical rain forests. Many marine invertebrates such as sponges, soft corals or shell-fewer mollusks are soft-bodied animals that are either sessile or slow moving and usually lack physical defenses like protective shells or spines, thus necessitating chemical defense mechanisms such as the ability to synthesize toxic and/or deterrent compounds[2]. The number of natural products isolated from marine organisms increases rapidly and now exceeds with hundreds of new compounds being discovered every year[3]. An important aspect of biodiversity conservation and sustainability of marine resources is the mitigation of non-indigenous species (NIS). To-date, there have been no reported extinctions of native marine species caused by exotic invaders[4]. Nonetheless, they pose a serious threat to the sustainability of aquaculture concerns[5] and they can alter the structure and composition of benthic communities[6], thereby threatening global marine biodiversity and resource sustainability. With only 16% of the worlds' marine eco regions free from NIS[7], invasive species are challenging pre- and post-border bio security strategies, and threatening biodiversity and ecosystem services around the world[8,9]. However, invasions can also provide insight into community ecology dynamics[10]; competitive interactions[11] and the resilience of native assemblages as NIS make their way into new ecosystems.

The combined availability of high-throughput molecular techniques and analyses of the resulting data based on explicit evolutionary models has caused a recent surge in the number of studies seeking to use genetic patterns to assess invasion pathways and the evolution of invasiveness[12,13]. Recent reviews of these molecular studies show that a wide array of taxa, geographic scales, and molecular markers have been covered over the past decade[14,15], with a range of results reported across both

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aquatic and terrestrial NIS[13]. A comprehensive analysis of molecular studies showed that conventional expectations of bottlenecks and reduced genetic variability for introduced populations do not always hold true for aquatic NIS, with only around 37% of studies reporting a significant loss of genetic variation in introduced populations[15]. However, most of these studies have sampled populations many years after the initial introductions and global spread. Marine taxa, also primarily sampled many years after introduction, exhibit a particularly wide range of molecular patterns. For example, high genetic diversity has been observed for the mitochondrial DNA gene cytochrome oxidase I (COI) in native and introduced populations of the ascidian *Microcosmus squamiger*. The first introduction of this species was recorded in1983 and it appears that extensive sharing of haplotypes has since occurred among populations[16].

The need for discovery of new and novel antibiotics is imperative because evidence suggests that development and spreads of resistance to any new antimicrobial agents is inevitable. Advances in molecular methods have shown that some symbiotic micro organisms are responsible for the production of secondary metabolites that serve as chemical defense for their hosts. Antibacterial activities have previously reported from extracts of some ascidians[17]. Marine organisms such as ascidians, sponges and soft corals, containing symbiotic microorganisms are a rich source of bioactive compounds. The ascidians are a rich source of compounds with cytotoxic proprieties; these marine natural products can be used for the discovery and development of novel chemotherapeutic agents[18]. Ascidians are conspicuous and important members of shallow benthic communities[19]. Therefore, information on their growth and biochemical and energetic composition[20] are important for evaluating their nutritional value and also in modeling the flow of materials and energy within marine benthos. Moreover, low nutrient levels could make some ascidians as unattractive prey while chemical defenses (acids, heavy metals, secondary metabolites *etc*) may make tissues distasteful to predators[21] and inhibit the settlement of fouling microorganisms[22]. To meet out the demand, marine products are playing an excellent role as they have high protein content. Marine invertebrates are being widely used as food around the world[23]. Seafood products are currently in high demand as they are considered healthy and nutritional[24]. Ascidians are also gaining importance as a source of animal protein. This creates the need for biochemical analysis of available native ascidians. The amount of carbohydrate, protein, lipid and minerals such phosphorous and calcium contents in few ascidians are previously reported by various authors[25].

## 2. Materials and methods

### 2.1. Collection and preparation of sample

Ten species of ascidians *Herdmania pallid* (*H. pallida*), *Microcosmus exasperates* (*M. exasperates*), *Microcosmus squamiger* (*M. squamiger*), *Microcosmus helleri* (*M. helleri*), *Didemnum psammathode* (*D. psammathode*), *Didemnum moseleyi* (*D. moseleyi*), *Lissoclinum fragile* (*L. fragile*), *Polyclinum madrasensis* (*P. madrasensis*), *Polyclinum indicum* (*P. indicum*), and *Polyclinum constellatum* (*P. constellatum*) were collected regularly during four seasons (Pre monsoon, Monsoon, Post monsoon and summer) in Palk Bay region, Southeast coast of India, from April 2010 to March 2011. The collected samples were immediately brought to laboratory in ice-cold condition. Species were identified as per the standard protocol[26-28]. In the laboratory, the collected ascidians were thoroughly washed with sea water to remove all the epiphytes and rinsed with distilled water to remove the salt and extraneous materials. Ascidians muscle samples were air dried for few days. The dried muscles were made in to powder for biochemical analysis such as protein, carbohydrate and lipids.

### 2.2. Moisture

To calculate the moisture content, 1 g of fresh tissue was oven dried at a constant temperature of 105 ℃ for 24 h[26]. The loss of weight was taken as moisture content.

### 2.3. Protein

The total protein was estimated using the Biuret method of Raymont *et al*[29]. The total carbohydrate in dried sample was estimated spectrophotometrically following the phenol-sulphuric acid method of Dubois *et al*[30].

### 2.4. Lipid

The lipid in the dried sample tissue was gravimetrically estimated following the chloroform-methanol mixture method of Tarjuelo *et al*[31].

### 2.5. Ash

Ash content was determined gravimetrically by incinerating 1 g dried sample in muffle furnace at about 550 ℃ for 6 h[32] and results are expressed in percentage.

### 2.6. Statistical analysis

Analysis of variance was performed to detect significant differences between the means of the aggregate and ascidians. Using the program Sigma-Stat, a Two-way ANOVA was calculated for multiple comparisons.

## 3. Results

The proximate composition of muscle tissue varied from species to species. In the present study, protein, carbohydrate and lipid contents of each ascidian species varied considerably. Seasonal variations in levels of proximate compositions in all the species were studied in this society. Each value in graph represents the mean of triplicate samples for each species. It was found very difficult to compare the monthly variations, as all the ten species, exhibited wide fluctuations in their proximate compositions. For the sake of convenience, average seasonal values were calculated by summing the monthly values.

### 3.1. Moisture

Variations of moisture content in different species of ascidians were ranged from 69.2% to 91.78%. The minimum (69.2%) content was noticed in *M. exasperatus* during monsoon season while maximum (91.78%) content was recorded in *D. psammatode* during summer season . The species significant ($P$<0.01) variations and seasons non–significant variations are as shown in the Figure 1.

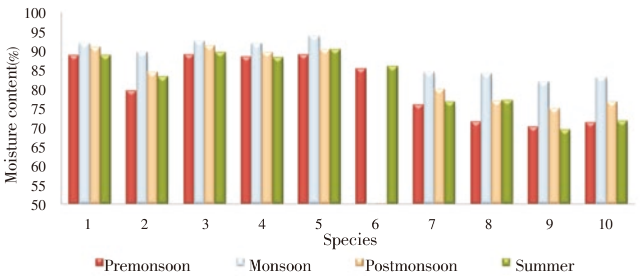


**Figure 1.** Seasonal variation of moisture content in different species of ascidians (% on dry weight basis).

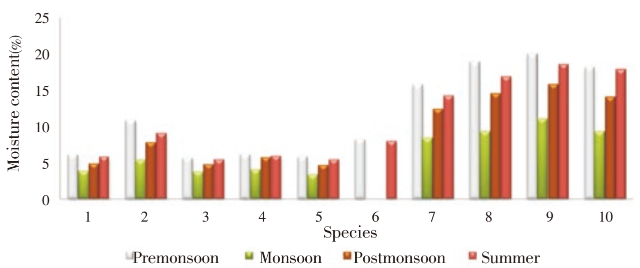


**Figure 2.** Seasonal variation of protein content in different species of ascidians (% on dry weight basis).

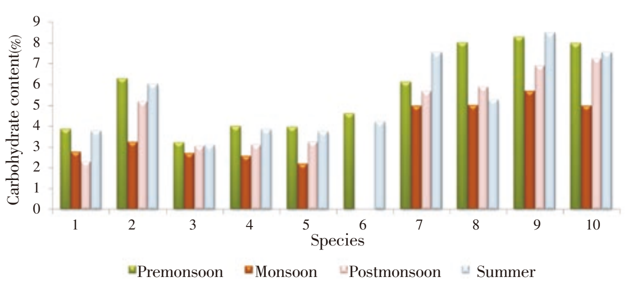


**Figure 3.** Seasonal variation of carbohydrate content in different species of ascidians (% on dry weight basis).

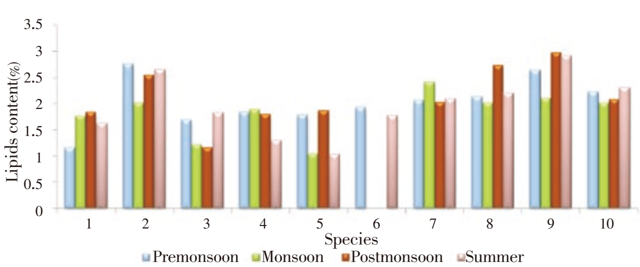


**Figure 4.** Seasonal variation of lipid content in different species of ascidians (% on dry weight basis).

### 3.2. Protein

The protein content in different species of ascidians varied from 3.8% to 20.01%. Minimum (3.8%) content of protein was observed in *D. psammatode* during monsoon season and maximum content of protein was (20.01%) recorded in *M. exasperatus* during premonsoon season (Figure 2). The two–way ANOVA showed highly significant variations between the species ($P$<0.01) and seasons ($P$ <0.01).

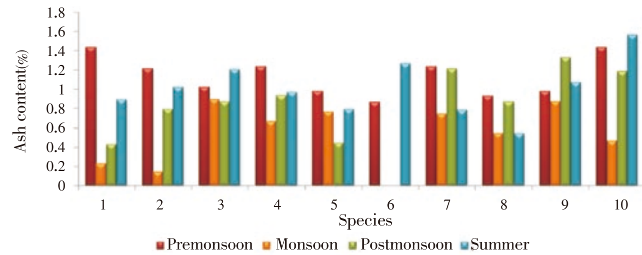


**Figure 5.** Seasonal variation of ash content in different species of ascidians (% on dry weight basis).

### 3.3. Carbohydrate

The variation of carbohydrate content in different species of ascidians was ranged from 2.2% to 8.29%. Minimum (2.2%) was observed in *P. indicum* during monsoon season and the maximum (8.29 %) was recorded in *M. exasperatus* during premonsoon season (Figure 3). The two–way ANOVA showed significant variations between the species ($P$<0.05) and seasons ($P$<0.05). In the present observation the carbohydrate contents was slightly higher than the lipids and lower than that of protein, carbohydrates constitute only a minor percentage of total biochemical composition.

### 3.4. Lipids

The lipid content in different species of ascidians was ranged from 1.05% to 2.97%. The minimum (1.05%) content was recorded in *P. indicum* during monsoon season while the maximum content was observed in *M. exasperatus* during postmonsoon season (Figure 4). The two–way ANOVA showed the species significant ($P$ <0.01) variations and seasons non–significant variations.

### 3.5. Ash

Ash content in different species of ascidians was ranged from 0.14% to 1.56%. The minimum (0.14%) was recorded in *D. moseleyi* during monsoon season. while the maximum (1.56%) was recorded in *M. hellari* during summer seasons (Figure 5). The two–way ANOVA showed the species non–significant variations and seasons significant ($P$ >0.05) variations. For comparison, the sum of ash, proteins, lipids, carbohydrates and chitin in crustacean zooplankton typically accounts for 90%–95% of the dry weight[33] investigated North Atlantic salp (Tunicata: Thaliacea) species and found the proteins to be the major contributor (82%) to the total. This corresponded to 6.6% of the dry weight. This value is substantially higher than that obtained in our study, *e.g.* 40%–50% of the total for aggregates and solitaries, respectively. This corresponded to 4.4% of the dry weight. Much higher protein contribution to the total measured by previous studies likely resulted from lower lipid content in the scalps when compared to present study.

## 4. Discussion

The protein content of ascidians showed great variation; these values are comparable with other earlier reports[20,34]. In the present estimation, the protein content was found to be the major biochemical composition in the solitary ascidians, *M. exasperates*. Similarly, high levels of protein occur in body components of Antarctic solitary ascidians *Cnemidocarpa verrucosa* and likely reflect the contribution of insoluble protein to structural materials including connective tissue[35]. The multitude of zooids that comprise large colonies of *D. cylindrical*, each possessing complements of longitudinal thoracic muscles[36] which may contribute to the high levels of protein (*e.g.* connective tissue). As protein was determined by subtraction, it is also possible some fraction of this insoluble material is attributable to a protein-polysaccharide complex of fibers (tunicin) known to occur in various concentrations in the tunic of ascidians[35,37]. The soft delicate nature of the tunic with embedded zooids did not allow biochemical analyses of the isolated tunic of *D. cylindrica*. Colonies comprised of much lower levels of soluble protein (4.9% dry weight), lipid (4.2% dry weight), and, especially, soluble carbohydrate (2.4% dry weight). These low percentages of biochemical constituents are similar to those measured in the outer tunic and endocarps, but two to three times lower than those measured in the body wall and ovitestes of the solitary Antarctic ascidian *Cnemidocarpa verrucosa*. The latter is not surprising as the discrete body components of solitary ascidians may be characterized by the localization of energy and materials in the body wall for energy reserves and in the ovitestes for gametogenesis . The protein content was positively related with salinity of the water[38]. Carbohydrates in fishery products contain no dietary fibre but only glucides, the majority of which consist of glycogen. They also contain traces of glucose, fructose, sucrose and other mono and disaccharides[39]. Determining the composition of lipids can be important in determining nutritional condition because cell membranes are made of polar lipids but energy is stored as neutral lipids, mostly triglycerides[40]. In general, lipid content has been very low in the ascidians as compared to protein and carbohydrate. The lower value lipids of ascidians (solitary) have been already reported by Park *et al*[34]. In general, ascidians do not feed when they are in spawning season. Their nutrient requirement is met by body reserves and as a result, the level of lipids falls steadily as spawning progress. When lipid is drawn from muscle reserves, it is replaced by water so, that the total mass of the fish remains much same[41]. The water content of muscle obviously rises and this makes the flesh weak. This is the reason for the poor eating equality of spawning ascidians. These tissue energy levels are similar to measurements for the tropical colonial ascidian, *Cystodytes lobatus* and nine species of ascidians from the *Canadian Arctic*. In all the species, higher protein, carbohydrate values in the muscle coincided with the spawning period and low values with post spawning months as observed in *Phallusia nigra*[42]. Seasonal changes in ash content have also been reported in other ascidians by Blum *et al*[6]. This study makes it crystal clear that the biochemical compositions which also can be interpreted as the nutritional representation of ascidians are significantly influenced by the varying seasons. This preliminary work on the biochemical composition of the Palk bay ascidians brings them to the light serving as a prelude for further research on secondary metabolite and pharmacological properties

In order to study its nutritive value, an attempt was made to estimate the protein, carbohydrate, lipid, moisture and ash contents in 10 species of ascidians. The results showed relatively higher nutritive values. The moisture content in all the ascidians was above 69%. The maximum moisture content was observed in *P. indicum* and the minimum in *M. exasperates*. The protein content varied considerably from 3.33% to 20.01%. The higher protein content was noticed in *M. exasperates* in pre monsoon season and the minimum in *P. indicum* in monsoon season. The carbohydrate content of the ascidians varied from 2.2% to 8.5%.The maximum value was found in *M. exasperates* in summer season and the lowest value was found in *P. indicum* in monsoon season. The lipid content varied between 1.03% and 2.91%. The lower and higher values were obtained from *P. indicum* in summer seasons and *M. exasperates* in summer respectively. The ash content varied between 0.23% and 1.56%. The higher value was found in *D. psammatode* and the lower value in *M. hellari.* In all the ten species, the percentage of moisture and ash content in muscle during maturity cycles was low in immature ascidians and high in mature ascidians. In relation to different seasons, moisture and ash content in muscle of all the ten species were low during post-spawning months and gradually increased during pre-spawning months leading to the highest values during spawning months carbohydrate and protein recorded higher values during post-spawning months and a gradual depletion was evident during pre-spawning months, leading to the low values during spawning months.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Natarajan K, Sathish R, Regupathi T, Riyaz A. Antibacterial

activity of crude extracts of marine invertebrate *Polyclinum madrasensis* Sebastian, *Ind J Sci Tech* 2010; **3**: 303-304.

[2] Sherif S, Edrada ERA, Lin WH, Proksch P. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates, *Nat Prot* 2008; **3**(12): 1820-1831.

[3] Jain R, Sonawane S, Mandrekar N. Marine organisms: Potential source for drug discovery. *Cur Sci* 2008: **94**(3): 292.

[4] Briggs JC. Marine biogeography and ecology: invasions and introductions. *J Biogeogr* 2007: **34**: 193-198.

[5] Davis M, Davis M. *Styela clava* (Tunicata, Ascidiacea) – a new threat to the Mediterranean shellfish industry. *Aquatic Invas* 2009: **4**: 283-289.

[6] Blum JC, Chang AL, Liljesthrom M, Schenk ME, Steinberg MK. The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. *J Exp Mar Biol Ecol* 2007: **342**: 5-14.

[7] Molnar J, Gamboa R, Revenga C, Spalding M. Assessing the global threat of invasive species to marine biodiversity. *Front Ecol Environ* 2008; **6**: 485-492.

[8] Coutts A, Forrest B. Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum*. *J Exp Mar Biol Ecol* 2007; **342**: 154-162.

[9] Dodgshun T, Taylor M, Forrest B. *Human-mediated pathways of spread for non-indigenous marine species in New Zealand*. Wellington, New Zealand: Department of Conservation; 2007, p. 43.

[10] Daleo P, Alberti J, Iribarne O. Biological invasions and the neutral theory. *Diversity Distrib* 2009; **15**: 547-553.

[11] Eastwood M, Donahue M, Fowler A. Reconstructing past biological invasions: niche shifts in response to invasive predators and competitors. *Biol Invasions* 2007; **9**: 397-407.

[12] Darling J, Blum M. DNA-based methods for monitoring invasive species: a review and prospectus. *Biol Invasions* 2007; **9**: 751-765.

[13] Dlugosch K, Parker IM. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol Ecol* 2008; **17**: 431-449.

[14] Dlugosch K, Hays C. Genotypes on the move: some things old and some things new shape the genetics of colonization during species invasions. *Mol Ecol* 2008; **17**: 4583-4585.

[15] Roman J, Darling JA. Paradox lost: genetic diversity and the success of aquatic invasions. *Trends Ecol Evol* 2007; **22**: 454-464.

[16] Rius M, Pascual M, Turon X. Phylogeography of the widespread marine invader *Microcosmus squamiger* (Ascidiacea) reveals high genetic diversity of introduced populations and non-independent colonizations. *Diversity Distrib* 2008; **14**: 818-828.

[17] Paul VJ, Williams RR. Marine chemical ecology. *Nat Prod Rep* 2008; **25**: 662-695.

[18] Ananthan G, Sivaperumal P, Mohamed Hussain S. Cytotoxicity of the crude extracts of marine ascidians (Tunicata: Ascidiacea) from Tuticorin, Southeast coast of India. *Arch App Sci Res* 2011; **3**(2):139-142.

[19] Monteiro SM, Chapman MG, Underwood AJ. Patches of the ascidian *Pyura stolonifera* (Heller, 1878): structure of habitat and associated intertidal assemblages. *J Exp Mar Biol Eco*,2002; **l270**: 171-189.

[20] Kowalke J, Tatian M, Sahade R, Arntz W. Production and respiration of Antarctic ascidians. *Polar Biol* 2001; **24**: 663-669.

[21] Pisut DP, Pawlik JR. Anti-predatory chemical defences of ascidians: secondary metabolites or inorganic acids. *J Exp Mar Biol Ecol* 2002; **207**: 203-214.

[22] Bryan PJ, McClintock JB, Slattery M, Rittschof DP. A comparative study of the non-acidic chemically mediated antifoulant properties of three sympatric species of ascidians associated with seagrass habitats. *Biofouling* 2003; **18**: 235-245.

[23] Karthikeyan MM, Ananthan G, Abdul Jaffar Ali. Nutritional values of solitary ascidian *Microcosmus exasperate* Heller, 1878 (Ascidiacea: Pyuridae). *Global Vet* 2010; **4** (3): 255-259.

[24] Skonberg DI, Perkins BL. Nutrient composition of green crab (*Carcinus maenas*) leg meat and claw meat. *Food Chem* 2002; **77**: 401-404.

[25] Rajesh M, Ali HAJ. Nutritional value and antimicrobial activity of marine ascidian species. *Ascidian News* 2008; **7**: 206-210.

[26] Kott P. The Australian ascidiacea. Part 1, Phlebobranchia and Stolidobranchia. *Mem Qd Mus* 1985; **23**: 1-440.

[27] Kott P. The Australian ascidiacea, part 2, Aplousobranchia (1). *Mem Qd Mus*,1990; **29**(1): 1-226.

[28] Kott P. The Australian ascidiacea, part 3, Aplousobranchia (2). *Mem Qd Mus* 1992; **32**(2): 37-620.

[29] Raymont JEG, Austin J, Linford E. Biochemical studies on marine zooplankton. I. The biochemical composition of *Neomysis integer*. *J Cons Perm Explor Mar* 1964; **28**: 354-363.

[30] Dubois M, Giles KA, Hamilton JK, Rebors PA, Smith F. Calorimetric method for determination of sugar and related substances. Analyt. *J Biol Chem.* 1956; **28**: 350-356.

[31] Folch J, Lees M, Sloane GSH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 1957; **226**: 497-509.

[32] AOAC. *Official method of analysis*. 15th ed. Washington DC: Association of official Analytical chemists; 1990.

[33] Clarke A, Holmes LJ, Gore DJ. Proximate and elemental composition of gelatinous zooplankton from the Southern-Ocean. *J Exp Mar Biol Ecol* 1992; **155**: 55-68.

[34] Park CK, Matsui T, Watanabe K, Yamaguchi K, Konosu S. Regional variation of extractive nitrogenous constituents in the ascidians *Halocynthia roretzi* muscle. *Nippon Suisan Gakkaishi* 1991; **57**: 169-174.

[35] Kott P. Form and function in the Ascidiacea. *Bull Mar Sci* 1989; **45**: 253-276.

[36] Kott P. Antarctic ascidiacea. A monographic account of the known species based on specimens collected under U.S. Government auspices 1947 to 1963. *Antarct Res Ser* 1969; **13**(I-XV): 1-239.

[37] Smith MJ, Dehnel PA. The chemical and enzymatic analyses of the tunic of the ascidian *Halocynthia aurantium* (Pallas). *Comp Biochem Physiol* 1970; **35**: 17-30.

[38] Raymont JEG, Conver RJ. Further investigation on the carbohydrate content of marine zooplankton .*Limno Ocenogr* 1961; **6**: 154-164.

[39] Okuzumi M, Fujii T. *Nutritional and functional properties of squid and cuttle fish*. 35th Anniversary of Commemorative Publication; 2000, p. 223.

[41] McClintock JB, Amsler MO, Amsler CD, Southworth KJ, Petrie C, Baker BJ. Biochemical composition, energy content and chemical antifeedant and antifoulant defenses of the colonial Antarctic ascidians, *Distaplia cylindrical*. *Mar Biol* 2004; **145**: 885-894.

[42] Abdul Jaffar Ali H. Comparative study on ecology of *Phallusia nigra* (Savigny, 1816) between Tuticorin (South east coast) and Vizhinjam (South west coast) of India. Ph.D. Thesis. V.O.C. College, India; 2004, p. 45-50.