Indian Journal of Geo Marine Sciences
Vol. 47 (07), July 2018, pp. 1391-1397

Natural diet of the alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) inhabiting Palk Bay, southeast coast of India

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Received 28 April 2016; revised 22 July 2016

A total of 56 guts of the alligator pipefish, *Syngnathoides biaculeatus* collected from Palk Bay, southeast coast of India were analysed for determining its natural diet composition. The gut fullness index as assessed by point method showed that the majority of fish (71.43%) either had half-full (37.5%) or quarter-full guts (33.93%). Mean values of relative growth length (RGL) and gonado-somatic index (GSI) were estimated to be 0.36±0.03 and 3.78±1.67%, respectively. Copepods and sand particles occurred with 100% frequency of occurrence (*%FO*) followed by amphipods (*%FO*=99.25) isopods (*%FO*=86.79), peracarids (*%FO*=70.76) and decapods (%*FO*=65.28). The results of the gut content analysis showed that *S. biaculeatus* largely preyed on crustaceans (~90%) which included amphipods (40.36%), copepods (35.27%), decapods (8.12%), isopods (3.48%) and peracarids (2.21%). Consistent occurrence of sand particles and algal pieces in the guts reflect the feeding behaviour of *S. biaculeatus* in seagrass habitat over the sandy bottom. No significance difference in prey numbers and composition between male and female specimens was observed (*P*>0.05).

[**Keywords**: Alligator pipefish, *Syngnathoides biaculeatus*, Palk Bay, diet, amphipods, copepods]

Introduction

The study on natural diet of fish plays a vital role in understanding its eco-biological processes and has implications for sustainable management and conservation of a population[1,2,3]. The growth, survival and reproduction of fish depend on the nutritional status and the energy assimilated by feeding activity[4]. Furthermore, distribution, migration and behaviour of fish are largely influenced by food availability and preferred prey items[5]. In view of the importance of fish in human nutrition, published literature[6,7] suggests that the biochemical composition of fish is strongly correlated to their food intake.

Due to trophic functions and bioactive properties, syngnathid fishes are ecologically and commercially important[8]. These fishes mostly inhabit fragile ecosystems and productive coastal areas such as seagrass meadows, corals, mangroves as well as seaweeds[9] and feed on benthic organisms at the bottom-water column interface[10,11]. Previously, feeding ecology of few syngnathid fishes inhabiting the seagrass beds has been studied[10,11,12,13,14].

The syngnathid fishes (seahorse, pipefish, pipehorse and seadragon) exhibit unusual suction feeding habit[15,16] which is known as '*pipette feeding*'[17] or '*pivot feeding*'[18]. These fishes are visual predators. With independently moving eyes, they wait until prey come close to their mouth and with a sudden attack (ambush predation), prey organisms are rapidly drawn inside the tubular snout with forcible intake of water[11,19]. Syngnathid fishes mostly prey upon live moving organisms that fit into their snout gape size such as amphipods, copepods, fish larvae and other small invertebrates[13,20, 21,22,23,24,25,26,27].

The alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) is dominant among the fish community in seagrass meadows[28] and is one of the most heavily exploited and traded pipefish species in traditional Chinese medicine (TCM). It is also listed as 'Data Deficient' in IUCN red list of threatened species, thus warranting extensive research on its biological aspects[29]. Perhaps, due to legal restrictions (Schedule I of Wild Life (Protection) Act, 1972) on wild collections, little information on biological aspects of Indian syngnathid species is available. Published literature on *S. biaculeatus*[30,31,32,33] suggests that a detailed study on diet composition of *S. biaculeatus* from its natural habitat is lacking. In view of this, an attempt has been made here to provide comprehensive information on the diet composition of *S. biaculeatus* inhabiting seagrass beds in Palk Bay, southeast coast of India.

Material and Methods

Altogether, 56 individuals (22 males and 34 females) of the alligator pipefish, *S. biaculeatus* (Bloch, 1785) caught as by-catch in wind-driven country trawl or '*Vallams*' (mainly operated for crab/shrimp fishing) at Thondi village in Palk Bay, southeast coast of India (09º46′35.46″N, 79º0′28.87″E) were used for diet analysis. Extensive seagrass beds mostly comprising of *Thalassia hemprichii*, *Cymodocea serrulata* and *Enhalus acoroides* in depths ranging between 2 and 6 m occupy the study area[34,35]. Immediately after collection, fishes were washed with ice cold distilled water and then placed in ice box completely filled with crushed ice and transported to the field laboratory. In the laboratory, fishes were weighed using electronic balance to the nearest 0.01 g. Total length (L_T, from tip of snout to the tip of caudal end) of each fish was measured to the nearest 0.01 mm with vernier calipers. Mean total length (L_T) and wet weight of the fishes were 195.7±2.83 mm and 5.39±0.94 g (males) and 190.9±2 mm and 5.56±0.74 g (females), respectively. Mean snout gape size recorded in *S. biaculeatus* was 1.9±1 mm.

Methods and formula described by various researchers were used for gut content analysis[10,23, 36,37,38]. After taking length-weight measurements, fishes were dissected by incision at ventral surface and gut was carefully removed. The total length (TL) of the gut was then measured (mm) from anterior to posterior with help of vernier calipers to the nearest 0.1 mm and guts were weighed using digital micro balance to the nearest 0.01 g. The relative gut length (RGL) was calculated by dividing TL by L_T of each fish. Fullness of the gut was estimated visually by measuring a portion of filled gut with vernier calipers and based on proportion of contents, the guts were categorised into six classes: empty (0%), moderately full (12%), quarter full (25%), half full (50%), full (75%) and very full (100%). After taking measurements, all guts were preserved in 10% buffered formalin with appropriate labels and transported to laboratory at CSIR-National Institute of Oceanography, Goa (India). In the laboratory, the guts were cut open by making a length-wise incision. All prey individuals were counted with help of counting chamber under stereo zoom microscope (Olympus SZX7, Japan) after washing the gut contents through 125 µm nylon mesh cloth. Prey items were identified to the lowest possible taxonomic level. Broken individuals of prey were identified up to group level and together listed as unidentified. Numerical methods were used for the gut content analysis as below:

$$\text{Frequency of occurrence } (\%FO) = \frac{Ni}{N\text{s}} \text{x} 100$$

Where, Ni = the number of guts in which prey item 'I' occurs

Ns = the number of total guts examined with food in the sample

$$\text{Percentage of prey } (\%N) = \frac{n'}{N\text{p}} \text{X } 100$$

Where, n'=the total number of individuals of a certain prey

Np = the total number of prey items.

Based on the number of empty guts, the vacuity coefficient index (I_v) in % was calculated

$$\text{Vacuity coefficient index } (\%Iv) = \frac{E\text{v}}{N\text{s}} \text{x } 100$$

Where, Ev = the number of empty guts

Ns = the number of total guts examined

Gastro-somatic index (%*GSI*) as a measure of feeding intensity was calculated by applying the following formula. Data were presented as mean ± SD.

$$\text{Gastro-somatic index } (\%GSI) = \frac{\text{weight of gut}}{\text{weight of fish}} \text{ x } 100$$

Nonparametric Man-Whitney *U* test[39] was used to assess the difference between mean numerical abundance of individuals of prey items and gut fullness index between male and female specimens of *S. biaculeatus*. Level of significance was judged as '*P*' = 0.05.

Result and Discussion

Studying the eco-biological aspects of syngnathid fishes has greater implications for better management of natural populations and improvement in captive rearing protocols. Due to limited mobility, these fishes are often seen attached to seagrasses, seaweeds, corals or to any submerged substratum with their prehensile tail[15]. Therefore, the source and availability of food material to these fishes is limited and restricted[11]. The alligator pipefish, *S. biaculeatus* has been considered to be a permanent resident of seagrass meadows with no reported migration[28]. The natural diet composition of *S. biaculeatus* inhabiting seagrass beds of Palk Bay, southeast coast of India has been described in this study.

Generally, the gut fullness index of fish provides insight into type of prey consumed as well as the process of digestion. In the present study, 5.36% of guts were empty, 16.07% were moderately full, 37.50% were half-full, 33.93% were quarter-full, while no gut belonged to very full gut (100%) category (Fig 1). Mean values of %I_v and %*GSI* recorded, respectively were 5.36% and 3.78±1.675% (males, 3.31±1.5%; females, 4.09±1.7%; P>0.05). Unfortunately, except a lone study by Gurkan *et al.*,[25] the detailed categorisation on feeding indices of other pipefish species is not available for comparison. In straight nose pipefish, *Nerophis ophidion,* the percentage of empty guts has been reported to be <10[25]. On the other hand, relatively higher percentage of empty guts in males (45.07%) compared to females (13.14%) has been reported in *Hippocampus guttulatus*[10]. Furthermore, Gurkan *et al.*,[26] observed that 16% and 27.27% of guts were empty in *H. hippocampus* and *H. guttulatus*, respectively. The vast differences in the gut fullness indices amongst syngnathid species might be due to ingestion of few larger preys (*Acetes* sp., amphipods) which may fill the gut completely[24]. For example, Nakamura *et al.*,[30] reported that the major items in guts of *S. biaculeatus* were shrimps (50%) and fish larvae (25%) by volume than other small crustaceans.

Furthermore, gender-specific significant differences in gut fullness index with higher values in females have been reported in few syngnathid fishes[10,40]. In contrast, no significant difference in gut fullness indices between genders were noticeable (P>0.05) in the present study. Gender dependent significant difference in feeding index (with lower proportion of empty guts in females compared to males) has been observed in two seahorse species (*H. guttulatus* and *H. hippocampus*) by Kitsos *et al.*,[10]. Similar observations have also been reported in deep snouted pipefish, *Syngnathus typhle* by Oliveira *et al.*,[40]. Such differences in feeding indices between two genders of syngnathid fishes could be attributable to lower feeding activity in males during reproductive period [10,40].

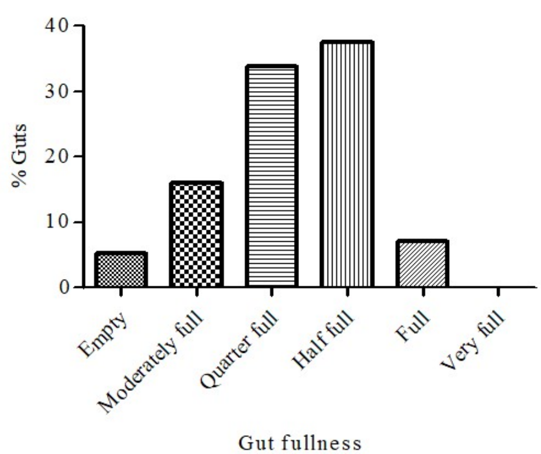

Fig. 1 — Gut fullness index based on six classes for *S. biaculeatus*; empty (0%), moderately full (12%), quarter-full (25%), half-full (50%), full (75%) and very full (100%)

Based on the present observation and some previous feeding ecological studies, the higher proportion of empty to half-full guts and lower percentage of full guts appear to be consistent amongst syngnathid fishes. In addition to reasons as explained above, the intervening period from the time of collection until fixing the gut contents might also reduce the gut fullness due to processes of digestion and food assimilation[23]. In syngnathid fishes, time required for food digestion has been estimated to about 3-4 hours[15] or may be even lower[41]. Enzymatic activity of bacterial flora associated with gut might be one of the factors for such lower digestion periods. The significant role of lipid degrading bacteria in digestion of the yellow seahorse, *H. kuda* has been documented by Tanu *et al.*,[42]. However, the possible role of similar enzymatic activity of bacterial gut flora in aiding the digestion in pipefish species cannot be ruled out. In the present study, 16.07% of guts were moderately full, 37.50% were half-full, while 33.93% were quarter-full and 5.36% were empty. Occurrence of all categories of gut fullness indices in *S. biaculeatus* indicates its continuous feeding pattern in natural habitat.

RGL as an indicator of feeding habit of fishes has been widely documented[43,44,45]. Relatively lower values of RGL (<1) are associated with in carnivore fishes, whereas the values >3 represent herbivory or detritivory feeding pattern. In the present study, mean RGL value of *S. biaculeatus* is quite low (0.36±0.03) which supports its carnivore feeding pattern. Carnivory feeding pattern (RGL~0.4) in two species of seahorses (*H. spinosissimus* and *H. trimaculatus*) from Malaysian coast has also been reported recently by Yip *et al.*[45].

Altogether, 10 major groups of prey items were identifiable from the guts of *S. biaculeatus* (Table 1). Numerical abundance of prey items ranged between 68 and 245 (mean±SD, 124.18±45.82). Although, the mean numbers of prey items in the guts of females

Table 1 — Mean ± SD prey numbers, frequency of occurrence (%*F*o) and percentage of prey (%*N*) in the guts of alligator pipefish, *S. biaculeatus*

Major group	Prey items	Mean nos. ± SD	%*FO*	%*N*
Crustacea				
Copepoda	*Paracalanus* spp.	16.91±7.26	100.00	13.56
	Acartia spp.	9.66±4.68	100.00	7.75
	Unidentified copepods	17.23±8.59	100.00	13.82
Peracarida	Mysids	1.23±1.37	64.15	0.99
	Cumacea	1.52±1.26	77.36	1.22
Decapoda	Megalopa larvae	0.16±0.37	18.87	0.13
	Shrimp larvae	1.98±1.57	79.25	1.59
	Acetes sp.	0.54±0.69	45.28	0.43
	Lucifer spp.	2.36±1.75	83.02	1.89
	Unidentified decapods	5.05±2.14	100.00	4.05
Amphipoda	*Eriopisa* spp.	17.61±7.52	100.00	14.12
	Hornellia spp.	5.45±3.32	100.00	4.39
	Hyale spp.	3.96±1.96	100.00	3.19
	Grandidierella spp.	2.38±1.78	96.23	1.90
	Unidentified amphipods	20.91±7.65	100.00	16.77
Isopoda	Isopods	4.32±2.68	92.45	3.47
Annelida	Polychaeta	0.16±0.37	16.98	0.13
Chordata	Pisces	0.16±0.46	13.21	0.13
Algae	Unidentified algae	0.36±0.64	28.30	0.29
Inorganic matter	Sand particles	11.00±4.60	100.00	8.82
Foraminifera	Foraminifera	1.41±1.36	67.92	1.14
	Mean±SD	124.18±45.82	–	–

(131.64±42.79) were relatively higher than males (112.63±48.90), the differences were insignificant (*P*>0.05). Mean prey numbers of 214.2 and 230, respectively in guts of female and males of southern pipefish, S*yngnathus folletti* from seagrass beds have been reported[24]. Furthermore, significant gender-dependent differences in feeding activity have been reported in other syngnathid fishes also[10,40,46]. On contrary, Taskavak *et al*.,[27] did not observe any significant difference in feeding pattern between male and females of *S. acus* collected from Izmir Bay, Turkey. The observed differential feeding behavior in two sexes of different pipefish species may be related to seasonality and reproductive status of natural populations. However, further studies in this direction are required to corroborate these initial findings.

Out of 21 prey items, 9 taxa consistently observed in individuals of *S. biaculeatus* having prey in their guts (Table 1). Copepods and sand particles occurred in the guts of *S. biaculeatus* with 100% frequency of occurrence (%*FO*) followed by amphipods (%*FO*=99.25) isopods (%*FO*=86.79), peracarids (%*FO*=70.76) and decapods (%*FO*=65.28). From the diet analysis, it is evident that crustaceans (amphipods, copepods and decapods) formed the major diet of *S. biaculeatus* inhabiting seagrass beds of Palk Bay. Amongst all prey items (Table 2), amphipods were the major group (40.61%) followed by copepods (35.13%), decapods (8.09%), sand particles (8.82%), isopods (3.47%), peracarids (2.21%) and others (1.68%). Amphipods were represented by *Eriopisa* spp., *Hornellia* spp., *Hyale* spp. and *Grandidierella* spp. while, *Paracalanus* spp. and *Acartia* spp. were major prey species amongst copepods.

Table 2 — Major groups of prey items (%) recorded in the guts of *S. biaculeatus*

Prey items	%
Amphipoda	40.50
Copepoda	35.59
Sand particles	8.89
Decapoda	7.79
Isopoda	3.49
Peracarida	2.22
Foraminifera	1.15
Algae	0.29
Polychaeta	0.13
Pisces	0.13

Decapods were represented by crab megalopa larvae, shrimp larvae, *Acetes* sp. and *Lucifer* sp. Among crustaceans, percent number (%*N*) of prey items such as *Eriopisa* sp., *Acartia* spp., unidentified amphipods and copepods were, 13.56, 7.75, 16.77 and 13.82, respectively higher. Other minor groups recorded were polychaete (%*N*=0.13), fish larvae (%*N*=0.13), unidentified algal matter (%*N*=0.29) and foraminifera (%*N*=1.14%) observed in the guts of *S. biaculeatus*. Based on the numerical abundance, frequency of occurrence and percent number, the prey items of *S. biaculeatus* were predominantly comprised of crustaceans (~90%). Micro-crustaceans, particularly amphipods, mysids and anomuran decapods have been reported to be the most dominating prey items in the stomachs of the long-snouted seahorse, *H. guttulatus* and short-snouted seahorse, *H. hippocampus* from Aegean sea, Turkey[10,26]. Amphipods, gastropods and harpacticoid copepods were also the dominant prey items in the guts of *N. ophidion* from Aegean sea, Turkey[25]. Although the crustaceans were the major prey items in the guts of 12 morphologically diverse syngnathid fishes from seagrass beds of south-western Australia, the size and composition of prey items, however, differed in individual fishes[13]. In a diet composition study of great pipefish, *S. acus*, Taskavak *et al*.,[27] reported that harpacticoid copepods, amphipods, cypris larvae and decapods crustaceans formed the major prey items. Dominance of copepods, amphipods, ostracods, crustacean eggs and caridean shrimps in the diets of Gulf pipefish, *S. scovelli* and dwarf seahorse, *H. zosterae* inhabiting in seagrass beds of Tampa Bay, Florida has been reported by Tipton and Bell[11]. Predominance of crustaceans in the diet of *S. biaculeatus* is comparable with the diet composition studies of other syngnathid fishes. Apparent differences in prey composition within crustaceans group observed in the guts of *S. biaculeatus* and other syngnathid fishes might be due to snout length, gape size, habitat and localities.

Habitat of syngnathid fishes has been reported to play a significant role in feeding behaviour and diet composition[46]. Teixeira and Vieira[47] reported that almost entire diet of southern pipefish, *S. folletti* consisted of crustaceans, particularly copepods, amphipods, ostracods and mysids. Interestingly, isopods along with copepods and amphipods were the dominant preys of same pipefish species inhabiting a Widgeon grass bed in the estuarine zone of the Patos Lagoon, southern Brazil[24] which were conspicuously absent in the previous study by Teixeira and Vieira[47]. Obvious difference in the diet composition of same species is reflected to the differences in habitats (open waters *vs* Wadgeon seagrass beds). During present study, all individuals of *S. biaculeatus* were collected from seagrass beds of single locality. Therefore, further studies are required with individuals collected from different habitats for upgrading the relationship between habitat and and diet composition in *S. biaculeatus.*

In this study, sand particles, pieces of algal matter and foraminifera were consistently observed in guts of *S. biaculeatus* (~10%). Occurrence of sand particles and algae in the guts of seahorses and pipefishes have been widely reported[10,13,23,24]. As mentioned previously, *S. biaculeatus* is permanent resident of seagrass beds which are mostly developed on sandy bottom of coastal sea. Therefore, it is possible that while attacking epiphytic prey growing on seagrass blades or feeding on sandy bottoms, sand particles, pieces of seagrass leaves and other algae might have also been sucked along with prey organisms.

Availability of food supply in the environment is very important for fish to meet their nutritional requirement in order to grow and survive. Abundance and composition of crustacean species in the ecosystem has been reported to influence the dominant prey items of syngnathid fishes[14]. In the present study, micro-crustaceans dominated (~90%) the prey organisms in *S. biaculeatus* which is similar to other syngnathid fishes. Coastal marine waters along the east coast of India support a greater abundance and rich biodiversity of micro-crustacean species[48,49]. In addition, a variety of prey organisms (larval fishes, shrimps, isopods, cumacea and mysids) have also been reported from the region in greater abundance[48]. Therefore, it appears that the natural diet composition of *S. biaculeatus* with dominance of micro-crustaceans more or less correlate with the composition of prey items in natural environment. Further studies delineating the differences in prey composition based on habitat, season, snout morphology etc are required for better understanding.

In conclusion, the results of the study provided first hand information for better understanding of natural feed preference by *S. biaculeatus*. Such information is vital for development of strategies for sustainable management of natural stocks, conservation measures and development of appropriate diets for further breeding and rearing programmes in captivity. This information is particularly important for such IUCN red list ‘Data Deficient’ species for understanding its biology as well as interactions between alligator pipefishes and its critical seagrass habitat.

Acknowledgements

Authors are grateful to the Director, CSIR-National Institute of Oceanography, Goa (India) and Goa University, Goa (India) for facilities and encouragement. Thanks are also due to the Principal Chief Conservator of Forests, Chennai and Chief Wildlife Warden, Ramanathapuram district, Tamil Nadu for necessary permissions. This is NIO contribution no. 5923.

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