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Multiple-choice feeding preference assay on two sea urchin species from the Gulf of Mannar, South East coast of India

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A multiple-choice feeding experiment was conducted on the two sea urchins, *viz*., *Salmacis virgulata* and *Temnopleurus toreumaticus,* in four feeding assays with 12 varieties of seagrass and seaweed. The results of the study have revealed that the species *Caulerpa peltata*, *C. racemosa*, *Kappaphycus alvarezii* and *Padina tetrastromatica* occupied the top feeding positions in the descending order for *S. virgulata*. But for *T. toreumaticus*, the order of preference was observed to decrease sequentially for *C. peltata*, *Cymodocea serrulata*, *C. racemosa* and *Syringodium isoetifolium*. This study further revealed that there was a leaning preference towards seagrass species in *T. toreumaticus* which was conspicuously absent in *S. virgulata*. This kind of studies would pave way to better understand sea urchin ecology and its urchin barren phenomenon.

[**Keywords**: Gulf of Mannar, Seagrass, Sea urchins, Seaweeds]

Introduction

Sea urchins are widely distributed throughout the world's oceans and often play major roles in controlling macroalgal populations and structuring the shallow subtidal communities[1]. The diets of these echinoderms consist largely of seaweed, but they also feed on microalgae, detritus, a variety of drift items, and small invertebrates[2,3]. The ecological roles of sea urchins worldwide are changing due to large-scale commercial harvests that reduce average body size and population density. Sea urchin grazing on reefs is important to control the population of macroalgae, which when dominant can result in "urchin barren" grounds. As sea urchin density can have such a significant effect on coral reefs through bioerosion, their population distribution and the subsequent impact on coral reefs are particularly important to understand[4].

The Gulf of Mannar is the richest marine biodiversity hotspot along the Southeast coast of India, encompassing the territorial waters from Dhanushkodi in the north to Kanyakumari in the south. It has a chain of 21 islands, located 2 to 10 km from the mainland along the 140 km stretch between Rameswaram and Thoothukudi. The area of the Gulf of Mannar under the Indian EEZ is about 15,000 km^2, where commercial fishing takes place only on about 5,500 km^2 and that too, only up to a depth of 50 m. A wide variety of fishing gear and crafts are used along the Gulf of Mannar coast for the exploitation of various pelagic and demersal fishery resources. Sea cucumbers and sea urchins are among the echinoderms that are commercially exploited around the world. In India, sea urchins are typically discarded by fishermen, and some sea urchin test is used in the souvenir industry. In the Gulf of Mannar region, four species of sea urchins are used as ornamentals and curios[5].

Materials and Methods

Two sea urchin species, *S. virgulata* and *T. toreumaticus* (Fig. 1), were collected from the Mandapam coast of the Gulf of Mannar and used in this study. A mini cage structure (Fig. 2) was designed with a slight modification to specifications given by Prince *et al*.[6] with an individual compartment (15×15 cm square cage) to avoid predation among individuals and a 1 mm mesh bottom to keep individuals and food in while allowing waste to pass through. This mini cage was set up in a one-ton FRP tank with adequate aeration and continuous water flow-through at a rate of 50 L/hr. In the plastic assemblies, five mini cage structures were attached in each row. 20 numbers juveniles of

S. virgulata and *T. toreumaticus* in the size range of 36.75±3.7 mm and 28.35±3.3 mm was used in each experiment. Sea urchins are herbivores in principle, but have feeding plasticity. In this study, both seaweed and seagrass species were tested for their preference by both species of sea urchin.

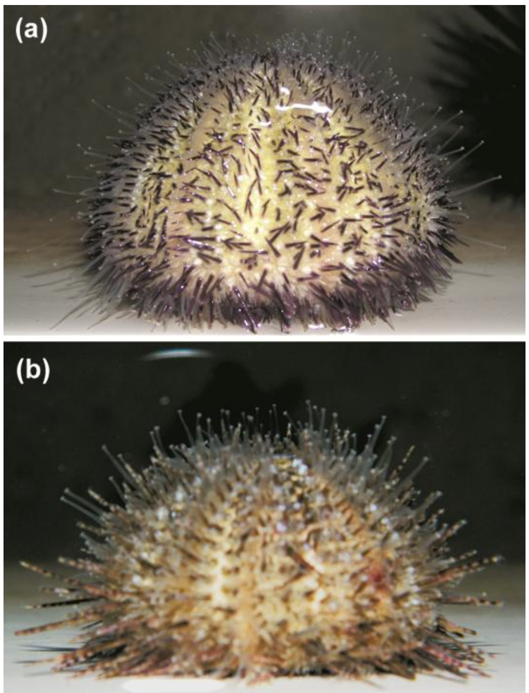

Fig. 1 — Two species of sea urchins [(a) *S. virgulata*, and (b) *T. toreumaticus*]

The sea urchins were starved for three days after collection in order to put these animals at their best feeding efficiency. Behaviourally, sea urchins are nocturnal animals; hence each feeding assay was performed overnight. In addition to that, there was a window of three days given between each feeding assay for both the species of sea urchins. In the first assay, three species of seaweeds *Caulerpa peltata, Halimeda gracilis, and Sargassum polycystum* and in the second assay *Ulva lactuca, Padina tetrastromatica and Caulerpa scalpelliformis*, and in the third assay *Kappaphycus alvarezii, Codium adhaerens and Cymodocea serrulata* and in the fourth assay *Caulerpa racemosa, Gracilaria corticata and Syringodium isoetifolium* were tested for the preference assay (Fig. 3). Among the seaweeds used *C. peltata, C. racemosa, C. scalpelliformis, C. adhaerens, H. gracilis* and *U. lactuca* were green seaweeds. The seaweeds *G. corticata* and *K. alvarezii* are red seaweeds. *P. tetrastromatica* and *S. polycystum* are brown seaweeds. The species *S. isoetifolium* and *C. serrulata* are seagrasses. Each feeding assay was started in the evening and continued for 12 hours period. 25 g of each feed material was given after dewatering 20 replicated animals at the start of the assay and after 12 hours the remaining feed material was weighed by pressing them in a tissue paper to remove excess water and to arrive at the quantity of feed material consumed over

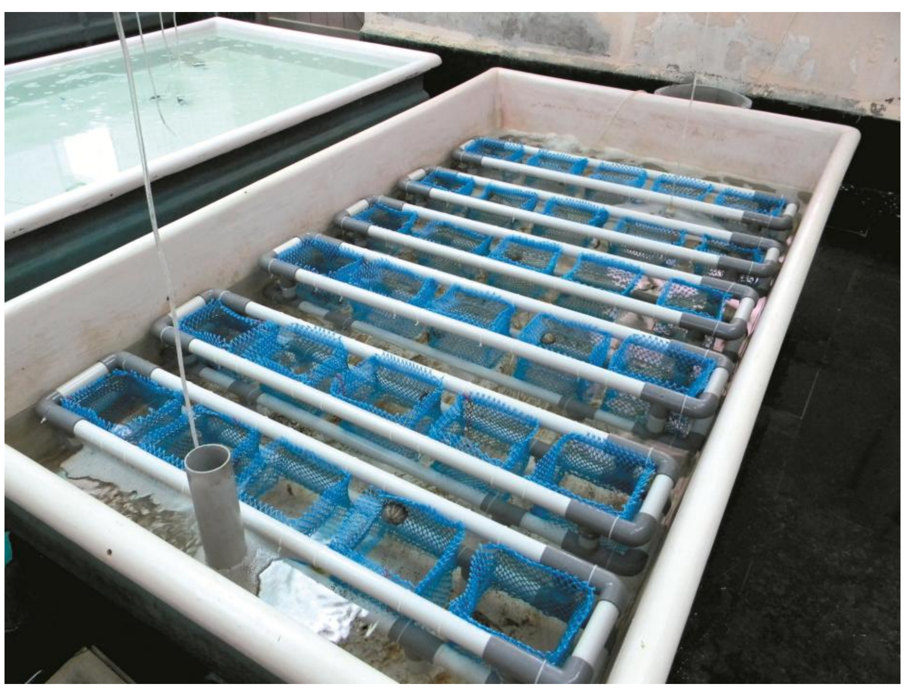

Fig. 2 — Mini experimental cage structure

Fig. 3 — Seaweeds and seagrass species used in the four experiments

this period. The percentage of feeding was calculated based on this analysis. The next feeding assay was started after starving the animals for 3 days. These feeding assays were run simultaneously for both the species of sea urchin with a similar set of fresh feed material. In each feeding assay, a combination of red, brown, green and seagrass species was offered to simulate the natural environmental condition where more than one species could be found in the grazing field. The data of the experiment was analyzed non-parametrically by Friedman test, treating the sea urchins as replicates in all the experiments to know the qualitative preference for different seaweeds and seagrass species and the results was box plotted.

Results

Among the six different green seaweed species offered *viz*., *C. peltata, C. racemosa, C. scalpelliformis, C. adhaerens, H. gracilis* and *U. lactuca* in four feeding assays, the top most preferred species was *C. peltata* followed by *C. racemosa* and *C. scalpelliformis* by both the sea urchin species. Among the two different red seaweeds offered in the feeding choice study, the sea urchin species *S. virgulata* showed a greater preference for *K. alvarezii* than *G. corticata;* however, this was vice-versa in the case of *T. toreumaticus*. Among the brown seaweeds offered in the study, both the sea urchin species have shown a greater preference for *P. tetrastromatica* compared to *S. polycystum*. In the third and fourth feeding assays, two seagrass species were added along with other seaweeds, and the results indicated *C. serrulata* was the choicer feed than *S. isoetifolium*. This analysis has been done after grouping them into the major natural groups.

When the overall consumption percentage of macroalgal feed offered in all the four feeding assays was compared (Figs. 4 & 5); the species *C. peltata*, *C. racemosa*, *K. alvarezii* and *P. tetrastromatica* occupied the top preference in the descending order for *S. virgulata*. But for *T. toreumaticus*, the feeding preferences when compared the order of preference decreased sequentially for *C. peltata*, *C. serrulata*, *C. racemosa* and *S. isoetifolium*. This study revealed that there was a leaning preference for seagrass species in the sea urchin *T. toreumaticus* which was conspicuously absent in *S. virgulata*.

Discussion

Animals are constantly making choices in response to their environment, whether those choices are related to food, habitat, or reproduction. In general, aquatic animals make choices, often simultaneously. It is assumed that certain factors have more weightage in the choice-making process than others[7]. In the present study, a multiple-choice feeding assay has been designed in which two species of sea urchins were offered a choice among ten different seaweed and two seagrass species.

Salmacis virgulata

The green seaweeds *C. peltata*, *C. scalpelliformis* and *C. racemosa* were the most sought-after food items by *S. virgulata* in the first feeding when offered 10 seaweed species and two seagrass species in various combinations in four feeding assays. In the second feeding assay, when green seaweed *C. scalpelliformis and* brown seaweed *P. tetrastromatica* were present, the sea urchin *S. virgulata* showed an equal preference for both the seaweeds. However, the preference for another green seaweed, *U. lactuca* was abysmally low. In the third and fourth feeding assays, when seagrass species *C. serrulata* and *S. isoetifolium* were offered along with seaweed species, the preference for seaweed species was comparatively higher than the preference for seagrass species. The present observation revealed that the sea urchin *S. virgulata* leaned more towards

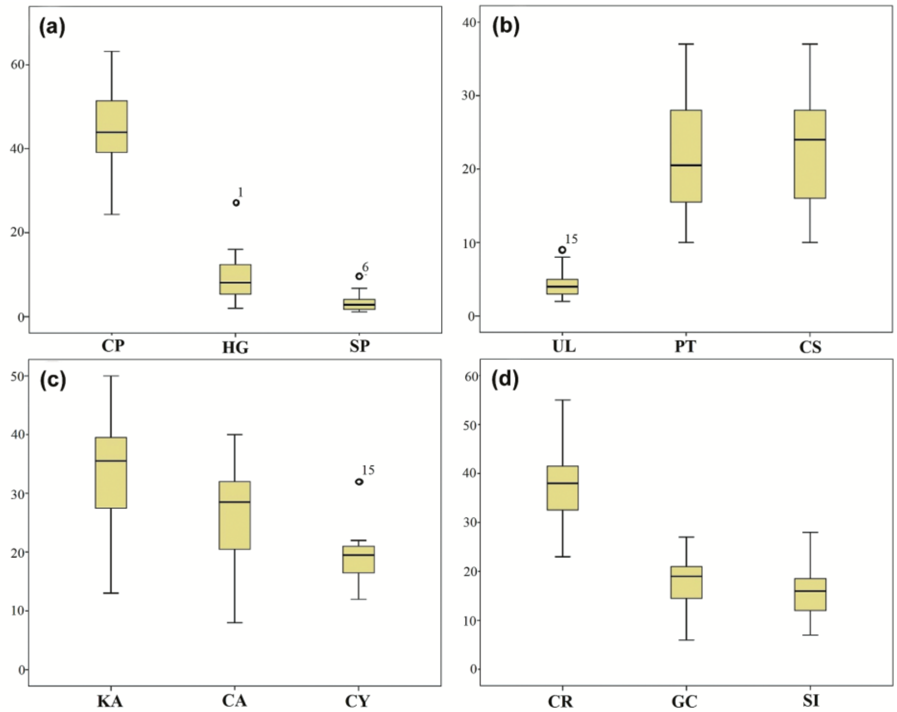

Fig. 4 — Box plot on multiple choices feeding preference experiment involving *S. virgulata* with sea grass and seaweed species in four different experiments: (a) Assay 1 - *Caulerpa peltata* (CP), *Halimeda gracilis* (HG), *Sargassum polycystum* (SP); (b) Assay 2 - *Ulva lactuca* (UL), *Padina tetrastromatica* (PT), *Caulerpa scalpelliformis* (CS); (c) Assay 3 - *Kappaphycus alvarezii* (KA), *Codium adhaerens* (CA), *Cymodocea serrulata* (CY); and (d) Assay 4 -*Caulerpa racemosa* (CR), *Gracilaria corticata* (GC), *Syringodium isoetifolium* (SI)

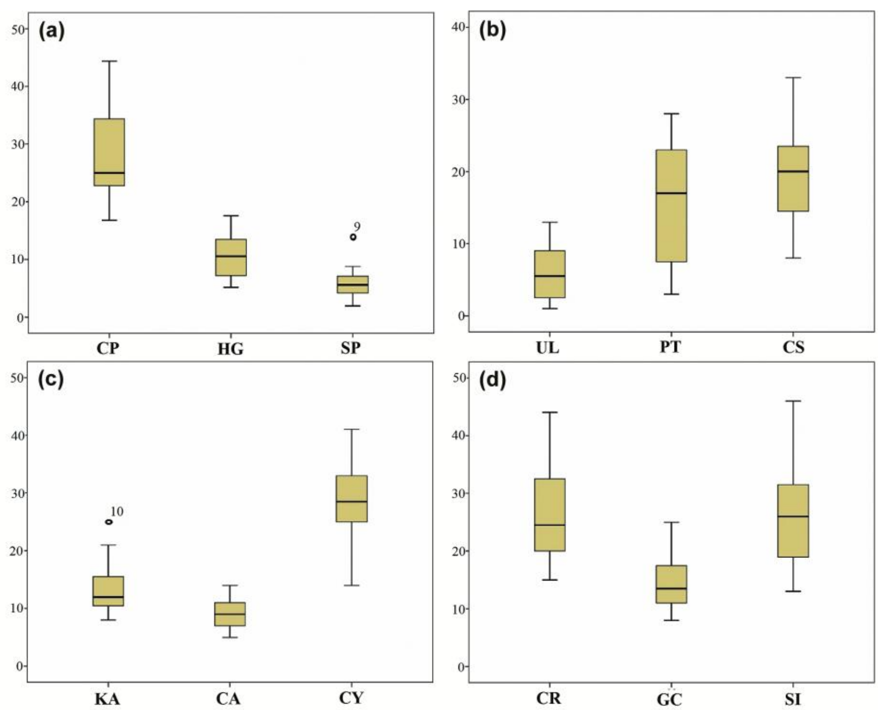

Fig. 5 — Box plot on multiple choices feeding preference experiment involving *T. toreumaticus* with sea grass and seaweed species in four different experiments: (a) Assay 1 - *Caulerpa peltata* (CP), *Halimeda gracilis* (HG), *Sargassum polycystum* (SP); (b) Assay 2 - *Ulva lactuca* (UL), *Padina tetrastromatica* (PT), *Caulerpa scalpelliformis* (CS); (c) Assay 3 - *Kappaphycus alvarezii* (KA), *Codium adhaerens* (CA), *Cymodocea serrulata* (CY); and (d) Assay 4 - *Caulerpa racemosa* (CR), *Gracilaria corticata* (GC), *Syringodium isoetifolium* (SI)

feeding on seaweed than seagrass. The green seaweed, *C. peltata* was the most preferred food item when the entire feeding assays were compared. The exotic seaweed *K. alvarezii* consumption was highest in the third feeding assay in spite of the presence of green seaweed *C. adhaerens*. This observation is comparable to that of Kitching & Thain[8], who stated that the *Codium* sp. was consumed by the sea urchin if its preferable seaweed, *Ulva* sp., was missing from the grazing ground. However, in the present experiments, *U. lactuca* and *S. polycystum* were the least preferred food items.

Temnopleurus toreumaticus

T. toreumaticus preferred green seaweeds, *C. peltata*, and two seagrass species, *C. serrulata* and *S. isoetifolium*, when presented with ten seaweed species and two seagrass species in various combinations in four feeding assays. In the second feeding assay, when green seaweed *C. scalpelliformis and* brown seaweed *P. tetrastromatica* were present, the sea urchin *T. toreumaticus* showed a higher preference for green seaweed *C. scalpelliformis*, but the preference for another green seaweed, *U. lactuca* was abysmally low. When seagrass species like *C. serrulata* and *S. isoetifolium* were offered alongside seaweed species in the third and fourth feeding assays, the preference for seagrass species was comparatively higher than the preference for seaweed species. This observation was diametrically opposite to that of *S. virgulata*. The current observation revealed that the sea urchin *T. toreumaticus* preferred to feed on sea grasses rather than seaweed. The green seaweed *C. peltata* and seagrass species *C. serrulata* were the most preferred food items when all the feeding assays were compared. The exotic seaweed *K. alvarezii* consumption was not as high as the level of consumption of *S. virgulata*. In the case of *T. toreumaticus*, *U. lactuca* and *S. polycystum* were found to be the least preferred food items. It is presumed that the protein content in seaweed is one of the important criteria for preference. A protein-rich diet is generally used to replenish the gonads of sea urchins to store energy[9].

In the habitats, where the predation and crevice space are not limited in the ecosystem, food availability is reported to govern the reef's carrying capacity of sea urchin populations[10]. Within habitat, sea urchin diets are reported to be diverse, with species of *Diadema* feeding extensively on *Thalassia* sp., *Syringodium* sp., and other brown and green algae[11]. Coppard & Campbell[12] reported that algal and seagrass species distributions in the reefs of Fiji appeared to have only a moderate influence on echinoid species distributions, as most species' diets appeared broad within a habitat. The food preference of *Arbacia punctulata* carried out by Hay *et al*.[13] revealed that when offered single species of seaweed in excess amounts, the sea urchin *A. punctulata* consumed significantly more *Gracilaria foliifera* and *Codium isthmocladium* than *Sargassum filipendula*, *Padina vickersiae* or *Dictyota dichotoma*. When all the above five species were offered to urchins simultaneously, patterns of palatability remained the same but absolute consumption of favoured species decreased significantly while consumption of less palatable species remained unchanged. Similar results were obtained in the present multiple-choice feeding assay for *S. virgulata* and *T. toreumaticus*.

Low food value species of seaweed are generally not much sought after by the sea urchins[14], and these species may have anti-nutritional factors to avoid being grazed, whereas high preference foods may not evoke such a response and thus may continue to be eaten. Some species of *Sargassum* produce polyphenols which may repel sea urchins. However, *Padina* is not known to produce unusual secondary metabolites[15]. The Mediterranean urchin, *Paracentrotus lividus* consumes less palatable species such as *Codium fragile* and encrusting algae when resources are limited[16]. In laboratory experiments, species of *Sargassum* and *Dictyota* are avoided by *A. punctulata*, presumably due to secondary metabolites, while species of *Gracilaria* and *Hypnea* are considered preferred foods[17]. Seaweeds and seagrass varieties used in the multiple-choice feeding assay could be grouped into three categories, *viz*., highly preferred, least preferred, and intermediate level preferred (Table 1). *C. peltata* and *C. racemosa* were the highly preferred seaweeds by the sea urchin species, and *H. gracilis*, *U. lactuca* and *S. polycystum* were the least preferred food items in the feeding assay for both the sea urchin species. Further studies conducted at Mandapam Regional Centre of CMFRI under the scope of the present investigation revealed that *S. virgulata* tends to consume large quantities of animal matter, while *T. toreumaticus* primarily consumes seagrass and seaweed. So, it can be inferred from the present investigation that inter-specific competition is therefore unlikely, except under conditions of extremely low floral availability.

Table 1 — Categories of preference of seaweeds and sea grass species in multiple choicesfeeding assay (SV - *Salmacis virgulata*, TT - *Temnopleurus toreumaticus*)

Sl. No	Seaweed/ Sea grass species	Highly preferred		Intermediate preferred		Least preferred	
		SV	TT	SV	TT	SV	TT
1	*Caulerpa racemosa*	■	■				
2	*Caulerpa peltata*	■	■				
3	*Caulerpa scalpelliformis*	■			■		
4	*Halimeda gracilis*					■	■
5	*Ulva lactuca*					■	■
6	*Codium adhaerens*	■					■
7	*Sargassum polycystum*					■	■
8	*Padina tetrastromatica*	■			■		
9	*Kappaphycus alvarezii*	■			■		
10	*Gracilaria corticata*			■	■		
11	*Cymodocea serrulata*		■	■			
12	*Syringodium isoetifolium*		■	■			

Table 2 — Seasonal variation in protein content (mg/g DW) of seaweeds from Hare Island off Tuticorin - reported by Lobo[29]

Seaweeds	Pre-monsoon	Monsoon	Post-monsoon
U. latuca	60.30 ± 1.36	68.20 ± 0.30	69.30 ± 4.54
C. tomentosum	32.10 ± 0.05	22.8 ± 3.20	37.43 ± 1.38
C. racemosa	26.60 ± 0.54	36.10 ± 0.29	43.42 ± 0.02
C. scalpelliformis	25.80 ± 0.98	32.5 ± 1.17	34.87 ± 1.38
H. tuna	7.46 ± 3.17	3.13 ± 0.63	5.28 ± 0.22
P. tetrastromatica	33.37 ± 1.21	48.2 ± 2.31	58.00 ± 4.18
S. polycystum	15.21 ± 2.36	21.96 ± 0.03	21.00 ± 0.91
G. corticata	47.55 ± 1.69	45.00 ± 2.39	55.29 ± 0.56
K. alvarezii	42.71 ± 0.39	48.20 ± 0.02	42.50 ± 2.54

The carbohydrates present in marine algae are different from those in higher land plants, and in addition, high protein content has been reported[18]. The seaweeds show great variation in the nutrient content from species to species, their geographical distribution, seawater temperature, salinity, light and nutrients[19]. The Indian coastline is endowed with 844 species of marine algae, comprising 216 species of chlorophyta, 191 species of Phaeophyta, 434 species of Rhodophyta and 3 species of Xanthophyta[20].

Jayasankar[21] studied the protein content of *Sargassum wightii* from the Pudumadam area and reported that the protein content varies from 3 to 7 percent. Kaliaperumal *et al*.[22] investigated the biochemical composition of Lakshadweep seaweeds and discovered that green algae had the highest protein content (18.9 %), followed by red algae (13.1 %) and brown algae (12.2 %). Murugaiyan *et al*.[23] reported that the maximum value for protein was found in *Caulerpa racemosa* (24.55±0.84) followed by *Caulerpa taxifolia* (23.78±0.37) and *Sargassum wightii* (16.59±0.86). The protein content in *Ulva lactuca* was recorded as high as 20 % by Kahiry & El-Shafay[24] from the Egyptian coast. Manivannan *et al*.[25] reported that the protein content of *Halimeda macroloba* and *Padina pavonica* was in the range of 28.94±0.68 % and 13.63±0.43 %, respectively. Kokilam *et al*.[26] reported that the protein content of *Padina tetrastromatica* to be in the range of 11.39±0.02 % from the Mandapam region. Paiva *et al*.[27] reported that the total protein content in *Codium adhaerens* in the range of 18.48±0.08 percent of dry weight from the Portugal waters. Athiperumalsami *et al*.[28] analyzed the seagrasses of the Gulf of Mannar and found that the protein content was in the range of 3.5±0.025 mg/g and 11.38±0.097 mg/g for *Cymodocea serrulata* and *Syringodium isoetifolium*. The multiple-choice feeding assay clearly shows that the protein richness of the macroalgae is an important criterion for its preference to both sea urchin species, which invariably require it for growth and gametogenesis. According to Lobo[29], the protein content of macroalgae along the Gulf of Mannar increases several folds after the monsoon (Table 2). These results reinforce the present findings that after the peak spawning season in September and October; the sea urchin foraging begins in the post-monsoon period until the appearance of the spawning period.

From this understanding, it is clear that the reproductive periodicity in the Gulf of Mannar is more dependent on the nutrient content of foraging algae and seagrass during the post-monsoon than on any other physicochemical factor.

Conclusion

The multiple-choice feeding preference experiment was designed to assess the feeding preference of sea urchins. When the overall consumption percentage of macroalgal feed offered in all the four feeding assays was compared, the species *Caulerpa peltata*, *C. racemosa*, *Kappaphycus alvarezii* and *Padina tetrastromatica* occupied the top feeding preference in the descending order for the species of sea urchin *S. virgulata*. But for the sea urchin species *T. toreumaticus*, the order of preference decreases sequentially from *C. peltata*, *Cymodocea serrulata, and C. racemosa* to *Syringodium isoetifolium*. This study revealed that there was a leaning preference for seagrass species in the sea urchin *T. toreumaticus* which was conspicuously absent in *S. virgulata*. From this understanding, it is clear that the reproductive periodicity in the Gulf of Mannar is more dependent on the nutritional content of foraging algae and sea grasses than on any other physicochemical factor. Further studies in this direction are required to understand the feeding strategy in the wild of sea urchin and to know its trophic interaction and its ecosystem services.

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Conflict of Interest

The authors declare no competing interests.

Ethical Statement

All of the experimental procedures involving sea urchins were conducted in an ethically responsible manner.

Author Contributions

The first author (RS) planned and executed the work and prepared the manuscript under the guidance and supervision of second author (PJ).

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