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Gut content analysis in *Holothuria leucospilota* and *Holothuria cinerascens* (Echinodermata: Holothuroidea: Holothuriidae) from Karachi coast

Quratulan Ahmed[1,*], Qadeer Mohammad Ali[1], Levent Bat[2], Ayşah Öztekin[2], Farhana Shafiq Ghory[1], Iqra Shaikh[1], Hafsa Qazi[1] and Ateeqa Baloch[1]

[1] *The Marine Reference Collection and Resources Centre, 75270, Karachi, Pakistan*
[2]*Sinop University, Fisheries Faculty, Department of Hydrobiology, 57000, Sinop, Türkiye*
**Corresponding author, Email address: quratulanahmed_ku@yahoo.com*

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Abstract: Holothurians are a prominent group of deposit feeders, they play an important role in sediment bioturbation in intertidal ecosystems. The sea cucumber *Holothuria leucospilota* (Brandt, 1835) and *Holothuria cinerascens* (Brandt, 1835) are an epibenthic deposit feeding species that mainly inhabits in intertidal region. Composition and diversity of microorganisms in the digestive tract of both species have scrutinized. Gut content analysis is a method that is used to study the diet and feeding habits of organisms. In the case of *H. leucospilota* and *H. cinerascens*, which are two species of sea cucumber, studying the gut content can provide important information about the role that these animals play in the ecosystem. This study may provide evidence about the feeding strategy of these animals, which may help reveal the association between their gut content and environmental habitat. Overall, studying gut content analysis in these selected species is important because it can provide valuable insights into the feeding habits and ecological roles of these animals, which can help to improve our understanding of marine ecosystems and the importance of sea cucumbers within them. Analyzing the gut content of these sea cucumbers can help to identify the types of food that they are consuming and how they are obtaining it. This information can be used to understand the ecological roles that these animals play and how they interact with other organisms in the ecosystem.

1. Introduction

Sea cucumbers are found in all marine environments throughout the world, from shallow to deep-sea environments. They are found in tropical and temperate zones, from the shallow waters to the deep sea, regardless of whether the rocks are sandy, muddy, or reef. They are fundamental component of marine ecosystems, serving a variety of tasks such as improving the sediment health, nutrient recycling, Influence on local water chemistry etc. (Velimirov *et al.*, 1977; Anderson *et al.*, 2010; Purcell *et al.*, 2016). *Holothuria leucospilota* are tropical sea cucumber widely distributed tropical in the Indo-Pacific region near boulders, corals, and seaweed clusters. This species is usually tolerant of salinity and temperature discrepancy and is commonly ascertained in between the rocky areas. It is also tolerant of long-term air exposure, and may be present along the shore (Iliyas, 2010). Also they play a vital role in near shore and marine ecosystem as sediment transporters and seabed scavengers, swallowing and passing a large amount of sand through their gut (Bonham and Held, 1962; Dzeroski and Drumm, 2003). The gut of *H. leucospilota* may take up the organic carbon present in organic carbon-rich sediments, conserve the constancy of seawater acid levels and the stability of coral reef ecosystems (Bonham and Held, 1962; Schneider *et al.,* 2011). Usually, the *H. leucospilota* can be around 30-40 cm long and can grow to be about up to 1m long. Body soft, long and cylindrical with no visible underneath or upper side. The colours are evenly dark brown or maroon to black having 20 large bushy tips feeding tentacles that positioned downwards It collects food scraps from the ground (Lane *et al.,* 2003). Cuvierian tubules are thin sticky white cylindrical tubes that can be released from its backside when it is disturbed. *Holothuria cinerascens* are cylinder-shaped purplish red-brown sea cucumber with a delicate, strong body wall and 20 tentacles (Smith, 1977). *H. cinerascens* are ectotherms and osmo-conformers species (Wang *et al.*, 2008; Zamora and Jeffs, 2012; Kamyab *et al.*, 2016) those live on rocky shores, where they are unevenly exposed to direct sunlight (James, 1982). This species lives in the outer reefover hard substratum, in crevices with strong wave action they extended their tentacles for organic particles suspensions from the water columns (Purcell *et al.,* 2012). Because of their suspension-feeding method and respiratory process, *H.cinerascens* may be susceptible to multiple microplastics absorption (Zamora and Jeffs, 2012; Purcell *et al.*, 2016; Iwalaye *et al.,* 2020). Recently Jia et al., (2022) reported comparative analysis of In situ eukaryotic food sources in three tropical sea cucumber *Stichopus monotuberculatus*, *S. chloronotus* and *Holothuria atra* species by Metabarcoding. An assessment of the gut eukaryotic community among the three species suggested that the feeding preference was different: *S. monotuberculatus* fed mainly on Diatomea and Arthropoda, and the other two species had higher *Apicomplexa* concentrations, which may be due to differences in the morphology of the tentacles and habitat preferences. Elakkermi *et al.,* (2021) studied the gut content of *Parastichopus regalis* from the west Algerian coast. The selected species preferred for very fine sediment particles that have high organic matter content. The residual part of its diet is constituent of a faunal fraction (foraminifera, annelids, fragments of mollusc shells, sponge and echinoderm ossicles, and undetermined fauna) and a floral fraction (diatoms and cyanobacteria).

Thus, the aim of the present study to investigate the gut content in *Holothuria leucospilota* and *Holothuria cinerascen* from Cape Monze beach Karachi coast. This information can be used to understand the ecological roles that these animals play and how they interact with other organisms in the ecosystem. In addition, studying the gut content of sea cucumbers can also help to identify any changes in their diet over time, which could be an indication of changes in the ecosystem or environmental conditions.

2. Methodology

Total twenty individuals of both *Holothuria leucospilota* (Fig. 1a) and *Holothuria cinerascen*(Fig. 1b) were collected from intertidal area of Cape Monze Beach (24°50'02''N66°39'24''E) (Fig. 2) during the low tide in September 2021 to March 2022. Physico-chemical parameters (water temperature, salinity, and pH) were recorded. After collection of all individuals were quickly transferred to laboratory to avoid evisceration (because to stress). Each individual of holothurian species was taken separately in a polyethylene bag. Fresh length and weight of specimens were taken in (cm) and (g). For taxonomic studies of species, morphological features were examined, ossicles were taken from three positions, dorsal, ventral body walls, and tentacles, a small skin of tissues were placing on a slide and adding a few drops of 3.5% bleach, and slides were rinsed with drops of distilled water and then were examined under a microscope at10 × 10 magnification.The gut of each individual was opened by a longitudinal incision and the digestive contents were carefully collected and placed separately in a small plastic bag containing 5 percent formalin. The gut contact method was used for the analysis of digestive contents described by Jones (1968) and Nédélec (1982).

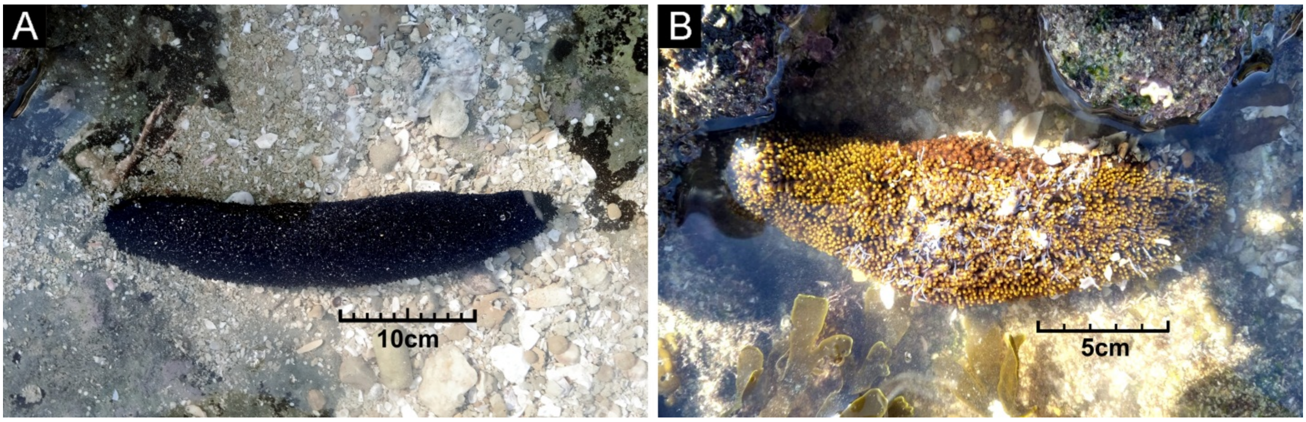

Figure 1. a. *Holothuria leucospilota, b. Holothuria cinerascens*

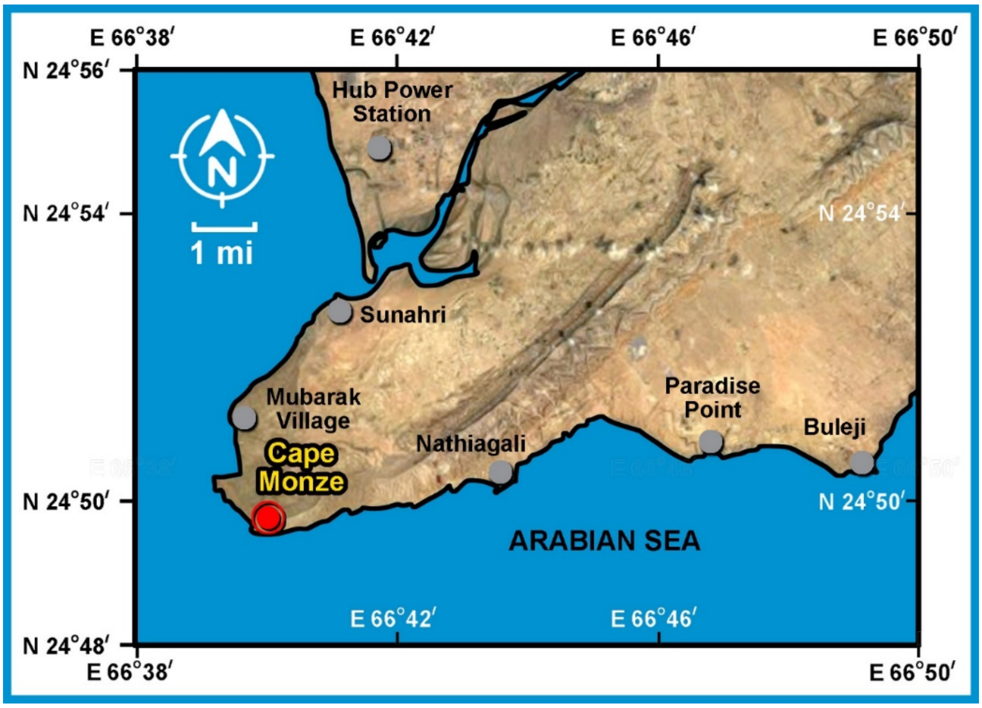

Figure 2. Map showing Sampling site Cape Monze.

The gut contents of the individuals were determined using a filter paper.The total contribution of each ingredient (digestive contents) was estimated. The sponges' ossicles, Polychaetes, mollusks shells, mollusks shell fragments, foraminifera, seaweed fragments, amphipods, copepods were identified under stereo zoom microscope (Nikon SMZ 10).ANOSIM (Analysis of similarity) and SIMPER (Similarity Percentage Analysis) were used to evaluate the differences in the prey composition of *H. leucospilota* and *H. cinerascens*. Bray-Curtis similarity was calculated on log (x + 1) transformation of the data set, the p-value was accepted as 0.05 and PAST v 4.04 software was used (Hammer *et al.,* 2001).

3. Results and Discussion

A total of twenty *H. leucospilota* and *H. cinerascens* individuals were measured, with a mean height of 31.40±4.83 and 17.80±1.98 cm, and a mean weight of 322.0±33.48 and 283±33.31 g, respectively. Highest water temperature (31°C) salinity (38ppt) was measured in October, and pH (8.2) in March. The analysis of the gut content of Holothurians shows that these sea cucumbers mostly ingest the same constituents (Fig. 3).

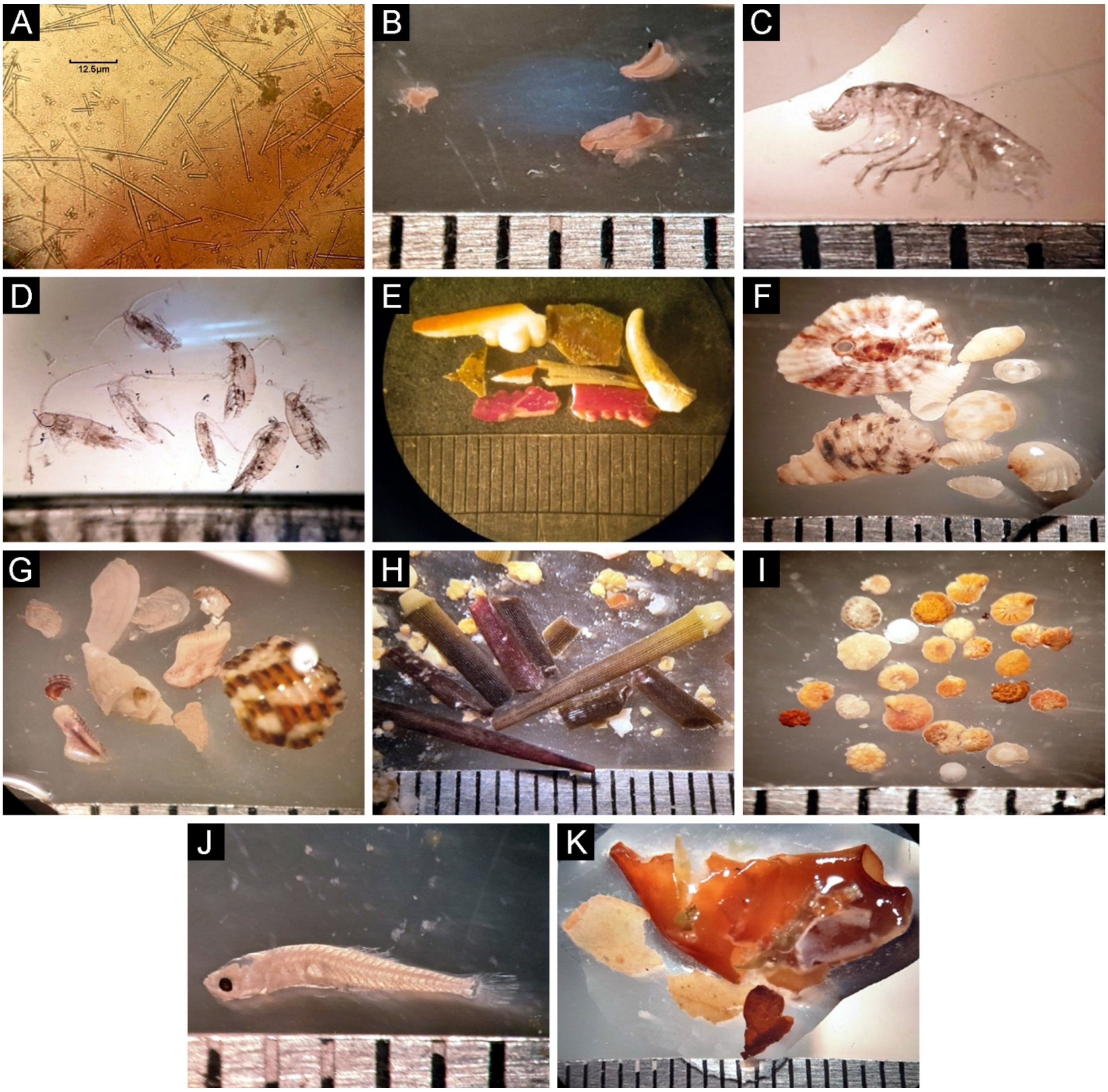

Figure 3. Gut contents of *Holothuria leucospilota* and *Holothuria cinerascens*. A. Sponge ossicles, B. Polychaetae larvae, C. Amphipod, D. Copepods, E. Crab shell fragments, F. Mollusks shells, G. Mollusksshell fragments, H. Sea urchin spines, I. Foraminifera, J. Fish larvae, K. Seaweed fragments

Total ten guts of *H. leucospilota* were analyzed, sponges' ossicles, Polychaetes, mollusks shell fragments, mollusks shells, foraminifera, seaweed fragments, amphipods, copepods, crab shells fragments, sea urchin spines fragments and fish were observed. The percentage distribution of prey groups in *H. leucospilota* was shown in (Fig. 4). Mollusks shell fragments (Msf) were observed in highest percentage in all individuals.

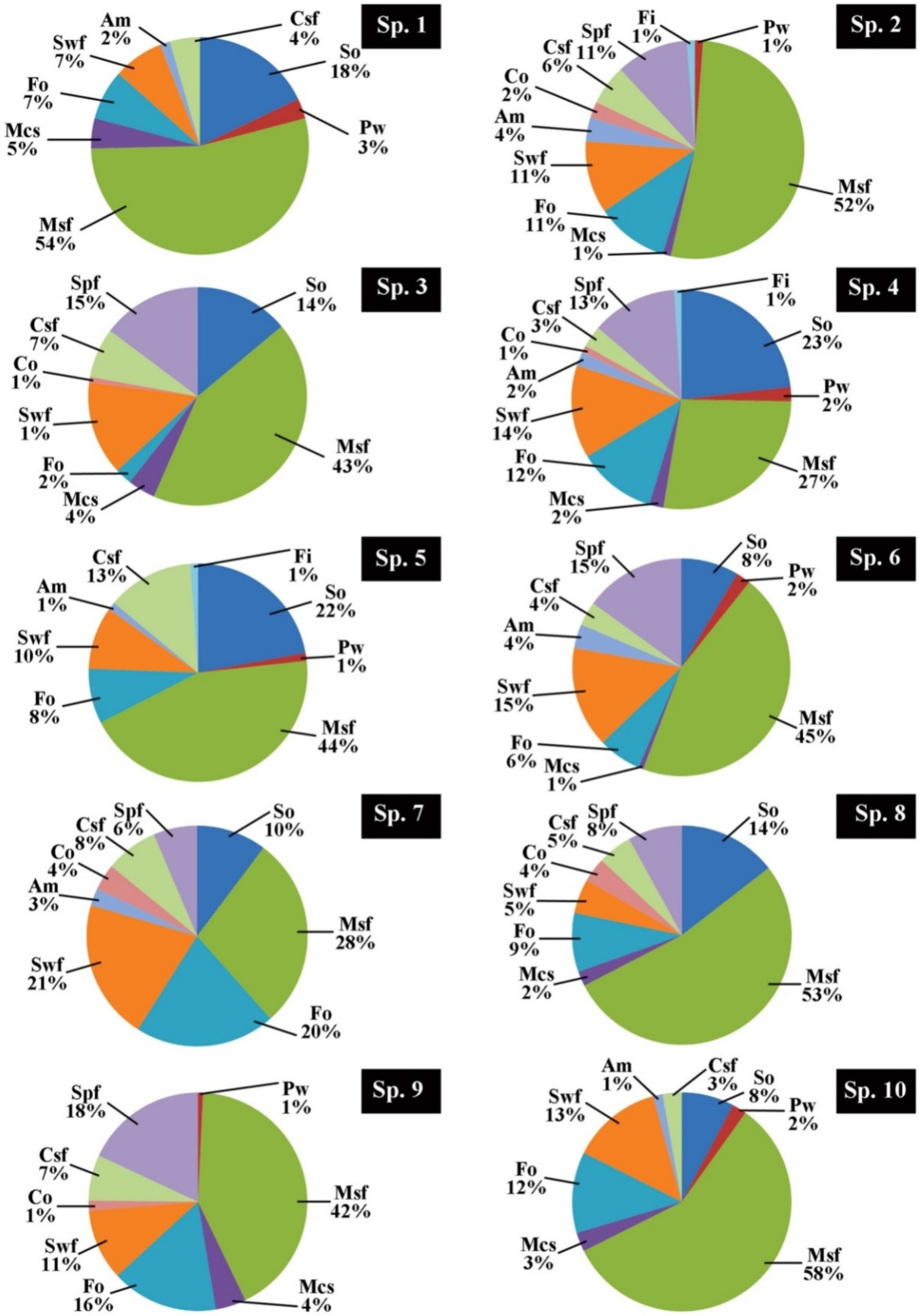

Figure 4.*Holothuria leucospilota*; So; Sponges ossicles Pw; Polychaetes, Msf; Mollusks shell fragments, Mcs;Molluscksshells, Fo; Foraminifera, Swf; Sea weed fragments, Am; Amphipods, Co; Copepods, Csf; Crab shells fragments, Spf; Sea urchin spines fragments and Fi; Fish.

Sponges' ossicles, Polychaetes, mollusks shells,mollusks, shell fragments, foraminifera, seaweed fragments, amphipods, copepods, crab shells fragments and sea urchin spines fragments were observed in analyzed ten guts contents *H. cinerascen.* The percentage distribution of prey groups in *H.*

cinerascen was shown in (Fig. 5). In *H. cinerascen,* also molluscan shell fragments (Msf) were observed in highest (%) in all individuals.

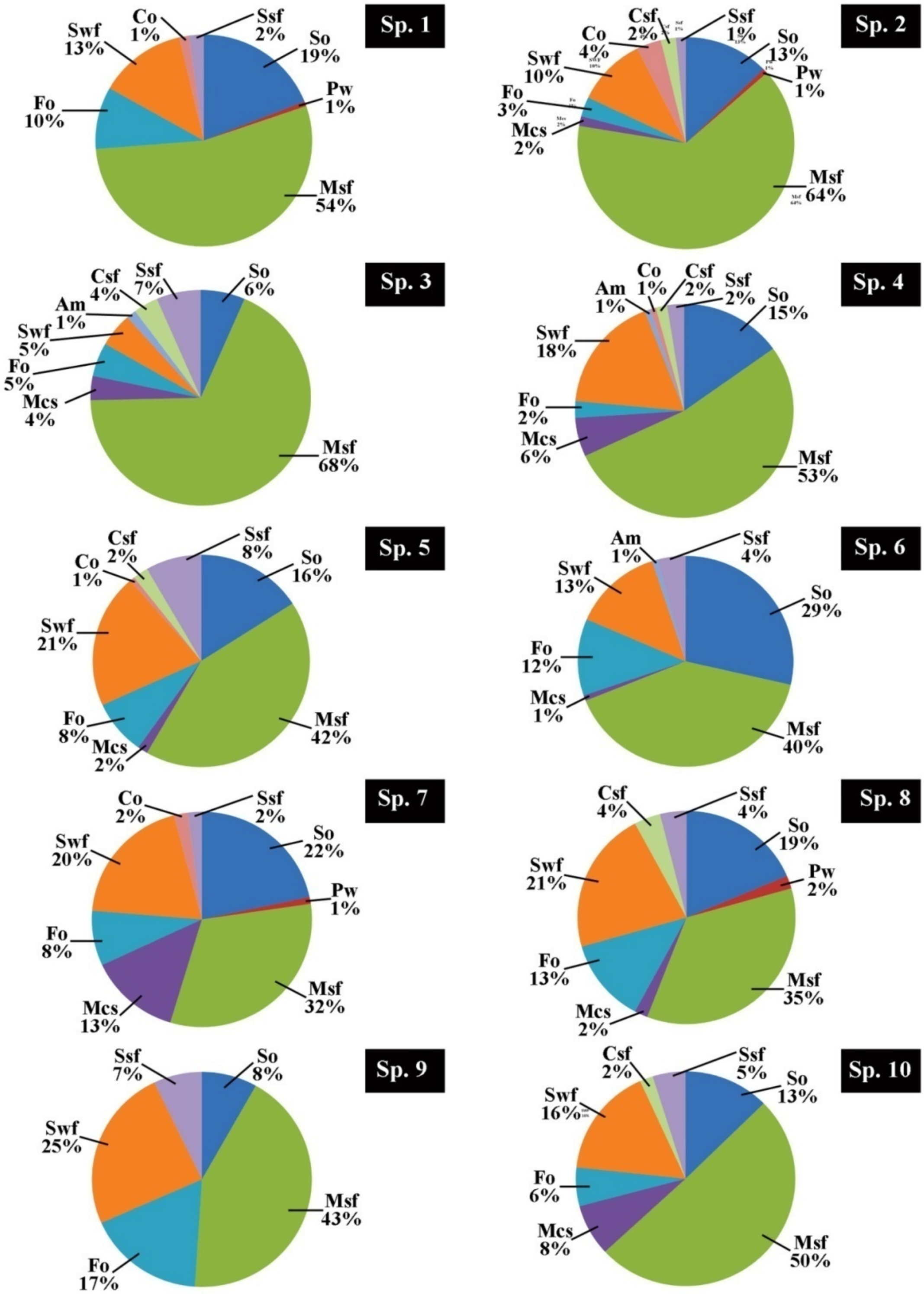

Figure 5. *Holothuria cinerascens*; So; Sponges ossicles Pw; Polychaetes, Msf; Mollusks shell fragments, Mcs; Mollusks shells, Fo; Foraminifera, Swf; Seaweed fragments, Am; Amphipods, Co; Copepods, Csf; Crab shells fragments, and Ssf; Sea urchin spines fragments.

Usually in both species mollusks shell fragments were observed in highest quantities. The differences in diet were statistically significant in *H. leucospilota* and *H. cinerascens* (ANOSIM: R =

0.27, $p<0.05$). The dissimilarity percentage of these species was 23% and the differences between species were mainly due to the change of the seaurchin spines, crab shells fragments, sponges' ossicles, and mollusks shells according to the SİMPER analysis.

Sea cucumber plays a significant role in benthic environment by affecting the composition of sea floor sediments. The decomposition of organic matter in sediments that accumulates in tidal flats and sheltered coastal areas often causes anaerobic conditions, as dissolved oxygen depletes as organic matter decomposes (Michio*et al.,* 2003).Holothurians play an important role in sediment bioturbation in intertidal ecosystems. Bioturbation refers to the process by which organisms mix and disturb the sediment on the ocean floor. This can include things like burrowing, digging, and churning the sediment. In intertidal ecosystems, sea cucumbers are among the many organisms that contribute to bioturbation. They do this by using their tentacles to collect food from the surrounding sediment and then moving through the sediment as they feed, which helps to mix and disturb it. This bioturbation helps to keep the sediment healthy by promoting the exchange of nutrients, oxygen, and other substances between the water and the sediment. It can also help to prevent the build-up of excess nutrients, which can lead to the overgrowth of algae and other organisms.

Traditionally the gut contents have been analyzed for the rationale of determining the feeding preferences of benthic animals (The´el, 1882). Holothurians' bulk diets have been studied extensively to find out what they prefer to eat (Hauksson, 1979; Khripounoff and Sibuet, 1980; Tyler *et al.,* 1992; Manship, 1995).

In the habitat, the availability of natural food strongly affected the abundance of sea cucumbers (Hartati *et al.,* 2017). There are many diverse food sources for sea cucumbers in the complex and heterogeneous habitat. Kashio *et al.*(2016) also affirmed that sea cucumber proved varying their microhabitat and activity level seasonally and when they found a suitable one, their feeding process continued. Using gut contents collected by traditional methods (trawling/dredging), feeding strategies are determined by studying animals collected in this manner (Roberts and Bryce, 1982; Billett, 1991; Roberts and Moore, 1997; Roberts *et al.,* 2000).

H. leucospilota and *H. cinerascens* are an omnivore, filtering through the sediment with its tentacles and feeding on detritus and additional organic matter (Setyastuti, 2014; Hartati*et al.,* 2017). These species make use of the organic matter that covered sediment and detrital particles as food.Therefore, particle size, organic matter, and microalgal biomass could be used to differentiate niches in optimal foraging strategies.

H. leucospilota and *H. cinerascens* are deposit-feeding sea cucumbers, digested and make use of organic component detached from the sediment (Viyakarn *et al.,* 2020), as shown in digesta of their alimentary canal.

Belbachir and Mezali (2018) reported that the prevalence of food items of animal origin can be obsessive and occasionally prefer these items more than other food sources. This discriminatory behavior is exercised on certain food items can only be valuable in stipulations of energy intake. The shape of the tentacles is usually modified to the size of the particles to be ingested detritus feeders that live on fine sediment have shorter tentacles, often peltate, constricting, and have a sticky substance on their surfaces. According to Belbachir *et al.,* (2014), marine deposits feeders adapt prefer foods those are simply comprehend quickly transfer in gut and for which the liberate of particles indispensable less attempt. In present study the gut constitutes of both species with notable (%) of foraminifera, in fact, according to Bakus (1973), foraminifera are one of the main food sources for holothurians. furthermore, this food resource is measured to be a vital part of the diet of holothurians, particularly in the early stages of their development. Gut content analysis in sea cucumbers can also help to identify any changes in their diet over time, which could be an indication of changes in the ecosystem or environmental conditions.

Conclusion

The main purpose of this study is to reveal the feeding regime of *H. Leucospilota* and *H. cinerascens* from Karachi coast of the Arabian Sea. It is important to know the stomach contents of Holothurians, for several reasons. For one, studying the stomach contents of sea cucumbers can give us insight into their feeding habits and dietary preferences. This information can be useful for understanding the role that sea cucumbers play in the marine ecosystem, as well as for determining the potential impacts that human activities, such as fishing and pollution, may have on their populations. In addition, studying the stomach contents of sea cucumbers can help us to identify the species of organisms that they are consuming, which can provide valuable information about the overall health and diversity of the marine ecosystem. Finally, studying the stomach contents of sea cucumbers can help us to identify potential sources of pollution or other environmental issues that may be affecting the health of the sea ecosystem.This study may provide evidence about the feeding strategy of these Holothurians, which may help reveal the association between their gut content and environmental habitat.It can also guide future studies on these shores.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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