Feeding habits and trophic level of *Himantura gerrardi* (Elasmobranchii; Dasyatidae) in northern Oman Sea: effects of sex and size class

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Abstract
The feeding habits and trophic level of *Himantura gerrardi* were studied according to sex and size classes of 80 individuals obtained using bottom trawl between May and December 2014 from the Oman Sea. Collected specimens were divided into three size classes (small<38 cm, medium 38-58 cm and large>58 cm) based on disk width. Prey was identified in seven main zoological categories, where crustacean and polychaetes were the dominant in diets, indicating this species is a demersal dwelling species. No significant differences were observed between sexes, but size classes showed significant differences in their diets. Also, results showed a decrease in feeding overlap between size classes with an increase in disk width, suggesting that intraspecific competition reduced, which allows the coexistence of different size classes. Trophic level was positively correlated with the disk width, which was lower than 4.0 for both sexes and different size classes, placing it in an intermediate trophic level.

Keywords: Dasyatidae, *Himantura gerrardi*, Feeding habits, Trophic level, Oman Sea.

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Introduction
The feeding habits of marine predators within the food web is critical to describe their ecological role within the ecosystem (Navarro et al., 2013). Also, the feeding habits of marine predators provide a comprehensive understanding of their ecological interactions such as feeding competition (O’Shea et al., 2013), predator-prey size relationships (Scharf et al., 2000), ontogenetic dietary shifts (Koen Alonso et al., 2001) and habitat selection (Heupel et al., 2007). In addition, feeding habit studies are necessary for conservation strategies, and ecosystem-based management through the estimation of trophic levels (Pauly and Christensen, 2000).

In marine environments, the trophic level (TL) represents the position of species within the food web (Pauly et al., 1998; Stergiou and Karpouzi, 2002). Most elasmobranch species are carnivores and tend to occupy higher TLs than other fishes. However, high TLs are not universal among this group (Vaudo and Heithaus, 2011). Batoid fishes usually occupy intermediate TLs in their communities (TL<4.0) and are also present in the diet of large sharks (Bornatowski et al., 2014). Therefore, they can be considered mesopredators with an important role to create a link between top-down TLs (Vaudo and Heithaus, 2011) and have the impact to control lower TL organisms in the marine ecosystems (Ebert and Bizzarro, 2007).

Coastal environments in tropical regions represent a high diversity and abundance of rays (Last and Stevens, 1994). The Oman Sea and Strait of Hormuz are in the subtropical region, a place in the Northwestern Indian Ocean, lying between latitudes 24° and 27°N and longitudes 55°50’ to 61°25’E. As expected, the highest abundance of species in these regions is rays, which have increased over the last two decades (Valinassab et al., 2006). Among these groups, sharpnose stingray, *Himantura gerrardi* (Gray, 1851) has a considerable total biomass, where it is one of the important members of the family Dasyatidae. This species also is widely distributed in the Indian Ocean from India to the south and east African coasts (Bianchi, 1985; Last and Compagno, 1999). This stingray inhabits demersal shallow waters on soft substrates, mostly found at depths of at least 60 m (Last and Compagno, 1999). Despite the abundance of this species in the Oman Sea and Strait of Hormuz, it is discarded by both traditional and industrial fishermen. So, information about feeding habits and trophic level of this species is very scarce. Also, in comparison with other marine ecosystems, information about the TL of the marine species inhabiting the Persian Gulf and Oman Sea is very scarce. For example, Vahabnejhad (2015) reported TL of 8 fish species using the analysis of their stomach contents in the Persian Gulf, which varied from 3.47 for *Argyrops spinifer*...
to 4.61 for *Saurida tumbil*. Similarly, Rastgoo (2015) reported TL of 6 elasmobranch species in the Oman Sea, which varied from 3.25 for *Pastinachus sephen* to 4.50 for *Torpedo sinuspersici*. The objectives of the current study were to analyze the stomach contents of *H. gerrardi* in the Oman Sea and Strait of Hormuz and trophic level estimation of this species in relation to sex and different size classes.

**Materials and methods**

Samples were obtained during spring, summer, autumn and winter through four cruises of bottom trawl R/V ‘Ferdows1’ between May and December 2014 from Strait of Hormuz and Oman Sea (from 56° 07’E 27°02’ N to 58° 01’ E 25°13’ N) (Fig. 1). Mesh size of cod end and headline of trawl net were 80 mm and 72 m, respectively.

Samples were collected between the sunrise and 14:00 hours from depths of 10 to 60 m. For all specimens, sex (based on the presence of claspers in males), weight (W) (nearest 10 g), disk length (DL) and width (DW) were recorded. Stomachs were removed and preserved in 5% formaldehyde for further examination in the laboratory. Stomach contents were identified to the lowest possible taxon (Sterrer and Schoepfer-Sterrer, 1986; Bosch et al., 1994; Asadi and Dehghani, 1996; Carpenter, 1997), counted and weighed (nearest 0.1 g). Due to the lack of knowledge about maturity size in *H. gerrardi* in the Oman Sea, to reach the ontogenetic trends, collected specimens were divided into three size classes, small (size class I) (<38 cm),
medium (size class II) (38–58 cm), and large (size class III) (>58 cm) based on disk width.

The length-weight relationship was determined using the equation $W = aDW^b$, where $W$ is weight (in g) and $DW$ is the disk width (in cm). The importance of various prey taxa was assessed by calculating the index of relative importance (IRI) (Hyslop, 1980) as:

$$\text{IRI} = (%N + %W) \times %FO$$

Where $%N$ is the number of a given prey type as a percentage of the total number of prey; $%W$ the weight of a given prey type as a percentage of the total weight of prey; and $%FO$ is the percentage of frequency of occurrence of each prey type (Hyslop, 1980). The IRI values were standardized in percentage values according to Cortés (1997):

$$\% \text{IRI} = \left( \frac{\text{IRI}}{\sum \text{IRI}} \right) \times 100$$

The trophic level (TL) for both sex and sizes was determined by using the percentage of weight for each prey in the diet using the program TrophLab (Pauly et al., 2000). We used only identified items. TrophLab estimates the TL with standard error (±SE) considering the diet composition and the TL of the different prey present in the diet, according to percentage of weight (Pauly et al., 2000):

$$TL_i = 1 + \sum_{j=1}^{G} DC_{ij} \times TL_j$$

Where $DC_{ij}$ is the fraction of prey (j) in the diet of consumer i; $TL_j$ is the trophic level of prey (j); while $G$ is the number of prey categories. The trophic level of each prey taxa was extracted from the FishBase dataset (Froese and Pauly, 2000).

Because of the large number of zeros in the dataset, data were pooled into seven taxonomic categories. Feeding strategy of different size classes were described by plotting the prey-specific abundance of the main zoological prey groups against $%FO$ (Amundsen et al., 1996). Prey-specific abundance was estimated as the number of prey I divided by the total number of prey in the stomachs that contained prey I, expressed as a percentage. The vertical axis represents the feeding strategy (specialization or generalization) of the predator. Points positioned in the upper part of the graph suggest specialist prey items, whereas points in the lower part indicate items that were only eaten occasionally. If no points are located in the upper right of the diagram and all points fall along or below the diagonal from the upper left to the lower right, the predator is thought to have a generalist diet and thus a broad niche width.

Pianka index with EcoSim 7.72 software and IRI of each prey was used to calculate niche overlap (Gotelli and Entsminger, 2005). Lower values suggest differences in diets or resource partitioning, while higher values suggest similar diets or strong resource competition. When values exceed 0.60, niche overlap was considered biologically significant (Zares and Rand, 1971).
Similarity matrix with the transformed estimated contribution values of food items, based on weight of main zoological prey groups was used to test variation between the diets of factors (sex and size classes). The test was then generated using the Bray–Curtis similarity coefficient. Diet similarity was analysed with non-metric multidimensional scaling analysis (nMDS). One-way analysis of similarity (ANOSIM) employed to investigate data. This test was used to verify similarities (distance) within defined factors and also calculates the statistic $R$, which varies between -1 and +1. The $R$ value close to zero represents there are no differences between factors, and $R$ value close to 1 indicates that the factors were dissimilar between the levels of each factor. Also, similarity of percentages (SIMPER) was used to estimate the contribution of each main zoological prey to differences in diets. The analyses were performed using the software PRIMER v.5 (Clarke and Gorley, 2001).

**Results**

A total of 80 specimens of *H. gerrardi* were collected from the Oman Sea and Strait of Hormuz. The length-weight relationships for all specimens are shown in Fig. 2.

In total, 19 different prey taxa were identified in all stomachs: eight different crustaceans, four teleosts, two echinoderms and one polychaete, bivalve, gastropod and cephalopod. In terms of %IRI, diets were dominated by crustaceans (%IRI=60.46), followed by polychaetes (%IRI=13.94). In contrast, cephalopoda, gastropodaand, echinodermata had lowest importance (%IRI <1; Table 2).

The samples were composed of 31 males and 49 females ($\chi^2, p<0.05$). Size distribution female and male of *H. gerrardi* sampled are shown in Fig. 3.
The range of DW was 18.5-76 cm (mean 38.7±16.7) for male and 18-80 cm (mean 41.8±14.1) for female. Of the total 80 stomachs examined, 71 (88.75%) stomachs contained prey (87.1% of male and 89.8% of female).

The ANOSIM did not find diet differences between males and females of *H. gerrardi* (*R*=0.038; *p*>0.05) (Fig.4). Both sexes mainly feed on crustaceans, followed by polychaetes (Table 1).

The numbers of small, medium and large individuals were 31, 43 and 6, respectively (*χ²*, *p*<0.05) and mean of DW (±SE) were 24.4±5.4 cm, 46.2±6.7 cm and 73.1±5.6 cm, respectively. Of the total 9 empty stomachs, 8 (88.8%) and 1 (11.1) belong to size classes I and III, respectively. In terms of %IRI, crustaceans were an important prey category for all size classes (%IRI<sub>small</sub>=78.79; %IRI<sub>medium</sub>=51.97 and %IRI<sub>large</sub>=50.77). In contrast, cephalopoda (%IRI<sub>medium</sub>=0.02), gastropoda(%IRI<sub>small</sub>=0.48 and %IRI<sub>large</sub>=0.67) had lowest importance for all size classes (Table 2).
Table 1: Values for dietary items and main zoological prey (in bold) based on sex of *Himantura gerrardi* in the Oman Sea were expressed by number (%N), weight (%W), percentages of occurrence (%FO), and index of relative importance (%IRI).

<table>
<thead>
<tr>
<th>Food items</th>
<th>Male (n= 31)</th>
<th>Female (n= 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%N</td>
<td>%W</td>
</tr>
<tr>
<td>Teleostei</td>
<td>2.25</td>
<td>15.18</td>
</tr>
<tr>
<td>Acropomatiidae</td>
<td>0.28</td>
<td>1.14</td>
</tr>
<tr>
<td>Haemulidae</td>
<td>0.21</td>
<td>0.79</td>
</tr>
<tr>
<td>Leioagnatidae</td>
<td>1.97</td>
<td>14.04</td>
</tr>
<tr>
<td>Unidentified teleostei</td>
<td>34.46</td>
<td>12.71</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>51.09</td>
<td>39.93</td>
</tr>
<tr>
<td>Crab</td>
<td>1.97</td>
<td>7.09</td>
</tr>
<tr>
<td>Portunidae</td>
<td>1.41</td>
<td>4.08</td>
</tr>
<tr>
<td>Xanthidae</td>
<td>0.84</td>
<td>2.29</td>
</tr>
<tr>
<td>Shrimp</td>
<td>12.71</td>
<td>51.61</td>
</tr>
<tr>
<td>Isopoda</td>
<td>1.69</td>
<td>0.21</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>2.82</td>
<td>0.50</td>
</tr>
<tr>
<td>Squillidae</td>
<td>1.69</td>
<td>1.64</td>
</tr>
<tr>
<td>Unidentified crustacea</td>
<td>6.21</td>
<td>11.38</td>
</tr>
<tr>
<td>Polychaetes</td>
<td>29.37</td>
<td>12.71</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>3.1</td>
<td>10.60</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>0.63</td>
<td>0.34</td>
</tr>
<tr>
<td>Cephalopoda</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>0.56</td>
<td>1.78</td>
</tr>
<tr>
<td>Holothuroidea</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Unidentified</td>
<td>13.55</td>
<td>19.77</td>
</tr>
</tbody>
</table>

The ANOSIM found significant differences in the diet composition among size classes ($R_{global}$=0.124; $p<0.05$). Pair-wise tests from the ANOSIM indicated that the diet of size
class I was dissimilar to size class III (R=0.749; p=0.001) (Fig. 4). Feeding strategy plots verified that crustaceans were an important prey category for all size classes. There was a high degree of specialization on this prey category by size class I, whereas a moderate level of specialization occurred by size class III on teleosts, polychaetas and bivalvia with a relatively narrow niche width (Fig.5).

The average Bray–Curtis dissimilarity in diet between size class I and III for H. gerrardi was 95.51 and this difference was predominantly due to crustaceans (44.41%), teleosts (27.08%), polychaetes (12.96%) and bivalvia (10.01).

Pianka’s index showed that there was a degree of dietary resource overlap between sexes (O=0.801), whereas a reducing dietary resource overlap was observed among size classes with an increase of DW (size class I and II= 0.842; size class I and III= 0.466; size class II and III=0.651), indicating that by increasing DW, intraspecific feeding competition is reduced, which allows the coexistence of two or more size classes.

Overall, the H. gerrardi occupied an intermediate trophic level (TL=3.69±0.59). The trophic level was similar among males and females with slight variation (male: TL=3.57±0.57,
female: TL=3.74±0.61), and size classes (small: TL=3.60±0.59; medium: TL=3.65±0.58; large: TL=3.80±0.63), and only a positive relationship between trophic level and DW was detected.

Discussion
According to the obtained results, *H. gerrardi* is a predator with a relatively wide trophic spectrum which feeds mainly on crustaceans, followed by polychaetes and teleosts. Other main zoological preys such as bivalvia, gastropoda and echinodermata were only found in several stomachs and contributed small proportions to the diet composition. Although the feeding habits of *H. gerrardi* has not been described in the Oman Sea, the preferential consumption of crustacean such as shrimp and crabs by this species has been reported by Compagno et al. (1989) in southern Africa. Also, to describe the diet of *H. gerrardi*, Manjaji et al. (2009) mentioned that the diet is presumably based on crustaceans, bivalve and small fishes. Higher up, similarity in the crustacean-diet is shared with one species of stingray of the genus Himantura from Australia (O'Shea et al., 2013). Also other species in the family of Dasyatidae such as *Pastinachus atrus*, *Taeniura lymma*, *Neotrygon kuhlii* and *Urogymnus asperrimus* had diets dominated by polychaetes (O'Shea et al., 2013). Therefore, this suggests that these two main zoological prey groups are the most important dietary categories for this family.

The similar diet exhibited by males and females of *H. gerrardi* can be explained by the fact that both sexes showed similar body size (Barbini et al., 2010; Navarro et al., 2013). Our results agree with previous studies conducted on several ray species (White and Potter, 2004; Navia et al., 2007; Jacobsen and Bennett, 2012), confirming no evidence for differences in diet of males and females for elasmobranch species was detected.

An analysis of similarity showed significant differences between the three size classes in their diet. Although results showed that crustaceans were important prey category in all classes, the individuals from size classes II and III had a wider trophic spectrum than size I, which according to Pianka index could help decrease intraspecific competition. Although disk width of mature individuals of *H. gerrardi* is not clearly determined in the Oman Sea, these measurements reported 47 and 54 cm for males and females, respectively (Manjaji et al., 2009). Therefore, it seems that the size class II included both mature and immature individuals, confirming feeding overlap with other size classes. These ontogenetic changes in feeding habits seem to be a common characteristic in elasmobranchs and have been reported in several species of rays and skates (Scharf et al., 2000; Brickle et al., 2003; White et al., 2004). The predominant proportions of crustaceans and the shares of many
marine benthic fauna in the diet confirm that the *H. gerrardi* is a demersal dwelling species. Nevertheless, in comparison with the high diversity of marine benthic fauna found in the other studies from the Oman Sea (Behzadi *et al.*, 2010), size class I had high presence of crustaceans especially shrimp in their diet, which could be due to specialization of the predator. Also the sample sizes for class III were only a few, but they could describe the general feature of the diet and feeding strategy. On the other hand, size class III had a relatively wide trophic spectrum and low percentage of empty stomachs found in larger specimens, which is in line with results reported by Lipej *et al.* (2013) from Adriatic Sea, suggesting it can be due to increase of swimming speed, movement patterns and enhanced ability to capture prey (Wetherbee and Cortes, 2004).

Most studies have been conducted to determine the trophic level based on the analysis of stomach contents, although new methods have been developed to determine the trophic level for marine species such as stable isotope. In marine ecosystems, trophic level varied from 2 for detritivorous/herbivorous species to 5 for carnivorous/piscivorous species (Pauly *et al.*, 1998). The relative moderate trophic level of *H. gerrardi* (3.5<TL<4.0) for both sex and size classes indicates that this species is a potentially important mesopredator within the Oman Sea food web. In addition, the results revealed that trophic level positively increases with body size. Demersal rays showed a slightly lower trophic level than sharks in the Oman Sea, where the group of demersal rays in this region showed a trophic level between 3.25 to 3.69 and that of sharks varied from 4.06 to 4.50 (Rastgoo and Navarro, 2017). Cortés (1999) calculated the trophic level for 149 elasmobranch species, which generally ranged between 3.10 and 4.70 and mentioned that sharks tend to be in the third level (TL>4.0), and with few exceptions, announced that the trophic levels of sharks are positively correlated with body size. Like sharks, skates also showed a positive relationship between body size and trophic level (Ebért and Bizzarro, 2007). The obtained differences in trophic levels between both sexes and size classes could be due to differences in size; larger fish tend to have higher TLs. Similar patterns were reported by Stergiou and Karpouzi (2002) for some teleost fishes in the Mediterranean Sea, which confirmed that the relative success of larger predators increases due ontogenetic changes in predatory capacities and tend to be much faster swimmers.

Although seasonal variations in elasmobranch diet have been reported for several species of ray (Muto *et al.*, 2001; Wetherbee and Cortes, 2004; Braccini and Perez, 2005; San Martin *et al.*, 2006), it is likely that *H. gerrardi* would show changes in feeding habits. Therefore, while the current study provides a baseline description of the diets of this species, further data from a
larger sample across seasons, spatial and temporal variation are required to provide a precise estimation of the feeding habits and trophic level. Also, Jabado et al. (2015) reported little overlap in the diets of two shark species in the Persian Gulf, this is especially important that future studies should focus on trophic interactions and mechanisms of coexistence within and among elasmobranch species to understand their important ecological role in the ecosystem.

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