Trophic level, food preference and feeding ecology of *Rachycentron canadum* (Linnaeus, 1766), in Hormuzgan Province waters (northern Persian Gulf and Oman Sea)

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Abstract

This study examined the ecological factors associated with feeding in *Rachycentron canadum* (cobia) in the waters of Persian Gulf and Oman Sea. Seasonally variation of stomach condition of cobia male, females, pooled and size wise were analyzed. In general, 577 cobia were collected (340 males, 237 females) (20–161 cm Total length) from Sept. 2014 to Sept. 2015 by the Ferdows-1 survey ship and from the landing areas in Bandar Abbas harbor seasonally. Our results provide the first estimates of trophic level (Tr.L) (4.32±0.76, mean±Se), consumption per biomass (Q/B) (6.13), and aspect ratio of the caudal fin ($AR_{c.f}$) (3.1) for *R. canadum* in the Hormuzgan Province. We also found that bony fish made up more than 80 percent of the relative importance of particular prey (IRI %), indicating *R. canadum* primarily preys on these species. The immature group (20–40 cm,T.L), tended to feed on Penaeidae family, small crabs, and mollusks. Hierarchical clustering revealed that the frequencies of different types of prey could be applied to categorize the length classes into three levels. The result of this study revealed that the immature group and the 20-40 cm length class tended to feed on Penaeidae family, crabs, and mollusks. Also, we found that males generally fed more than females in all seasons and that *R. canadum* had an average dietary intake. The findings of this study can be used to prepare a food formulation for cobia that resembles the native diet.

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Introduction

The ecosystem of the Persian Gulf and Oman Sea is unique and includes species that are both commercially and ecologically important. Studies of aquatic ecosystems have shown that species feeding interactions can be affected by fishing and that when fishing pressure is increased, these relationships may collapse (Walters and Martell, 2004). Thus, analysis of the feeding habits of specific species can be useful for determining the status of the food web, as existence of a species in an ecosystem depends on the availability of prey. Also, feeding studies are essential for understanding different phases in the fish life cycle, such as growth, breeding, and migration (Bal and Rao, 1984). Piscivorous fish are top predators in many aquatic systems and the knowledge on the size of their prey is essential to identify their potential impact in structuring populations at lower trophic levels(Pavlovic *et al.*, 2015), also access to the diet of a species has many uses in aquaculture (Bahrami Babaheydari *et al.*, 2015)

Results from these studies can be useful for making modeling software such as EwE (Ecopath with Ecosim). In Ecopath, the modeled ecosystem was partitioned into groups of similar species and estimates of biomass, diet, production and food consumption were provided (Christensen *et al.*, 2014). This model consists of three main routines: 1. Ecopath (a static, mass-balanced snapshot of the ecosystem food-web), 2. Ecosim (a time-dynamic simulation module for exploring fisheries management) and 3. Ecospace (spatial and temporal dynamic module) (Walters *et al.*, 2010).

In addition, a suite of modules or plug-ins have also been developed and added to the EwE package, such as EcoTroph, which is a representation of the biomass distribution across trophic levels in the ecosystem (Gascuel *et al.*, 2011). Trophic levels are the relative position of a species in the food web. Also, several studies have been done in the Persian Gulf by using Ecopath model (Taghavi Motlagh *et al.*, 2013; Vahabnejhad *et al.*, 2013; Vahabnejhad, 2015). Cobia is the only species in the Rachycentridae family that is a pelagic, migratory fish. It is widely distributed across the tropical and subtropical seas of the world, except in the central and eastern Pacific Ocean (Fisher and Bianchi, 1984)**.** Cobia is considered one of the most important species and the average catch was 1782.27 tonnes per year (2004-2014) (Fishstatj, 2014). Dietary studies of this species have shown that it feeds from benthic and demersal communities, including on Chnodrichthies, stomatopods, and crab, although cobia has been reported to be a pelagic fish (Fry and Griffiths, 2010).

The aim of the present study was to analyze some of the ecological factors associated with *R. canadum* feeding in the north region of the Persian Gulf and Oman Sea. Although several studies have been performed on the fish diet habitats in the Persian Gulf and Oman

Sea, this study is the first to analyze feeding ecology of cobia in this region.

Materials and methods
Sample collection and analysis
This study was performed in the northern parts of the Persian Gulf and Oman Sea (Hormuzgan Province region), which is located between latitudes 25º 23' N and 28º 57' N and longitudes 52º 41' E and 58º 00' E (Fig.1). The Ferdows-1 survey ship (a stern trawler) was used for data collection in the landing areas in Bandar Abbas harbor seasonally. Identification of cobia and its prey (to the greatest degree possible) was performed based on standard references (Fisher and Bianchi, 1984; Sterrer and Schoepfer-Sterrer, 1986; Bruyne, 2003; Smith *et al.*, 2003). During this study, 577 cobias were collected (340 males, 237 females) (20–161 cm total length). Biometric measurements, including total length (using a measuring board to the nearest 1 mm), total weight (using a portable digital balance to the nearest 0.01 g), and caudal fin height, were collected. Photographs of the caudal fins were taken for cobia samples collected.

After taking biometric measurements, the samples were dissected and digestive system was carefully separated for stomach contents analyzing. Specimens collected from the landing areas of Bandar Abbas were kept on ice, and while onboard, samples were frozen immediately. Both types of samples

were transported to the zoology laboratory in the Persian Gulf and Oman Sea Ecological Research Institute. Extracted stomachs were weighed (to the nearest 0.01 g), and contents were poured into a petri dish containing distilled water. To determine the type of prey, the stomach contents were viewed using an Olympus stereo microscope at 0.8–5.6× (zoom) and 10× resolution. Using the point method (Costello,1990), each stomach was visually sorted and categorized as empty, one-quarter full, half full, three-quarters full or full (Berra *et al.*,1987). After identification of the stomach contents, numbers of prey and their weights (±0.001 g) were recorded in a sampling form.

Trophic level
The software used to calculate trophic level is available for download on the Fishbase site (Froese and Pauly, 2000). Trophic level (T.L) was calculated based on the proportion (by weight) of each prey component in the cobia diet. Trophlab estimates the trophic level (TROPH) and standard error (SE) of a fish by considering the diet composition and TROPH of its prey, based on the fraction of prey items by volume or weight in the predator's diet plus one (Froese and Pauly, 2000). In this study, the weight method was used to estimate prey proportions. Trophlab expresses TROPH as:

$$TROPHi = 1 + \sum_{j=1}^{G} DCij.\text{TROPHj},$$

Where DC_{ij} is the fraction of prey j (in the diet of consumer i), $TROPH_j$ is the

trophic level of j, and G represents the number of prey categories.

Consumption per biomass ratio (Q/B)
Many approaches exist to estimate Q/B

Log Q/B = 7.964 + 0.204 log W_∞ − 1.965 T' + 0.083 Ar +0.532h+0.398 d (R^2=0.53,98df)

Where T' is an expression mean annual water temperature and defined as T' =1000/Kelvin (Kelvin=°C+273.15). A CTD apparatus was used to determine water temperature. A.R is defined as the aspect ratio of the caudal fin ($AR_{c.f}$) and was measured using Image J software; h is a dummy variable expressing food type (1 for herbivores, 0 for detritivores and carnivores); d is a dummy variable also expressing food type (1 for detritivores, 0 for herbivores and carnivores); and W_∞ is the asymptotic weight (calculated by insertion of infinite length into the length-weight relationship). Q/B was used to measure the level of ecological efficiency of feed used. In other words, the coefficient of efficiency of ecological food consumption is estimated by this factor. Aspect ratio of the caudal fin (AR_{cf}) measurements can be used to better understand propulsive efficiency. $AR_{c.f}$ was measured in at least 50 percent of the samples from each length class due to the change in shape of the caudal fin over the lifetime of cobia. $AR_{c.f}$ was estimated for each length class using the below equation (Abramoff *et al.*, 2004):
Aspect ratio=h^2/S

(Christensen *et al.*, 2008). In this study, the following equation was used (Christensen and Pauly, 1992):

Where h is caudal fin height, and S is caudal fin surface area (measured using Image J software). Class $AR_{c.f}$ values represent the average of the $AR_{c.f}$ values obtained for each length class.

Feeding indices
The Stomach emptiness index (C.V), gastro-somatic index (GaSI), and index of relative importance of primary food sources (IRI%) were calculated to quantify cobia feeding behavior. C.V estimates the voracity of predator fish. In more voracious fish species, a lower percentage of empty stomachs are observed. C.V can be calculated using the following equation:
CV = ES × 100/TS
Where ES is the numbers of empty stomachs, and T.S is the total number of stomachs analyzed. It is important to note that the C.V index may not be very robust, as increases in empty stomachs can also be due to regurgitation of prey. Therefore, GaSI and IRI% were also used to describe the importance of various preys in the diet. GaSI is another method for estimating the feeding intensity of fish and can be determined using the following equation:

GaSI= (weight of stomach/weight of fish) $\times 100$

IRI% estimates the frequencies of different types of prey and is calculated as follows:

IRI%= F%$\times$(N%+W%)

Where N% is defined as the numerical composition, W% is defined as the weight composition, and F% is defined as the frequency of occurrence (Ugwumba and Ugwumba, 2007). Feeding intensity was compared in different length groups to better understand changes in cobia feeding behavior in different life stages. Additionally, to assess changes in diet due to maturation, fish were also divided into two size-based classes according to length at first maturity (L_{m50}). This index was previously reported to be 81.25 cm in Hormuzgan province waters (total length) (Daghooghi *et al*, 2007). Statistical analyses, including one-way analysis of variance (ANOVA) and compare means tests were performed to compare the frequencies of different prey by number and weight across different length classes and also between the immature and mature groups. Differences in the diet composition of cobia were also compared among different length classes using the Bray-Curtis similarity index.

Results

T.L (4.32±0.76, mean±SE), Q/B (6.13), and $AR_{c.f}$ (3.1) values for cobia in the Hormuzgan Province waters. Analysis of stomach contents revealed a total of 406 individual prey from 577 cobia (males and females), resulting in a total prey weight of 6310.16 g. IRI% is commonly used in studies of fish diet, and IRI% values, as well as the factors used to calculate this index, N% (numerical composition), W% (weight composition), and F% (frequency of occurrence), are shown in Table 1. Assessment of IRI% revealed that prey items could be divided into three categories. Bony fishes made up more than 82%, digested matter made up 11–14.27%, and other groups made up less than 5% of the total contents (Table 1). Calculation of the average annual IRI% indicated that bony fishes (83.28%), digested matter (13.04%), Penaeidae family (2.395%), mantis shrimp (0.29%), Chnodrichthies (0.225%), molluscs (0.185%), *Thenus orientalists* (0.1575%), *S. pharaonis* (0.145%), crabs (0.1275%), *U. duvaucelii* (0.11%), and octopus (0.0425%) were the preferred foods for cobia living in the Hormuzgan Province waters (Table 1).

Analysis of the frequencies of the number and weight of various prey by length class revealed that bony fishes was the only group that was observed in the all length classes (Figs.1 and 2). Results of a one-way ANOVA test revealed a difference between prey frequencies (by number and weight) for different length classes ($p > 0.05$). Comparison of bony fish number and weight frequencies also revealed a difference for all length classes ($p > 0.05$), except between the 41–60 and 61–80 groups ($p < 0.05$).

Table 1: Percentage numerical composition (N%), percentage weight composition (W%), frequency of occurrence (F%) and percentage index of relative importance (IRI%) of main prey groups by seasons for *Rachycentron canadum* in the Hormuzgan Province waters.

| Pre items	Sep-Dec 2014				Dec 2014 – Mar 2015				Mar-Jun 2015				Jun-Sep 2015			
	N%	W%	F%	IRI%	N%	W%	F%	IRI%	N%	W%	F%	IRI%	N%	W%	F%	IRI%
Bony fish	66.6	49.15	53.74	83.74	63.86	55.39	55.42	84.2	47.61	51.18	54.04	82.42	38.24	51.25	56.95	82.78
Chondrichthies	3.57	6.57	3.15	0.44	0	0	0	0	4.69	4.09	3.39	0.46	0	0	0	0
Crab	0.89	1.24	3.12	0.09	2	3.79	1.66	0.12	0.78	1.64	1.69	0.06	1.54	4.72	2.32	0.24
Mantis shrimp	0.89	0.68	1.56	0.03	2	1.72	3.33	0.16	0.78	0.81	1.69	1.04	4.62	3.59	6.97	0.93
Penaeidae family	3.57	3.57	6.25	0.62	5	5	8.33	1.06	10.16	10.16	11.86	3.72	9.23	9.23	13.95	4.18
Thenus orientalis	1.79	6.55	3.12	0.36	1	3.05	1.66	0.09	1.56	1.95	3.39	0.18	0	0	0	0
Molluscs	2.69	2.33	4.68	0.32	0	0	0	0	0	0	0	0	3.08	2.47	4.65	0.42
S. pharaonis	0	0	0	0	2	2.96	3.33	0.21	1.56	3.26	3.39	0.25	1.54	1.61	2.32	0.12
U. duvaucelii	0.89	0.73	1.56	0.03	1	1.15	1.66	0.05	1.56	2.76	3.39	0.23	1.54	1.81	2.32	0.13
Octopus	0.89	1.87	1.56	0.06	1	0.77	1.66	0.04	0.78	2.02	1.69	0.07	0	0	0	0
Digested matter	18.22	30.31	21.26	14.27	22.14	26.17	22.95	14.1	30.52	221.3	15.47	15.57	40.21	25.32	10.52	11.2
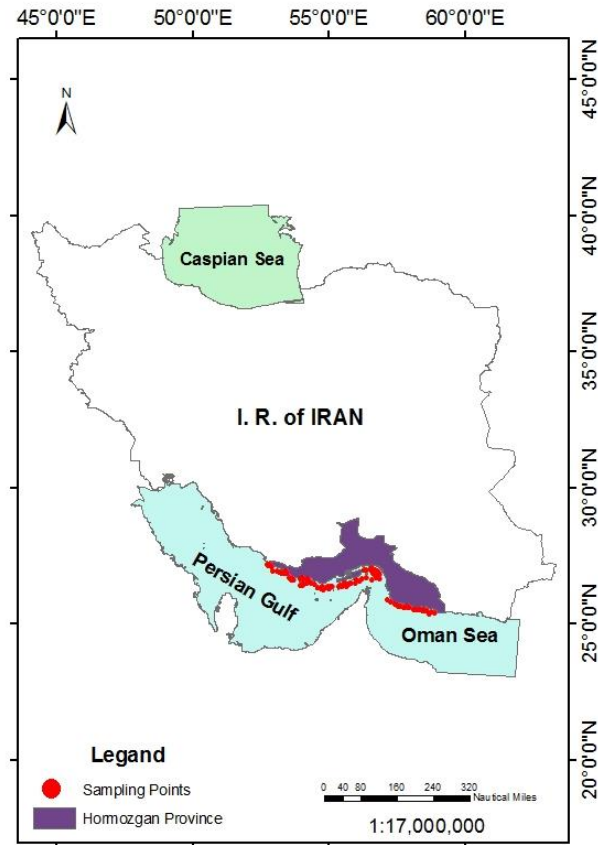
Figure 1: The sampling area in this survey (Hormuzgan Province waters).

Additionally, a predominance of Penaeidae family, crabs, and mollusks were observed in the stomach contents of fish belonging to the 20–40 cm length class (Figs. 2 and 3).

Results of a comparison of means test revealed that the frequency

of prey number was similar only for bony fishes in the mature and immature groups ($p<0.05$), but that the frequency of prey weight differed between these groups.

The immature group tended to feed on Penaeidae family, crabs and mollusks, based on the frequencies of prey by weight and number, but these frequencies differed from that of the mature group ($p>0.05$, Table 2).

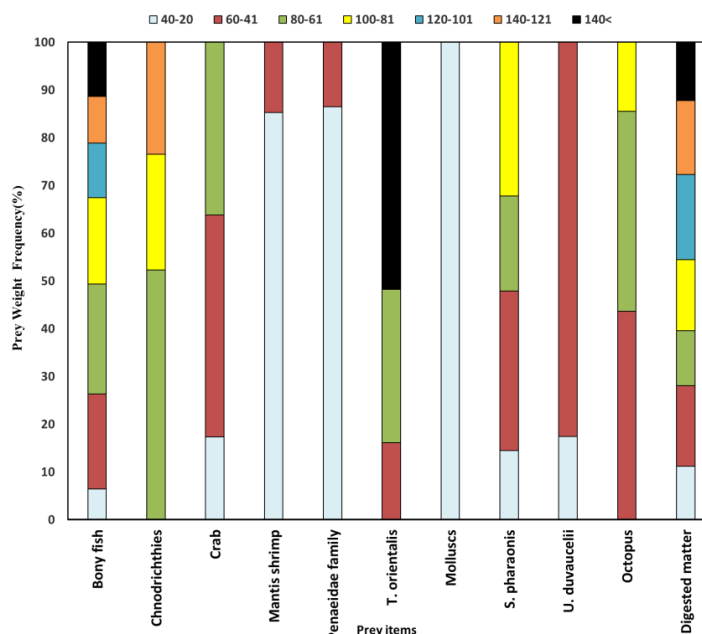Hierarchical clustering was used to compare the frequencies of prey by number between length classes (Fig. 4).

Figure 2: Frequency of prey number in stomach cobia based on length-class (cm) groups (2014-2015).

Table 2: Frequency of number and weight of prey in cobia stomach based on maturity groups (2014-2015).

Prey items	Numbers (%)		Weight (%)	
	Under maturity	Upper maturity	Under maturity	Upper maturity
Bony fish	46.42	43.04	39.72	48.71
Chondrichthies	0.49	0.49	1.41	1.28
Crab	1.23	-	2.67	-
Mantis shrimp	1.73	-	1.53	-
Penaeidae family	6.91	-	3.29	-
Thenus oreintalis	0.99	0.25	1.54	1.65
Molluscs	1.23	-	1.17	-
S. pharaonis	1.23	0.25	1.57	0.71
U. duvaucelii	0.99	0.00	1.49	0.00
Octopus	0.49	0.25	1.09	0.18

Results from this analysis revealed that the length classes could be divided into three groups based on frequency of prey by number. The first group was comprised of the 121–140 length class; the second group was comprised of the

21–40, 81–100, and 101–120 classes; and the third group was comprised of the 41–60, 61–80, and>140 classes (Fig. 4).

The graph in Fig. 5 shows the GaSI values for male and female cobia. GaSI differed between males (3.1–4.9) and females (2.9–4.3) in this survey, indicating that males generally fed more than females across all seasons. The lowest GaSI values for both males (3.1) and females (2.9) were observed in the summer, and the highest values were observed in the winter (4.9 and 4.3 for males and females, respectively).
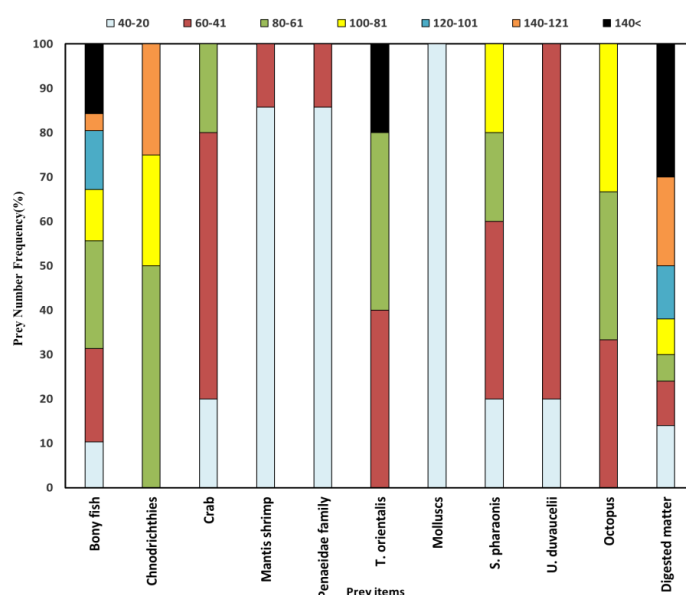

Figure 3: Percentage of prey weight in stomach cobia based on Length-class (cm) groups (2014-2015).
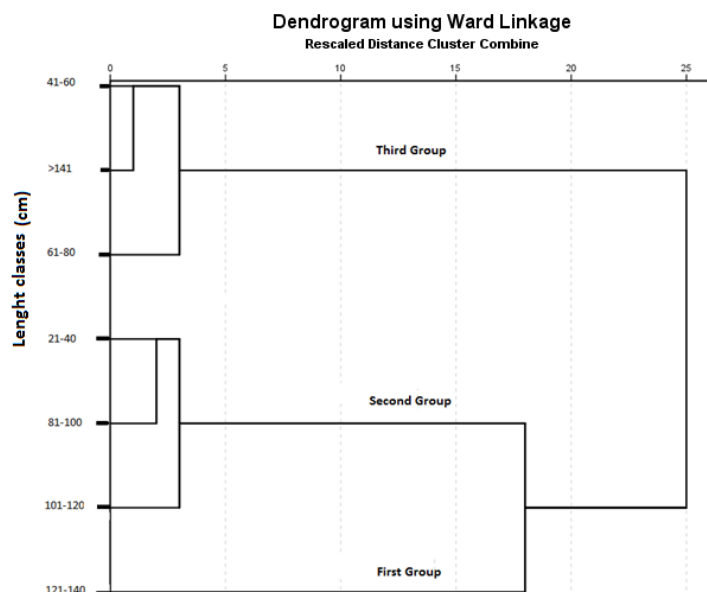

Figure 4: Dendrogram of cluster analysis of prey number of cobia for length classes (2014-2015).

CV showed similar seasonal variations, also empty stomachs were more frequent in the spring and summer seasons (60.58% and 60.19%, respectively), and the lowest values were observed in the winter (48.03%) and autumn (53.33%).
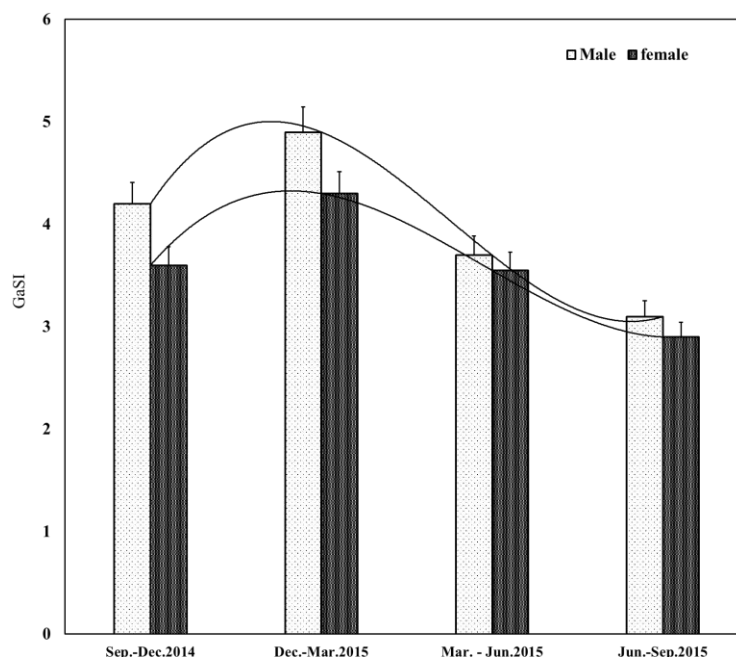

Figure 5: Seasonally variation in gastrosomatic index of males and females of cobia (2014-2015).

Discussion

In this study, we have presented quantitative data describing the diet of *Rachycentron canadum* in the waters of Hormuzgan Province. T.L was reported 3.96 in the Persian Gulf and Oman Sea (Moniri *et al.*, 2015), Also 4.35, 4.38, 4, 4.42, 2.3 and 3.9 were reported in Gulf of Carpentaria, Caribbean coral reefs, northwest coast of India waters, Northwest Atlantic, Northeastern Gulf of Mexico and the Gulf of Carpentaria respectively (Salini *et al*. 1994; Opitz, 1996; Franks *et al*., 2000; Van der Velde *et al*., 2010; Sajeevan, 2011; Froese and Pauly, 2000). The T.L was calculated as 4.32 ± 0.76 (mean±Se) in this study. Higher trophic level may be

due to higher level species richness or a larger number of available preys in this region (Vahabnezhad *et al*., 2013). Thus, based on the similarities of T.L values across the different seasons, we can conclude that the cobia diet had a similar variety of prey throughout the year in this region. Trophic levels range from 2 for herbivores and detritivores to 5 for carnivores (Pauly *et al*., 1998). Therefore, our results demonstrate that *R. canadum* is a top level predator in this ecosystem and has a high rate of food consumption. In addition to seasonal variations, T.L can also vary due to changes in the availability of prey and geographical differences (Arendt *et al*., 2001) and due to

differences in feeding intensity (Hassler and Rainville, 1975). Most fish have a lower trophic level when they are smaller and younger compared to when they are large and old (Pauly, 2010), indicating that can change with the size and age of fish (Pauly and Watson, 2005). In this study, T.L was not compared for different age groups, but it is likely that this index would vary based on changes in feeding habitats associated with the transition from the larval stage to more mature stages. Similarly, Q/B may also vary by maturity and season. Although Q/B is determined by year, consumption is not realistically constant during the year. The general decrease in the Q/B ratio could be due to a greater proportion of large fish in the historical system. When prey is scarce, fish feeding time will increase, resulting in a higher Q/B value (Christensen *et al.*, 2008).

Changes in body structure can also impact fish feeding. The shape of the caudal fin has a large impact on fish propulsion, so changes in caudal fin shape during the fish lifetime may increase the efficiency of propulsion. In species with high swimming activity and generally high metabolic rates, $AR_{c.f}$ is frequently higher, while in sedentary fish that presumably have a relatively lower food intake, $AR_{c.f}$ is frequently lower (Palomares and Pauly, 1989). Normally, demersal fishes have a low aspect ratio (Christensen and Pauly, 1992). Estimates of $AR_{c.f}$ can vary greatly in cobia due to changes in the tail shape throughout the lifespan, in

which the cobia caudal fin shape changes from round to truncate, and then from emerginate to forked. For this reason, specimens were selected from all length classes in equal proportions in this study. Previous feeding studies of cobia in this area have indicated that their primary food source is bony fishes (76%), followed by crustaceans (25%), and mollusks (11%) (Daghooghi *et al.*, 2007). Results from a study of cobia in the northwest coast of India revealed that the major food sources for cobia is this region were fish (72%), crabs (17%), squilla (3%), shrimp (2%), squid (1%), and digested matters (1%) (Sajeevan, 2011). Our IRI% results confirmed the findings of these studies (Daghooghi *et al.*, 2007; Sajeevan, 2011) (Table 1). Results from this study and previous studies indicate that the primary food source for cobia is bony fishes and that the other groups are likely accidental foods. Because the cobia is an opportunistic feeder, the key factor that determines the cobia diet is the availability of prey in the habitat. However, ontogenetic diet shifts have been observed in a variety of marine fish species (Karpouzi and Stergiou, 2003), and stated that cobia have a great interest in eating crab and shrimp. Because IRI% depends on the numerical percentage, the weighted percentage, and the frequency of occurrence, the relative importance of different food sources can be inferred from the IRI% value (Pinkas *et al.*, 1971). This index is used to reduce the bias inherent to each one of its

components (Rosecchi and Nouaze, 1987). Thus, the use of IRI% lends more confidence to the data revealing the prevalence of prey items in the diet found in this study.

In the present study, Bony fishes were observed in the prey of all length classes of cobia, although their frequency was higher in the 41–60, 61–80, and 81–100 cm length classes, compared to other classes. One-way ANOVA revealed that the frequency of bony fishes by weight and number was only similar in the 41–60 and 61–80 cm classes. Consistent with this observation, it has been reported that when *R. canadum* reach a full length of 1 meter, they stop feeding on crustaceans and begin consuming Bony fishes, mollusks, and batoid fish (Daghooghi *et al*., 2007). Comparison of means test results revealed that the frequencies of prey numbers between immature and mature groups was similar only for the bony fishes group ($p<0.05$).Together, comparison of means testing of the immature and mature groups and one-way ANOVA comparisons of the length classes suggest that immature fish tended to feed on Penaeidae family, crabs, and mollusks and that benthic groups (Penaeidae family, crabs, and mollusks) also dominated in the 20–40 length class. Analysis of the IRI% by length class confirmed that cobia tended to consume Bony fishes across all length classes. Thus, feeding on benthic groups, such as Penaeidae family and crab, may be due to inadequate growth

of the jaw bones, coupled with the fact that cobia appear to be demersal during this stage. Changes in cobia feeding behavior were also observed for different length groups in the northwest coast of India (Sajeevan, 2011) and the frequencies of mollusks and crabs were increased in the stomachs of immature cobia.

Results from this study show that *R. canadum* exhibited an average diet, although cobia has been reported to be gluttonous fish in most studies. Cobia may feed on chondrichthies because they share the same hunting grounds. Therefore, when chondrichthies are feeding from the bottom, cobia sharing the same space hunts chondrichthies (Takamatsu, 1967). The study demonstrating an association between cobia and sting rays suggest that this relationship might be due to an increase in food availability near areas inhabited by rays. Chondrichthies stir up benthic prey in their vicinity to find organisms, and this movement may help cobia to recognize its prey location so that cobia can feed more effectively (Takamatsu, 1967). Another study concluded that cobia tend to move to areas with greater food abundance, particularly crustaceans (Darracott, 1977), suggesting that cobia prefer crustaceans over other food items.

Results from hierarchical clustering in this study reveal that cobia length is not the primary determinant for selectivity of prey numbers, although similarities in prey numbers were observed between the 81–100 and 101–

120 classes in the second group (Total groups except chondrichthies) and the 41–60 and 61–80 cm classes in the third group (Bony fish, *T. orientalis* and Octopus). Previous results have shown a decrease in prey numbers in higher length classes in cobia (Sajeevan, 2011). Variations in feeding intensity in cobia may also be due to stage of life, as feeding has been shown to be decreased during spawning. In agreement, we found that males fed more than females in all seasons (Daghooghi *et al.*, 2007 and Sajeevan, 2011). Furthermore, March–June has been reported to be the cobia spawning season in the Hormuzgan Province (Daghooghi *et al.*, 2007), and we observed maximum and minimum feeding before and during this season, respectively. Additionally, analysis of cobia stomach contents revealed that the vacuity index was affected by seasonal fluctuations. The C.V was higher than 60% during spring and summer, reached 53.33% in autumn, and decreased to 48.03% in winter. Thus, spawning appeared to impact feeding intensity. However, samples collected from the landing areas may be a source of bias in estimated C.V, due to autolysis of stomach contents. In general, however, these results are consistent with the feeding behavior of the most fish species, which varies considerably during the year because of physiological changes that occur during reproduction.

In conclusion, this study reports quantitative data describing the feeding ecology of *R. canadum* in the northern Persian Gulf and Oman Sea. Our results suggest that the removal of top predators, such as cobia, can disturb the entire ecosystem because they have a fundamental role in maintaining a balance in the population of their prey. Additionally, because determining an optimal food source plays an important role in aquaculture, the findings of this study can also be used to develop food formulation for farmed cobia.

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