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Natural diet of *Macrobrachium nipponense* shrimp from three habitats in Anzali Wetland, Iran

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ABSTRACT
The freshwater shrimp, *Macrobrachium nipponense* is an invasive species which has recently been reported in Anzali Wetland, Iran. It exhibited good tolerance and adaption in this wetland ecosystem. This study examined certain aspects of feeding of *M. nipponense* in three habitats of this wetland. Shrimps were randomly sampled from April 2016 to March 2017. The stomach contents were obtained from 367 specimens ranging in length from 4.2 cm to 6.9 cm. The empty stomach index (VI) showed that this shrimp was a voracious ($0 \leq VI < 20$) species in all seasons expect winter, when 99% of the specimens had empty stomachs. Fourteen dietary items were categorized in the three habitats of the wetland, with phytoplankton, mollusks and detritus forms being the dominant food items in the western, central and eastern habitats respectively. The feeding precedence index (FP) revealed that the most abundant portion of food was subsidiary one ($50 \geq FP \geq 10$) and the highest proportions of subsidiary food were phytoplankton (24.5%), gastropods (34%) and detritus (29.11%) in the western, central and eastern habitats, respectively. Omnivorous feeding is one of the reasons for the success, high tolerance and adaptation of *M. nipponense* in the Anzali Wetland ecosystem.

Key words: *Macrobrachium nipponense*, Food behavior, Habitat, Anzali Wetland.

INTRODUCTION
Transitional waters such as wetlands are important but fragile ecosystems in the costal landscape. These water basins provide useful services to society such as water purification, erosion control and fishing resources, as well as habitat and food for migratory and resident animals. They are also an attractive site for recreational activities (Ewel 1997; Jafari 2009). Anzali Wetland is a large, shallow, eutrophic freshwater wetland, marshes, and seasonally- flooded grasslands and tourism ecosystem at the southwestern coast of the Caspian Sea in Iran (Nazarhaghighi *et al*. 2014; Ganjali *et al.* 2014). Preserving these natural habitats and this extremely diverse wetland flora and fauna is an issue of great importance (Mehrvarz & Ashouri nodehi 2015). The protection and sustainable management of all aquatic habitats requires a thorough understanding of how conditions of the habitats influence their aquatic organisms. The interactions between prey and predators are fundamental to the survival of aquatic organisms in a specific area (Domenici *et al*. 2007).
Macrobrachium nipponense (de Hann 1986), a species of Palaemonid shrimp, originated in mainland China (Chen *et al.* 2009) and is broadly distributed over East Asian (Japan, Korea, Vietnam, Myanmar, and Taiwan) (Chen *et al*. 2015). This species has been recorded from Anzali Wetland (Iran), with the first recorded specimens encountered in 1998 (Grave & Ghane 2006). Around the world, this shrimp inhabits most inland freshwater areas

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including lakes, rivers, wetlands, canals, ponds and estuarine areas (New 2002; Ma *et al*. 2012). In Anzali Wetland it exhibits high larval tolerance, a fast growth rate and rapid reproduction (Grave & Ghane 2006).

Environmental factors such as temperature, salinity, oxygen solubility and salinity play an important role in the feeding behavior and survival of aquatic organisms (Domenici *et al*. 2007). Temperature has a great influence on metabolic rate, food conversion efficiency and energy flow, and hence the growth of crustaceans (Montagna 2011). Studies on *M. nipponense* revealed that temperature and salinity strongly influence the dynamics of these species (Zoughi shalmani *et al*. 2017). Examining feeding habits and food availability is an indispensable part of the biological and functional study of any species, since the main functions of an organism (e.g., growth, development and reproduction) are dependent on its energy sources, i.e. its prey (Wootton 1992; Silva *et al*. 2007; Lima *et al*. 2014). A useful index for assessing the importance of dietary items is the feeding index (FI), which assesses the proportions of frequency of occurrence and volume of each item consumed (Kawakami & Vazzoler 1980; Lima *et al.* 2014).

The majority of studies on feeding habits of shrimp have focused on the family Penaidae (Cortes & Criales 1989/1990). Information concerning the feeding biology of *Macrobrachium* spp. (Palaemonidae) remains scarce, being limited to some reports by Collins & Paggi (1998) on *M. borelli,* Albertoni *et al.* (2003) on *M. acanthurus,* Abayomi *et al*. (2011) on *M. vollenbovenii* & Lima *et al*. (2014) on *M. caracinus*. The knowledge of the natural diet and feeding habits of an animal species is important for the establishment of its nutritional needs and interactions with other organisms (Albertoni *et al*. 2003). Nutrition studies is extremely important to make a shrimp farm profitable (Ayisi *et al*. 2017). Besides, studies of stomach contents can provide useful information about a species' feeding habits, trophic position, identifying and quantifying its food resources, and detecting preferred food items or the most used food in the environment (Taranam *et al*. 1993). As mentioned above, the knowledge of the feeding habits of *M. nipponense* is very limited. The objective of the present study was to describe the natural diet of *M. nipponense* at three habitats in Anzali Wetland.

MATERIAL AND METHODS

Study area

The natural diet of *M. nipponense* was studied in three different habitats in the western (Abkanar; 358690 'N, 4145610'E), eastern (Sheijan; 367986'N, 4139484'E), and central (Sorkhankol; 358969'N, 419513'E) basins of Anzali Wetland (37°28'N, 49°28'E), in Guilan Province, Iran. The flora and fauna of these three habitats are extremely diverse and all these water basins have different physico-chemical, morphological and phyto-ecological characteristics (Ayati 2003).

Sampling

The *M. nipponense* specimens were sampled in Anzali Wetland from spring 2016 to winter 2017. Samplings were performed during the day time, using electrofishing gear (150V) (permission granted by Department of Environment in Guilan Province, License letter code, A.P. 5830) and the shrimps gathered with small fishing hand net. A total of 367 specimens were analyzed for gut content: 126 from the western, 126 from the central and 115 from the eastern habitats. The sampled shrimps were immediately preserved in ice and sent to the laboratory at the Inland Waters Research Institute in Anzali Port City for further analysis. Total length was measured with a Collis-Vernier to the nearest mm, while the body weight was taken to the nearest 0.01 g with a digital scale. Guts were extracted with dorsal cuts in the cephalothorax and preserved in ethanol 70% for further analyses (Albertoni *et al*. 2003). Gut contents were emptied in petri dishes containing freshwater, and a large amount of the full gut content of the shrimps was fragmented. Therefore, the categorization of the dietary items were made by analyzing the hard parts of the content (carapaces, exoskeletons, shells, fish eyes, scales and spines, parts of plants), while other organisms digested quickly and without hard parts were categorized as detritus. All food items were examined using a stereomicroscope to the lowest possible taxonomic level, and the volume occupied by each item was estimated through the points (volumetric) method (Pillay 1952) in which, each food item in the stomach is allotted a certain number of points based on its volume. Empty stomachs were not considered for the estimation of frequency of dietary items and all the estimated stomachs were full. The food precedence index (FP) was used to determine the type of food (Biswas 1993) with Formula 1:

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$$FP = NSj / NS \times 100 \quad (1)$$

where: NSj = Number of stomachs with prey j, NS = Number of full stomachs

FP < 10 = random food, 50 ≥ FP ≥ 10 = subsidiary food, 50 ≤ FP = principal food.
The percentage of filled space in the stomach was estimated according to the total volume of each stomach, and categorized as follows (Euzen 1987) by Formula 2:

$$VI = ES/TS \times 100 \quad (2)$$

where: VI = Empty stomach index, ES = Number of empty stomachs, TS = Total stomachs.
If 0 ≤ VI < 20, the logical conclusion is that the shrimp is voracious; 20 ≤ VI < 40, the shrimp is fairly voracious; 40 ≤ VI < 60, shrimp is middle alimentary; 60 ≤ VI <80, shrimp is comparatively hypo alimentative and 80 ≤ VI < 100, shrimp is hypo alimentative.
Parallelograms showing the importance of the groups of dietary items in the three habitats in Anzali Wetland were constructed with the data obtained. Kawakami & Vazzoler (1980) suggested that a figure with a combination of the volume percentage on the *x*-axis and frequency of occurrence percentage on the *y*-axis represents the relative importance of the food items, as shown by Formula 3:

$$FI = Fi \times Vi / \Sigma (Fi \times Vi) \quad (3)$$

where: F_i = frequency of occurrence (percentage) of "i" item, V_i = volume (percentage) of "i" item

Physico-chemical factors
During each sampling period, water temperature was measured with a thermometer with a sensitivity of 0.1ºC. pH, total dissolved solids and electrical conductivity were measured using a pH/ EC/ TDS meter (HANNA 3100 Model) by dipping the probes into the 1 meter of water depth until the screen showed a fixed reading, in accordance with the manufacturer's instructions.
Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD**)** were measured after collecting the samples in a labeled 250 ml brown bottle. Samples were kept inside an incubator in the research laboratory at 21°C for 5 days, and then dissolved oxygen (DO) was measured. BOD_5 was obtained by subtracting the 5-day DO reading from the 0-day DO reading (American Public Health Association) (APHA 2005). The open reflex was used for determination of COD (APHA 2005).

Data analysis
Normality of data was assessed using the Kolmogorov-Smirnov test (Zar 1996). Means and standard deviations were calculated using Excel 2010.
The significant differences were calculated by one-way analysis of variance (ANOVA) using SPSS version 17.

RESULTS
The physico-chemical parameters are given in Table 1. The mean ± SD annual water temperatures during the present study were 17.4 ± 7.3 ºC, 18.1 ± 8.3 ºC, and 18.7 ± 9 ºC in western, central and eastern habitats of Anzali Wetland respectively.
DO was insufficient in summer and spring in all three habitats.
BOD and COD showed considerable decreases in the autumn in the western habitat (Table 1).
Total length, weight and seasonal variations in degree of stomach fullness of *M. nipponense* between spring 2016 and winter 2017 are given in Table 2. The highest occurrence of empty stomachs in *M. nipponense* was 99%, in winter. The VI index showed that *M. nipponense* can be a hypo alimentative (80 ≤ VI < 100) species in winter and a voracious (0 ≤ VI < 20) one in other seasons (Table 2).

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There were significant differences in VI between seasons (One-Way ANOVA, $P < 0.05$). The highest occurrence of VI in the three habitats was found in the winter, while the lowest in spring and autumn. The total length (cm) and weight (g) of the shrimps in the three habitats in winter were also lower than in the other seasons.

Table 1. Seasonal physico-chemical parameters of water recorded in the three habitats of Anzali Wetland. (DO: dissolved oxygen, T: temperature, BOD: biochemical oxygen demand, COD: chemical oxygen demand).

Habitat	Season	DO	Water T (°C)	Conductivity (µS cm^{-1})	pH	BOD (ppm)	COD (ppm)
West	Spring	7.81	19	246.67	7.76	12.33	25.67
	Summer	5.76	26.4	265.67	8.11	6.83	18.71
	Autumn	8.18	15.3	294	8.43	5.8	14.8
	Winter	8.62	9	293	8.96	10.21	20.57
Center	Spring	8.22	22	519.33	8.14	15.33	31.33
	Summer	7.56	27.7	546.07	8.11	12.47	23.73
	Autumn	8.01	13.5	443.33	7.84	7.5	16.42
	Winter	8.86	9.2	388	8.2	10.3	22.4
East	Spring	7.19	26	623.33	7.39	12.33	27.6
	Summer	5.04	26.8	638.47	7.15	7.17	20.9
	Autumn	6.46	13	702	7.52	11.64	19.9
	Winter	7.63	9.1	831.33	8.05	23.3	49.1

Table 2. Total length (cm), weight (g), number (N) and empty stomach (%) of *M. nipponense* recorded in four seasons, in the three habitats of Anzali Wetland.

Habitat	Season	TL (cm)	W (g)	N	Empty (%)
West	Spring	5.2 ± 0.03	2.9 ± 0.03	30	8.8^{b}
	Summer	4.8 ± 0.02	2.6 ± 0.01	32	12.1^{b}
	Autumn	6.6 ± 0.01	4.1 ± 0.03	44	0^{c}
	Winter	5.1 ± 0.03	1.8 ± 0.02	20	99^{a}
Center	Spring	5.7 ± 0.03	3.3 ± 0.02	30	8.6^{b}
	Summer	6.1 ± 0.02	2.6 ± 0.02	44	10.6^{b}
	Autumn	6.9 ± 0.03	4.1 ± 0.03	32	7.4^{b}
	Winter	4.8 ± 0.02	1.4 ± 0.03	20	99^{a}
East	Spring	4.6 ± 0.02	2.6 ± 0.02	30	7.6^{b}
	Summer	4.9 ± 0.01	2.5 ± 0.01	30	9.2^{b}
	Autumn	5.4 ± 0.02	2.2 ± 0.02	37	8.3^{b}
	Winter	4.2 ± 0.03	1.1 ± 0.01	18	99^{a}

Fourteen dietary items were categorized for *M. nipponense* at the three habitats in Anzali Wetland. Stomach contents also varied with respect to the habitat. The FP indices of *M. nipponense* stomachs in Anzali Wetland are illustrated in Table. 3. The most abundant portion of food in *M. nipponense* was subsidiary one (FP more than 10 and less than 50) and the most common subsidiary foods ($50 \geq FP \geq 10$) were phytoplankton 24.5%, gastropods 34%, and detritus 29.11% in the western, central and eastern habitats respectively.

The estimated feeding indices (the proportion of frequency of occurrence and volume of each dietary item in *M. nipponense* stomachs) are represented as parallelograms (Figs. 1, 2 and 3) and indicate the relative importance of each dietary item as food for *M. nipponense*.

There were significant differences in the frequency of occurrence of dietary items in *M. nipponense* between the three habitats (One-Way ANOVA, $F = 2.41$; $p < 0.05$). The food items i.e. plants, crustaceans, detritus, mollusks, bryozoans, insects and sponges showed the most variations in terms of frequency according to both habitat and season.

Our results showed that species such as phytoplankton, mollusks and detritus were the dominant items, with FI of 34.7%, 79.6% and 37.18% in western, central and eastern habitats respectively.

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Table 3. The feeding precedence index (Fb) of stomachs of *M. nipponense* recorded in three habitats of Anzali Wetland. (SF, subsidiary food; RF, random food).

Items	WestFb (%) SF	WestFb (%) RF	CenterFb (%) SF	CenterFb (%) RF	EastFb (%) SF	EastFb (%) RF
Fish	15.5	-	-	7	12.2	-
Plant	12	-	15.5	-	-	8.5
Phytoplankton	24.5	-	-	9.5	-	4.5
Gastropoda	-	9	34	-	23.5	-
Insect egg	-	7	-	-	-	-
Detritus	12	-	13.5	-	29.11	-
Ostracoda	-	4	-	-	-	-
Bivalvae	-	2	-	8	-	-
Plumatella	10	-	-	-	-	-
Shrimp	-	2	10.5	-	18.16	-
Diptera	-	2	-	-	-	-
Sponge	-	-	-	2	-	-
Cloptera	-	-	-	-	-	3.03
Azolla	-	-	-	-	-	1
Total	74	26	73.5	26.5	82.97	17.03

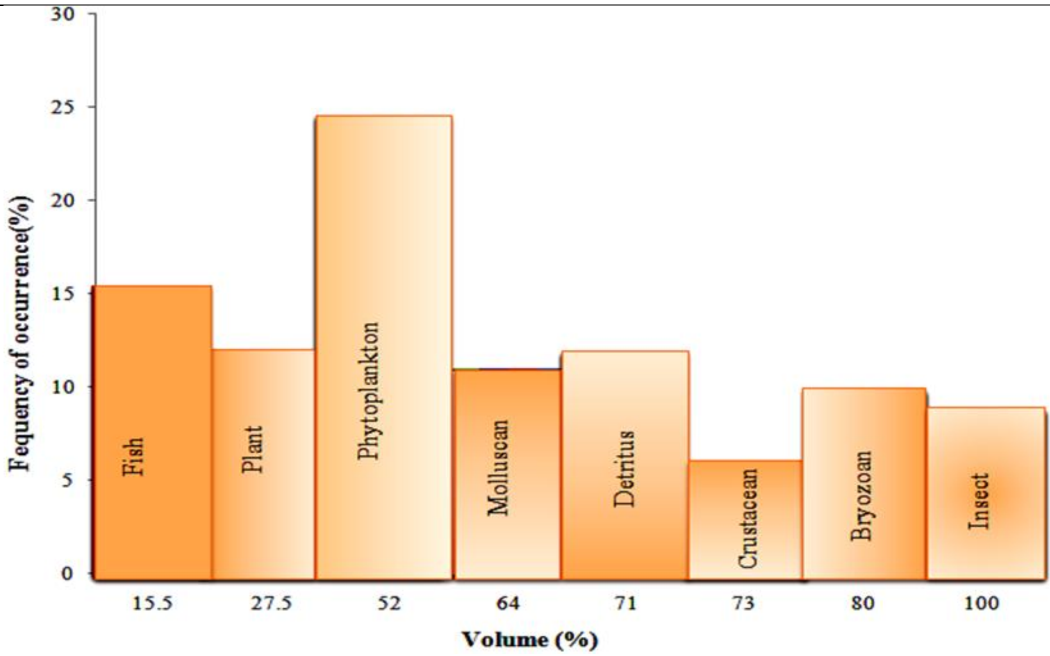

Fig. 1. Parallelograms of feeding index of *M. nipponense* samples from the western habitat of Anzali Wetland.

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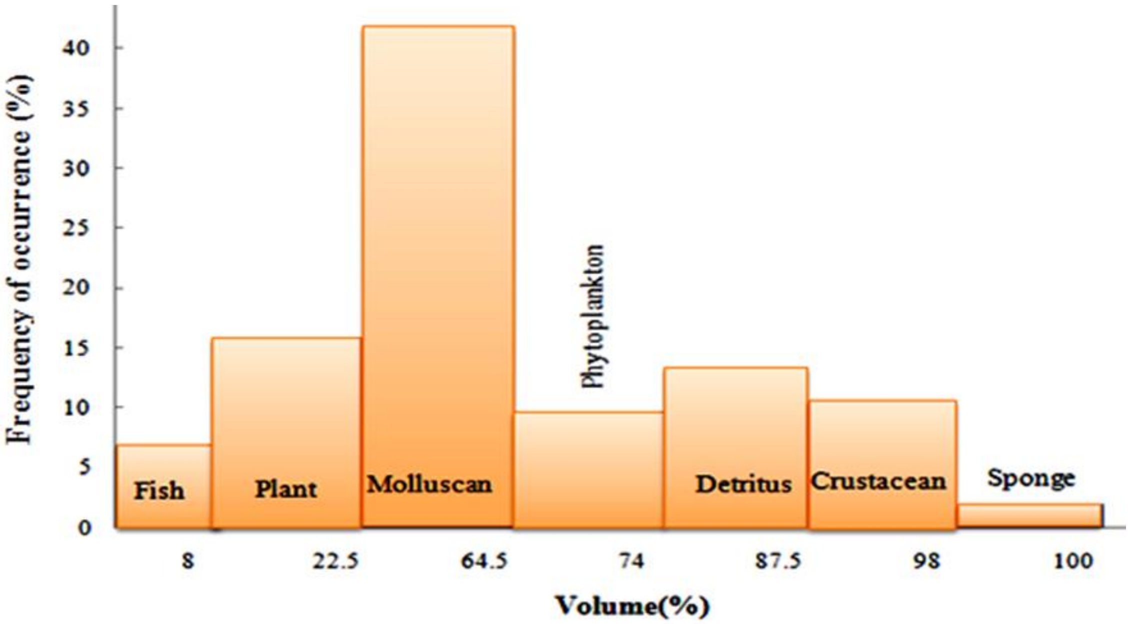

Fig. 2. Parallelograms of feeding index of *M. nipponense* samples from the central habitat of Anzali Wetland.

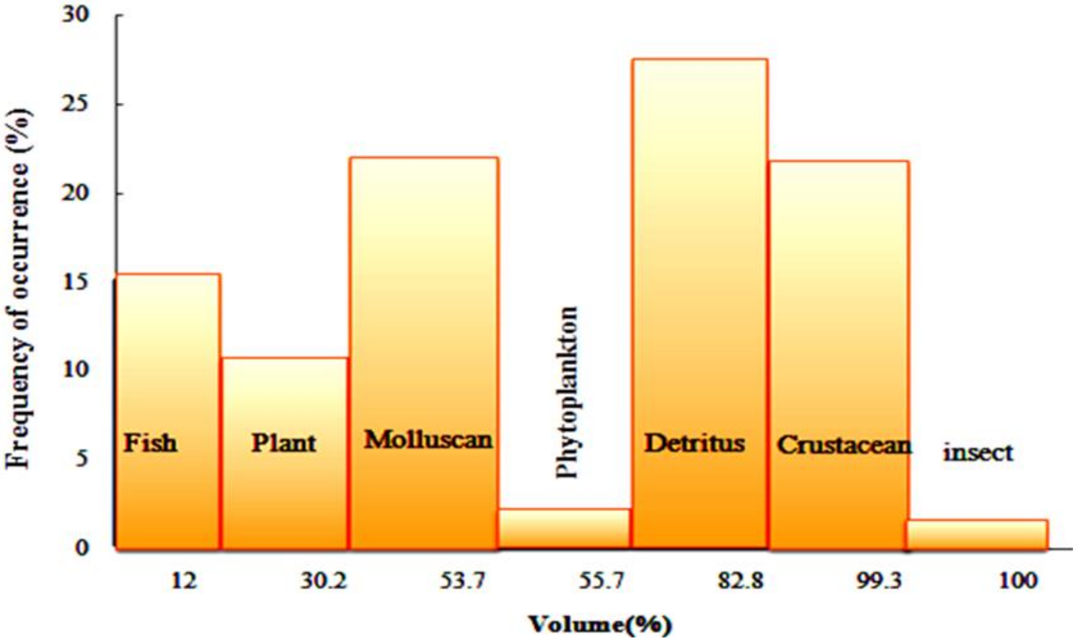

Fig. 3. Parallelograms of feeding index of *M. nipponense* samples from the eastern habitat of Anzali Wetland.

DISCUSSION

Understanding shrimp nutrition habits requires extensive field and laboratory studies in order to infer the main sources of nutrition for particular species. Feeding studies can identify the prevalence of food items, but it is still not possible to assess the diet preferences of the shrimp without detailed complementary studies capable of estimating the range and abundance of potential food items available in their natural environment. Studies on the natural diet of a species are important for the establishment of its nutritional needs and its interaction with other organisms, as well as for the evaluation of the ecosystem function and structure (Lima *et al*. 2014). Decapod crustaceans are known to be opportunistic omnivores (Albertoni *et al*. 2003) and it has been demonstrated that Palaemonids consume a variety of diets (Collins & Paggi 1998; Lima *et al*. 2014). Analyses of stomach contents of *M. nipponense* in Anzali Wetland indicate that this species is omnivorous, feeding on a wide variety of organisms including algae, aquatic insects, fish, aquatic plants, benthos, crustaceans and large quantities of organic detritus.

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The results of the present study are in line with those of previous works including Subrahmanyam (1975) on *Palaemonsty liferus*, Lee *et al.* (1980) on *M. rosenbergii*, Murthy & Rajagopal (1990) on *M. equidens,* Roy & Singh (1997) on *M. choprai,* Collins & Paggi (1998) on *M. borelli,* Sharma & Subba (2005) on *M. lamarrei,* Bello-Olusoji *et al*. (1995) on *M. macrobrachion,* Jimoh *et al*. (2011) on *M. vollenhovenii*, Sethi *et al*. (2013) on *M. lar* and Lima *et al*. (2014) on *M. acantrus*.

In the present study, phytoplankton, mollusks and detritus represented the highest percentage of natural diet of *M. nipponense* in the western, central and eastern habitats of Anzali Wetland respectively. Local availability of food and prey is probably one of the major factors influencing *M. nipponense* feeding behavior in this setting. For example, aquatic plants, phytoplankton and filamentous algae have an important impact on physico-chemical water components especially in the western habitat; they can trap excessive nutrients, increase DO through photosynthesis, decrease BOD and COD, protect larvae and other organisms as a refuge and provide food for organisms (Alonso-Rodrıguez & Paez-Osuna 2003). The high percentage of phytoplankton observed in the stomach content of *M. nipponense* in the western habitat suggested that this species may visit the upper water column more often to fulfill its dietary requirements than *Metapenaeus monoceros* (Rao 1988), *Macrobrachium choprai* (Roy & Singh 1997) or *M. lar* (Sethi *et al*. 2013). It has been suggested that aquatic plant species are not particularly rich in the central habitat of Anzali Wetland. In addition, the mean depth of water varies in this area according to season, with highest during winter and spring (i.e., above 2 m) due to river inflow, and lowest in summer due to higher evaporation and exploit for farming and other human activities (Mehrvarz & Ashouri nodehi 2015). River inflow events increase water mixing; so, currents have major effects on phytoplankton and other organisms in the water column, which can be offered to *M. nipponense* shrimp taking their food from the bottom of their habitats, especially in the central habitat (Gentien *et al*. 2007). Insufficient food and the presence of high amounts of detritus (in terms of both frequency and relative volume) are the reasons why *M. nipponense* feeds on dead material in the eastern habitat. A large amount of organic matter in its gut may suggest that this species feeds on the bottom when other more ideal alimentary substances are lacking (Roy & Singh 1997; Sethi *et al*. 2013). Thus, like other decapod species, *M. nipponense* are able to take their food from the bottom of their habitats or from the fauna associated with submerged and shore vegetation in the body of water (Williams 1981; Albertoni 2003). The presence of shrimp particles, i.e., the head, rostrum or legs of *M. nipponense* in this study might suggest that this species becomes cannibalistic when food is insufficient or when they feed on molted shrimp. The larger shrimps do not attack the smaller ones, but when one dies or becomes weak, it will be eaten by others (Sharma & Subba 2005). Quantitative consumption of the food items of animals was not stable (Azizov *et al.* 2015). Since the composition of food in *M. nipponense* depends on the seasonal dynamics of food organisms in Anzali Wetland, its higher percentage of empty stomachs in the present study may be due to a decrease in metabolic function in cold water, which reduces its prey-searching behavior in winter. Changes in abiotic factors such as temperature can significantly alter the functional and physiological response of predators (Claireaux & Lefrancois 2007). It has been reported that low temperatures shorten the season of the shrimp high activity and affect its feeding rate (Lavajoo *et al*. 2011). Wang *et al*. (2016) suggested that feeding behavior in *Homarus americanus* increased by upraising temperature. The distribution of animals and interaction with predators and food are also affected by temperature alterations (Wang *et al*. 2016). According to Niu *et al*. (2003), food consumption behavior in *Macrobrachium rosenbergii,* increased directly with temperature. In this study the lowest total length and weight in *M. nipponense* were recorded in winter. According to Lavajoo *et al*. (2013), abiotic factors such as temperature, food availability, organic matter and plant distribution influence the growth of crustaceans. The varying physiological responses by predators and prey to altered water temperature tend to decreased or increased predation efficiency (Tylor & Collie 2003). The recorded dissolved oxygen (ranged between 5 and 9) and pH (ranged between 7 and 9) in Anzali Wetland in the study interval exhibited little variation and were within the ranges reported by Santos *et al*. (2006) for *M. amazonicum,* Valenti (1996) for freshwater prawns and by Sampaio & Valenti (1996) for *M. rosenbergii.* It may therefore be assumed that in the present study, the impacts of these parameters upon the *M. nipponense* feeding behavior were negligible. The analyses of feeding items by the points (volumetric) method and frequency of occurrence have been employed in several studies on natural feeding, providing an indication of the preference for one or more categories within the population studied (Hyslop 1980). The percentage of frequency of occurrence is a measurement of the regularity of a specific food item in the diet of a sampled population.

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The present study exhibits an agreement between the percentage of volumes and the frequency of occurrence of food items, indicating that these items most frequently ingested by *M. nipponense* were also the ones found in the highest numbers in that area. However, the percentage of volumes and frequency of occurrence of food items in the stomach of *M. nipponense* differed in the three habitats.

CONCLUSION

M. nipponense is an omnivorous species, and subsidiary food makes up the main part of its diet in Anzali Wetland. Its feeding habit was dependent on the frequency of food items in the three habitats. Certain abiotic factors and the availability of food items in these habitats influenced the feeding behavior of *M. nipponense* in this study. Thus, omnivorous feeding appears to be one of the reasons for the species' success and survival in the Anzali Wetland ecosystems, increasing its tolerance and adaptation. The impact of water temperature in winter on the availability of food and the predator-prey relationship increases the VI to 99%.

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تغذیه طبیعی میگو *Macrobrachium nipponense* از سه زیستگاه تالاب انزلی، ایران

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چکیده

میگو آب شیرین *Macrobrachium nipponense* به عنوان یک گونه مهاجم که اخیرا در تالاب انزلی در ایران گزارش شده است، محسوب می‌شود. این گونه سازگاری و مقاومت زیادی را با اکوسیستم تالاب انزلی دارد. مطالعه حاضر در ارتباط با جنبه‌های خاصی از تغذیه میگو *M. nipponense* در سه زیستگاه تالاب انزلی می‌باشد. میگوها به صورت تصادفی از اپریل ۲۰۱۶ تا مارچ ۲۰۱۷ نمونه‌برداری شدند. محتویات معده ۳۶۷ نمونه با طول بین ۴/۲ تا ۶/۹ سانتی‌متر بررسی شد. شاخص تهی بودن معده (VI) نشان داد که، گونه مذکور به عنوان گونه حریص با شاخص تهی بودن معده ($20 > VI \leq 0$) در همه فصول به جز فصل زمستان با ٪۹۹ معده‌های خالی، بود. چهارده رقم غذایی در معده این میگو از سه زیستگاه شناسایی شد که فیتوپلانکتون، نرم تن و دتریت به عنوان ارقام غذایی غالب به ترتیب در ناحیه غربی، مرکزی و شرقی تالاب انزلی محسوب شدند. شاخص فراوانی غذایی (FP) نشان داد که، بیشترین سهم غذایی را غذای کمکی ($10 \leq FP \leq 50$) و بیشترین سهم غذای کمکی فیتوپلانکتون (٪۲۴/۵)، گاسترووپود (٪۳۴) و دتریت (٪۲۹/۱۱) به ترتیب در زیستگاه‌های ناحیه غربی، مرکزی و شرقی داشتند. تغذیه همه چیز خواری یکی از دلایل موفقیت، تحمل بالا و سازگاری میگو *M. nipponense* با اکوسیستم تالاب انزلی می‌باشد.

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