Knowledge and Management of Aquatic Ecosystems (2012) 405, 07
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DOI: 10.1051/kmae/2012013 http://www.kmae-journal.org

Food preference of the giant mudskipper *Periophthalmodon schlosseri* (Teleostei: Gobiidae)

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Received September 20, 2011
Revised April 25, 2012
Accepted April 30, 2012

ABSTRACT

Key-words:
P. schlosseri,
stomach content analysis,
stable isotope analysis,
δ^{13}C and δ^{15}N ratios,
food preference

The giant mudskipper (*Periophthalmodon schlosseri*) is one of the commonly found mudskipper species living and it makes a significant biomass value in the mangrove ecosystem. Samples of this mudskipper species were collected and analysed for stomach content and stable isotope ratios (δ^{13}C and δ^{15}N) to determine their food preference. The stomach content analysis showed four groups of food items: fiddler crabs (*Uca* sp.), medaka fish (*Oryzias* sp.), juveniles of indeterminate fish species and indeterminate remains of prey items. *P. schlosseri* females prefer to prey on *Oryzias* sp. (57.8%), *Uca* sp. (26.7%) and juveniles of indeterminate fish species (6.7%), while the males prefer to prey on *Uca* sp. (84.6%) and *Oryzias* sp. (7.7%). The indeterminate remaining prey items were 8.9% and 7.7% for respective sexes. The stable isotope analysis showed *Uca* sp. and *Oryzias* sp. being the main food items for *P. schlosseri*. The values of δ^{13}C and δ^{15}N ratios also showed differences in food preference among sexes, where females of all life stages prefer to prey more on *Oryzias* sp. and little *Uca* sp. In contrast, the male *P. schlosseri* prefer to prey only on *Uca* sp. throughout their life, with the exception of juvenile male *P. schlosseri,* which suggested they also consume a small amount of *Oryzias* sp. Behavioural differences among the sexes and life stages were suggested to cause differences in food selection. The size of the food items also influences food preference.

RÉSUMÉ

Préférences alimentaires du périophthalme géant *Periophthalmodon schlosseri* (Teleostei : Gobiidae)

Mots-clés :
P. schlosseri,
analyse des

Le périophthalme géant (*Periophthalmodon schlosseri*) est l'une des espèces de périophthalme couramment rencontrée et il représente une importante biomasse dans l'écosystème de la mangrove. Des échantillons de cette espèce ont été récoltés et analysés pour leur contenu stomacal et les rapports des isotopes stables (δ^{13}C et δ^{15}N) afin de déterminer leur préférence alimentaire. L'analyse du contenu

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Article published by EDP Sciences

contenus stomacaux, analyse des isotopes stables, δ^{13}C et δ^{15}N, préférence alimentaire

stomacal a montré quatre groupes de ressources alimentaires : crabes violonistes (*Uca* sp.), poissons médaka (*Oryzias* sp.), juvéniles d'espèces de poissons non identifiées et des restes indéterminés de proies. Les femelles de *P. schlosseri* préfèrent comme proies *Oryzias* sp. (57,8 %), *Uca* sp. (26,7 %) et des juvéniles d'espèces de poissons non identifiées (6,7 %), tandis que les mâles préfèrent *Uca* sp. (84,6 %) et *Oryzias* sp. (7,7 %). Les proies restantes indéterminées ont été de 8,9 % et 7,7 % pour les deux sexes respectivement. L'analyse des isotopes stables a montré que *Uca* sp. et *Oryzias* sp. étaient les principales ressources alimentaires pour *P. schlosseri*. Les valeurs de δ^{13}C et δ^{15}N ont également montré des différences dans les préférences alimentaires chez les deux sexes, où les femmes de tous les stades de vie se nourrissent plus sur *Oryzias* sp. que sur *Uca* sp. En revanche, les mâles de *P. schlosseri* préfèrent s'attaquer à *Uca* sp. tout au long de leur vie, à l'exception des mâles juvéniles de *P. schlosseri* qui consomment aussi une petite quantité de *Oryzias* sp. Les différences de comportement entre les sexes et leurs stades sont supposées causer des différences dans le choix des aliments. La taille des ressources alimentaires influe également sur les préférences alimentaires.

INTRODUCTION

The mangrove ecosystem can be considered as an important area because it supports a large diversity of terrestrial and aquatic biota. It is important in many aspects, including environmental roles (sheltering coastlines and estuaries through storm protection, shore stabilisation, and the control of coastal soil erosion and flooding), a breeding ground and nursery habitat for many biota, as well as sources for basic commercial and subsistence commodities (including foods, medicines, wood fuel and wood products) (Sathirathai and Barbier, 2001). In Malaysia, the mangrove area is estimated to be around 530 000 ha.

The mudskippers can be found on tidal flats and in mangrove swamps that formed in creeks, estuaries and coastal waters during low tide (Tytler and Vaughan, 1983; Murdy, 1989; Graham, 1997; Takita *et al*., 1999). They spend extensive periods of time out of water and have numerous physiological, morphological and behavioural specialisations for amphibious life (Clayton, 1993; Lee and Graham, 2002). In Peninsular Malaysia, there are more than eight mudskipper species that can be found in the intertidal area (Takita *et al*., 1999; Khaironizam and Norma-Rashid, 2002). The distribution of each species depends on several factors, including abundance of food items, habitat preference, human disturbance and others. The giant mudskipper (*Periophthalmodon schlosseri*) is one of the commonly found mudskipper species living and it makes a significant biomass value in the mangrove ecosystem. It is one of the biggest mudskippers and can grow up to about 24 cm in standard body length (Swennen *et al*., 1995). The dorsal part is the ground colour, dark to pale brown laterally and whitish to grey ventrally. It can easily be distinguished by a typical black stripe coursing from the eye posterior across the dorsal opercular edge, continuing from the dorsal to pectoral fin, and up to the caudal peduncle. There could be six to eight dark brown saddle-like diagonal bars visible, especially at night (Polgar, 2009).

There are several studies on foraging behaviour of mudskippers, ranging from descriptions of feeding behaviour in the field to stomach content analysis. Studies on feeding behaviour of *P. schlosseri* are very limited and are only based on field observation. It is reported as a carnivore and feeds mainly on crabs and other locally abundant animals, including syntopic species of mudskippers living on the mudflat and mangrove area (Sasekumar *et al*., 1984; Murdy, 1989; Clayton, 1993; Larson and Lim, 2005). Mazlan *et al*. (2006) reported the foraging behaviour and food selection of *P. schlosseri* mainly on the fiddler crabs (*Uca* sp.). The study also describes the frequency and time allocated for several feeding processes prior to consuming.

The stable isotope analysis has emerged as a powerful tool to also indicate the feeding habits of organisms (DeNiro and Epstein, 1978). The analysis of naturally occurring stable isotopes has been used as a complementary tool for animal diet analysis in many food-web studies (Michener and Schell, 1994; Cocheret de la Morinière *et al*., 2003; Melville and Connolly, 2003). This can be performed by analysing stable isotope ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$). Hobson *et al*. (1997) reported how changes in these ratios have been used to explain trophic relationships within marine food webs. The $\delta^{13}C$ is used to indicate relative contributions to the diet of different potential primary sources in a trophic network, indicating the aquatic *vs.* terrestrial, inshore *vs.* offshore, or pelagic *vs.* benthic contribution to food intake (Dauby *et al*., 1998; Asante *et al*., 2008). An animal is on average enriched in $\delta^{13}C$ by about 1‰ relative to its diet (DeNiro and Epstein, 1978; Michener and Schell, 1994). The $\delta^{15}N$ can be used to indicate trophic levels of organisms in the food webs. Minagawa and Wada (1984) and Post (2002) showed that the mean enrichment in $\delta^{15}N$ at a single feeding process is about 3.4‰. According to Cocheret de la Morinière *et al*. (2003), simultaneous measurements of C and N stable isotopes can provide information on source materials and trophic relationships, even though a significant temporal and spatial within-group variation in the stable isotope composition of food sources has been found, and trophic levels should be assigned and understood carefully. The objective of the present study is to investigate food preference of *P. schlosseri* according to their life stages and sexes by using stomach content analysis and stable isotope analysis.

MATERIALS AND METHODS

> STOMACH CONTENT ANALYSIS

Sampling activities were conducted from 14–16 September 2009 along the intertidal mudflat of Puloh River during lowtide. This sampling area was located at the Global Positioning System (GPS) coordinate of 3° 4.108′ N 101° 23.062′ E. This river is among the main tributaries flowing into the Klang Strait, located at the north of Port Klang, Malaysia. The types of samples that have been collected during the sampling activities were listed in Table I. The *P. schlosseri* mudskippers were observed and classified into three life stages (juvenile, subadult and adult). These stages were determined based on their standard body length, gonad presence and gonad maturity. The sex of each individual was identified by examining the shape of the urogenital papilla (King and Udo, 1998). The male mudskipper has a pointed urogenital papilla, whereas a female has a square and tapered urogenital papilla. Since the stomach content analysis is one of the standard analytical methods for studying the diets and food habits of fish and other marine invertebrates, this method was adopted into this study based on methods described by Hyslop (1980) with minor modifications. Briefly, all collected mudskippers were injected with 10% formalin and kept frozen at low temperature in an ice-box to prevent gastric digestion of the consumed foods. All samples were brought back to the laboratory for stomach content analysis. Each individual was cut ventrally along the abdomen using the dissecting tools. The gastro-intestine tract was further cut and the stomach contents were removed and immediately fixed in a 4% buffered formalin solution. All stomach content was identified using a stereomicroscope (Stemi, 2000, Carl Zeiss, Göttingen, Germany) for identification up to genus, measurement of length and body count. As for the indeterminate remains, body parts were collected to construct a complete individual of food items. It was then analysed for $\delta^{13}C$ and $\delta^{15}N$ to determine the type of sample they are. All individuals of *P. schlosseri* were also observed under a dissecting microscope to determine their sex and maturity status.

> STABLE ISOTOPE ANALYSIS

Analysis of $\delta^{13}C$ and $\delta^{15}N$ values of collected organisms was based on a method reported by Nakamura *et al*. (2008). In general, the fillet tissue of the giant mudskipper, fiddler crabs, medaka fish and juveniles of indeterminate fish species (all collected from the field), and indeterminate remains (obtained from the stomach content analysis) were washed with Mili-Q water and dried in an oven (Advantec SP650, Japan) at 60 °C for at least 24 hours or until a constant weight was obtained. A dried sample was grinded separately to a fine powder using a ceramic mortar and pestle. Each sample was placed in a dry and clean centrifuge tube. Elimination of the lipid component in the sample was conducted by adding 3 mL of a mixture of chloroform: methanol (2:1 ratio) and the extracting was conducted for 3 h. The mixture was then centrifuged at 760× g (4 °C) for 10 min using a high-speed refrigerated centrifuge (himac CR20, Hitachi Koki Co., Ltd., Tokyo, Japan). Once finished, the supernatant was discarded and the remaining pellet was dried in a vacuum desiccator for 1 h. All samples (fish tissues and the food items) were then fumed with 12 M HCl for 10 h to remove inorganic carbonates. The excess acid was subsequently removed in a vacuum desiccator with some pellets of NaOH for 3 h. The samples were dried at 60 °C before analysis. Carbon (C) and nitrogen (N) stable isotope compositions were measured with an elemental analyser connected on-line to an isotope-ratio mass spectrometer (FLASH EA/Conflo III / DELTA plus XP, ThermoFisher, Tokyo, Japan). Isotopic compositions of C and N were expressed in δ notation (δ^{13}C, δ^{15}N) as part per thousand (‰) differences from an international standard (Vienna- PeeDee Belemnite (V-PDB) for carbon; atmospheric N_2 for nitrogen). The analytical precision for the isotopic analyses was better than ±0.2‰ for both δ^{13}C and δ^{15}N.

> *DATA AND STATISTICAL ANALYSIS*

Stable isotope data were reported as the relative difference between ratios of a sample and standards in standard notation as:

$$\delta X(‰) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{std}}}\right) - 1\right] \times 1000$$

where, R is ^{13}C/^{12}C or ^{15}N/^{14}N of sample or standard, X is δ^{13}C or δ^{15}N in per-mil (‰) deviation of that sample from the recognised isotope standards. All data were analysed statistically using the SPSS Statistical Analysis Package (Version 17). One-way analysis of variance (ANOVA) was used to assess whether the stable isotope ratios varied significantly among organism sample types, life stages and sexes.

RESULTS

> *IDENTIFICATION OF* P. SCHLOSSERI *FOOD PREFERENCE BY STOMACH CONTENT ANALYSIS*

In general, the female *P. schlosseri* was larger than the male. However, the body length was not significantly different between the two sexes ($p > 0.05$), even by comparing based on their life stages (Table I). From the total individual number of fish and crabs, 53.6% of the stomach contents were identified as *Uca* sp.; 34.5% were *Oryzias* sp.; 3.6% were juveniles of indeterminate fish species, and 8.9% were the indeterminate remains, respectively. Among the female *P*. *schlosseri*, about 64% of the consumed food items were fish, including *Oryzias* sp. and juveniles of indeterminate fish species (57% and 7%, respectively). At least 27% of the food items of the female mudskipper were identified as fiddler crabs (*Uca* sp.) and 9% were the indeterminate remains. As for the male *P. schlosseri*, about 84% of the consumed food items were identified as *Uca* sp.; 8% were *Oryzias* sp., and 8% were the indeterminate remains.

> *IDENTIFICATION OF* P. SCHLOSSERI *FOOD PREFERENCE BY STABLE ISOTOPE ANALYSIS*

Stable isotope analysis was conducted on samples of *P. schlosseri* (different sexes and life stages), *Oryzias* sp., *Uca* sp. (different sizes), juveniles of indeterminate fish species, indeterminate remains (collected from stomach content analysis) and *Rhizophora* sp. leaves. The

Table I
Information of samples collected from Puloh River.

Sample type		No. of individuals (*n*)	Size (mm)	Gonad
P. schlosseri	**Male (Adult)**	5	154.75–219.83	Mature male testis
	Male (Subadult)	5	113.72–139.64	Immature male testis
	Male (Juvenile)	4	95.53–105.49	Testis absent
	Female (Adult)	6	163.28–221.93	Mature female ovary
	Female (Subadult)	5	115.03–145.62	Immature female ovary
	Female (Juvenile)	5	101.05–107.56	Ovary absent
Indeterminate remains from male *P. schlosseri*		7	–	–
Indeterminate remains from female *P. schlosseri*		6	–	–
Small size *Uca* sp.		10	<25	–
Big size *Uca* sp.		10	≥25	–
Oryzias sp.		10	21.52–33.26	–
Juvenile of indeterminate **fish species**		10	18.62–34.57	–
Rhizophora sp.		10	–	–

Table II
Stable isotope ratios of δ^{13}C and δ^{15}N in selected biota from Puloh River.

Sample	δ^{13}C (‰)	δ^{15}N (‰)
***P. schlosseri* (♂; Adult)**	–20.27	9.45
***P. schlosseri* (♂; Sub-adult)**	–20.31	9.47
***P. schlosseri* (♂; Juvenile)**	–20.73	8.87
***P. schlosseri* (♀; Adult)**	–20.36	7.60
***P. schlosseri* (♀; Sub-adult)**	–20.52	7.49
***P. schlosseri* (♀; Juvenile)**	–20.68	7.33
***Uca* sp. (Small; carapes < 25 mm)**	–21.05	5.44
***Uca* sp. (Big; carapes > 25 mm)**	–16.38	6.88
Indeterminate remains (from ♂ *P. schlosseri*)	–21.12	5.47
Indeterminate remains (from ♀ *P. schlosseri*)	–21.33	4.15
***Oryzias* sp.**	–21.40	3.82
Juvenile of indeterminate fish species	–23.27	7.41
***Rhizophora* sp.**	–26.46	2.85

values of all δ^{13}C and δ^{15}N ranged from –26.55 to –16.03‰ and 2.71 to 9.62‰, respectively. For each type of sample, the average δ^{13}C and δ^{15}N values are listed in Table II. The highest δ^{13}C was obtained from the big size *Uca* sp. with an average of –16.38 ± 0.24‰. The lowest δ^{13}C was obtained in *Rhizophora* sp. leaf samples (–27.79 ± 0.51‰). There was a significant difference of δ^{13}C value for *Rhizophora* sp., juveniles of indeterminate fish species and big-size *Uca* sp. with all other sample types ($p < 0.05$). For the food preference of different life stages of *P. schlosseri*, δ^{13}C values of juvenile, subadult and adult females demonstrated that there was no significant difference between one another ($p > 0.05$). In contrast, δ^{13}C for juveniles of male *P. schlosseri* was significantly different from subadult and adult males ($p < 0.05$).

DISCUSSION

In this study, there are four groups of food items for *P. schlosseri* collected from the Puloh River area, which include fiddler crabs (*Uca* sp.), medaka (*Oryzias* sp.), other fish (juveniles of unknown fish species) and indeterminate remains. The indeterminate remains consisted of body parts of food items. Their presence was counted based on discoveries of body parts that construct a complete body of an organism. In this study, the food preference of *P. schlosseri* based on sexes showed that the female individuals prefer to prey on small fish rather than

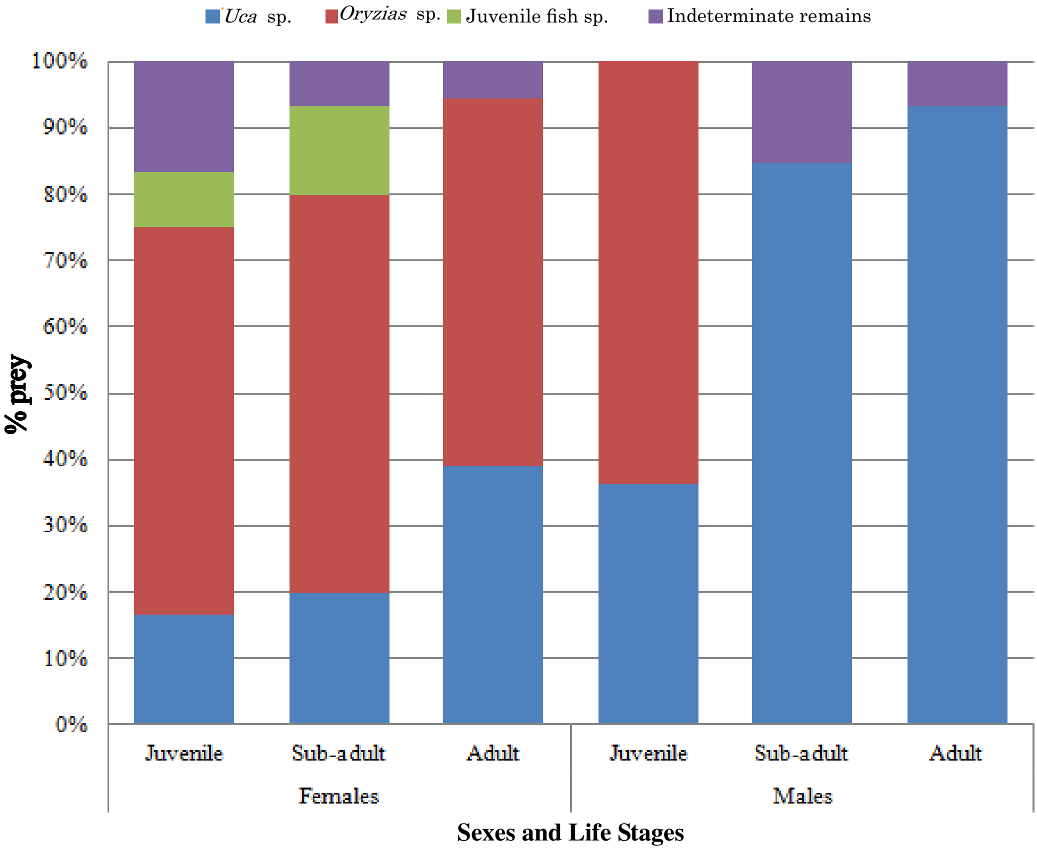
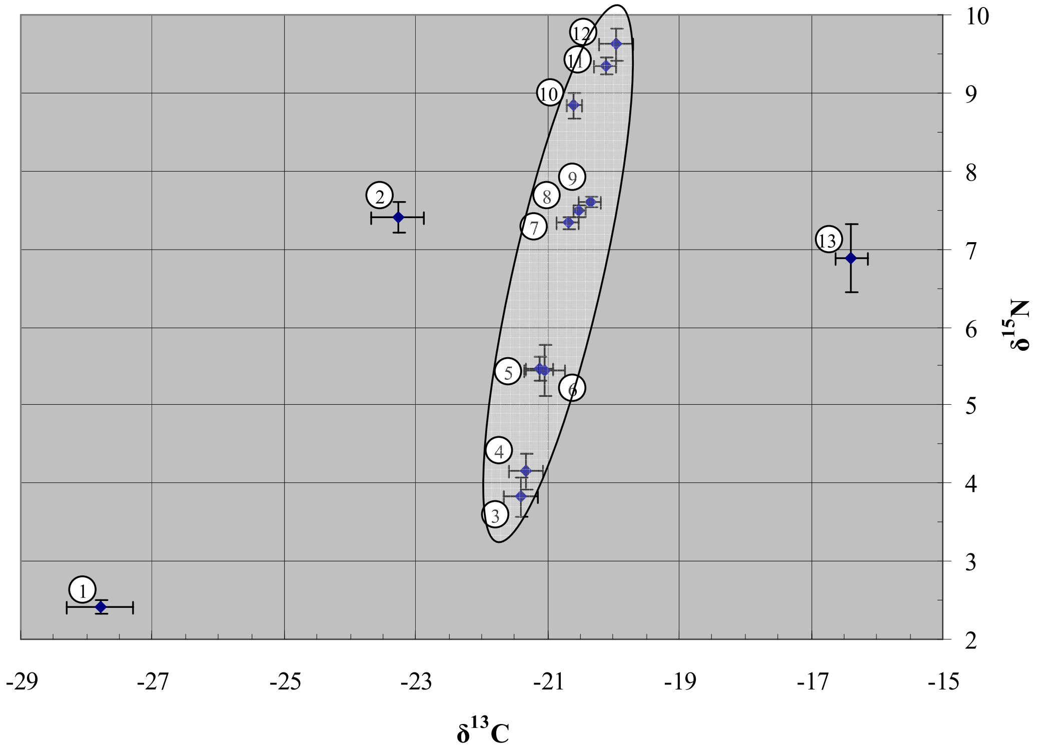


Figure 1
Percentage of prey items according life stages of female and male P. schlosseri*.*

fiddler crabs. Figure 1 showed the percentage of food items being consumed for every life stage of female and male *P. schlosseri*. At every live stage of the female *P. schlosseri*, almost 60% of the total food items were the *Oryzias* sp., thus demonstrating medaka being the main food item for the female *P. schlosseri* throughout their life. The foraging behaviour of the females tends to maintain a similar pattern throughout their lives. Individuals of *Uca* sp. were consumed in different amounts at every life stage of female *P. schlosseri*. In contrast, the male *P. schlosseri* individuals were found to prefer to prey only on *Uca* sp. However, during the juvenile life stage, the male *P. schlosseri* also prey on medaka, although not in a significant amount. The indeterminate remains in both female and male *P. schlosseri* were unable to be determined under the stereomicroscope. This type of remaining was usually found in the intestine, where parts of consumed prey/s have generally been digested. Further analysis with stable isotope analysis proposed the indeterminate remains of female *P. schlosseri* being a mixture of *Oryzias* sp. and *Uca* sp., while samples from male *P. schlosseri* demonstrated that it consisted only of *Uca* sp. (Figure 2).

The findings of this study also suggested that food preference between male and female *P. schlosseri* is generally controlled by their different behaviour. In the wild, male mudskippers were found to prefer to catch and consume *Uca* sp. than other food types. This could be due to their exploring and territorial behaviour (Murdy, 1989; Mazlan *et al*., 2006). Since *Uca* sp. could be found abundant on the mudflat and mangrove ecosystem, they are reasonable to be the main food items for male *P. schlosseri*. Preying on this crab would be an easy task for *P. schlosseri*, since they can move fast in the mud as well as their ability to dig into the mud or the crab's hole for catching their targeted prey. The male *P. schlosseri* have also been seen to spend lesser time in water or tidal pools (*pers. obs.*). This event could be the reason



Remark:

1 - *Rhizophora* sp. leaves
2 - Juvenile of indeterminate fish species
3 - *Oryzias* sp.
4 - Indeterminate remains (female *P. schlosseri*)
5 - Indeterminate remains (male *P. schlosseri*)
6 - Small *Uca* sp. (<25mm)
7 - Juvenile female *P. schlosseri*
8 - Sub-adult female *P. schlosseri*
9 - Adult female *P. schlosseri*
10 - Juvenile male *P. schlosseri*
11 - Sub-adult male *P. schlosseri*
12 - Adult male *P. schlosseri*
13 - Big *Uca* sp. (>25mm)

Figure 2
δ^{13}C and δ^{15}N to demonstrate food preference of P. schlosseri *from Puloh River.*

why no complete body or body parts of *Oryzias* sp. or juveniles of indeterminate fish species were inside the male stomach. *P. schlosseri* were reported to have a more efficient ability to obtain oxygen through their skin rather than their gills. However, they will still have to keep their gills wet by keeping a certain volume of water inside their buccal and pharyngeal cavities (Graham, 1997).

As for female *P. schlosseri*, most of the food items were mainly *Oryzias* sp. and a few juveniles of indeterminate fish species. According to Mazlan and Rohaya (2008), the breeding season of *P. schlosseri* is between June and October. Since sampling activities for the present study were conducted within the breeding season, it could be a reason for explaining the female mudskippers dwelling at the water's edge, tidal pools or nearby their burrow for protection and laying their eggs, while the males explore and guard their territory (*pers. obs.*). If there are prey items trapped in the tidal pools or swamped near the water's edge, they could be easily caught and eaten. Another possibility is female *P. schlosseri* has been chased off from the male's burrow after laying their eggs. According to Sayer (2005) and Ishimatsu *et al.* (2007), the female will be driven out from the burrow after mating. The male *P. schlosseri* assumes broodcare responsibilities. However, in other fish species, a cohabitation period of variable length occurs. In this regard they are much like the majority of gobies, with broodcare by the male being typical. The driven off female will dwelling at the water's edge and tidal pools.

Oryzias sp. is a small size fish species existing in the sampling area. Due to the body-size limitation, individuals of this species prefer to live in a slow water current area (*e.g.* near the water's edge, under a boat, etc.), water surface and semi-enclosed area (Ismail, 2011). Swimming in a big group nearby the water's edge exposes themselves to females of *P. schlosseri,* who occupy the similar aquatic area. Moreover, some individuals of *Oryzias* sp. could be trapped in the tidal pools, crab holes and mudskipper burrows during the tidal process. Once this occurs, they are exposed to predation by *P. schlosseri* females. Juveniles of indeterminate fish species were also found in the stomach of *P. schlosseri* females. However, the number was very low. This result suggests that juveniles of indeterminate fish species could coincidentally be prey.

The food preference, according to the life stages of *P. schlosseri,* demonstrated that female *P. schlosseri* have almost a similar preference throughout their life (juvenile to adult) (Figure 1). Their main food item consists of small-size fish. As for male *P. schlosseri*, the juvenile male individuals were also found preying on *Oryzias* sp., but at a lower amount. The male *P. schlosseri* were preying on small fiddler crabs as the main food item. This finding could suggest that male *P. schlosseri* have started their crab preference since the juvenile stage. As for the sub-adult and adult stages of male *P. schlosseri*, individuals at these stages are territorial and aggressively exploring the mangrove and mudflat areas. Therefore, they prefer to prey on fiddler crabs that are abundant in these areas.

In general, data on δ^{13}C and δ^{15}N from the present study is comparable to that reported by Rodelli *et al*. (1984). The carbon isotope signature (δ^{13}C) of consumers can reveal the relative contribution of a diet as potential primary sources in a trophic network. The lowest δ^{13}C was obtained in *Rhizophora* sp. leaf samples. As a plant group, *Rhizophora* sp. samples are expected to have the least δ^{13}C among all sample types of this study. Kuramoto and Minagawa (2001) also reported almost a similar value of δ^{13}C for *Rhizophora* sp. In this study, the highest δ^{13}C value was obtained from the big-size *Uca* sp. ($>$25 mm carapes length) with an average of -16.38 ± 0.24‰. In the case of δ^{15}N, the lowest value was obtained from *Rhizophora* sp. samples (2.42 ± 0.09‰), while the adult male *P. schlosseri* showed the highest (9.62 ± 0.20‰).

Results from the stable isotope analysis revealed that the *Rhizophora* sp. leaves, big-size *Uca* sp. ($>$25 mm carapes length) and juveniles of indeterminate fish species were not the main food items for *P. schlosseri,* since their δ^{13}C ratios were larger than 1‰, as reported by DeNiro and Epstein (1978) and Michener and Schell (1994). The juveniles of indeterminate fish species can be said to be a minor food item of *P. schlosseri,* since it was found in the stomach content analysis but in small numbers, particularly among the female *P. schlosseri*. It could have been preyed on after being trapped in water pools on the mudflat during low tide. Therefore, the remaining organisms to be included within the *P. schlosseri* food chain are *Oryzias* sp., small *Uca* sp. ($<$25 mm carapes length) and *P. schlosseri* itself (shaded oval in Figure 2). Food preference according to sexes and life stages of *P. schlosseri* showed that female *P. schlosseri* (juvenile, sub-adult and adult) were predicted to prey mainly on *Oryzias* sp., with occasionally consuming *Uca* sp. The δ^{13}C values of these samples were found significantly correlated through statistical analysis. Furthermore, the δ^{13}C value for the indeterminate remains collected from the intestine of female *P. schlosseri* proposed that it was a mixture of *Oryzias* sp. and *Uca* sp. The δ^{13}C value of indeterminate remains of female *P. schlosseri* was closer to the *Oryzias* sp. δ^{13}C ratio, thus proposing that the mixture mainly consisted of *Oryzias* digested tissues and a small portion of *Uca* tissues. As for male *P. schlosseri*, the δ^{13}C ratios demonstrated that adult and sub-adult male *P. schlosseri* could only have preyed on small-size *Uca* sp. However, the juvenile male *P. schlosseri* is predicted to consume a small amount of *Oryzias* sp. or some other small fish, which have caused the δ^{13}C for juvenile male *P. schlosseri* to be lower than the other male life stages.

Results of δ^{15}N ratios also showed a similar pattern to the δ^{13}C ratios for *P. schlosseri* food preference, according to sexes and life stages. The increment of the δ^{15}N ratio in about 3‰ to 3.4‰ (Minagawa and Wada, 1984; Cabana and Rasmussen, 1994; Post, 2002) between each trophic level clearly demonstrated a food preference for each sex and life stage. Results in the present study showed that the $\delta^{15}N$ value for the lower trophic level in the *P. schlosseri* food chain is about 3.93‰ (female mudskipper) and 5.44‰ (male mudskipper), respectively. The *Rhizophora* sp. $\delta^{15}N$ value was lower than the minimum range of food items for female and male *P. schlosseri*, thus confirming field observation reports (Sasekumar *et al.,* 1984; Murdy, 1989; Clayton, 1993; Larson and Lim, 2005; Mazlan *et al.*, 2006) claiming that this species is a carnivorous fish. In the case of juveniles of indeterminate fish species and big-size *Uca* sp. ($>$25 mm carapes length), although both have $\delta^{15}N$ ratios within the acceptable range, $\delta^{13}C$ ratios suggested that they are not the acceptable food choice for *P. schlosseri*.

The size of the food items could also affect food preference of any organism. In the present study, the maximum mouth opening of *P. schlosseri* was measured to be less than 25 mm. The big-size *Uca* sp. ($>$25 mm carapes length) was not found in the stomach content analysis and the $\delta^{13}C$ value from the stable isotope analysis showed that this group was out of range of an acceptable $\delta^{13}C$ ratio for a potential prey item. On the other hand, the small-size *Uca* sp. ($<$25 mm carapes length) were found in many samples of *P. schlosseri* and the $\delta^{13}C$ ratio was within the acceptable range. Elton (1927) suggested that body size is central to the structure and function of food webs. According to Layman *et al.* (2005), prey selection is influenced by size-based morphological constraints. The relationship between body size of predator and trophic position of the involved organisms in a food chain will vary as a function of predator-prey body size ratios and minimum body size of prey.

ACKNOWLEDGEMENTS

This study was jointly supported by a grant-in-aid from the Scientific Program (No. 19380224), the Japan Society for Promotion of Science (Asian CORE Program), the Research University Grant Scheme (Project No. 03-01-11-1155RU) from Universiti Putra Malaysia and the Fundamental Research Grant Scheme (Project No. FRGS/1/11/ST/UPM/02/12) from the Ministry of Higher Education, Malaysia.

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