Diet and ecological roles of the endangered big-headed turtle *Platysternon megacephalum*

Yik-Hei Sung Corresp., [1, 2] , **Billy C.H. Hau** [1] , **Nancy E Karraker** [1, 3]

[1] School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China
[2] Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China
[3] Department of Natural Resources Science, University of Rhode Island, Kingston, Rhode Island, United States of America

Corresponding Author: Yik-Hei Sung
Email address: heisyh@gmail.com

Populations of the big-headed turtle *Platysternon megacephalum* are declining at unprecedented rates across most of its distribution in Southeast Asia owing to unsustainable harvest for pet, food, and Chinese medicine markets. It was proposed for upgrading from endangered to critically endangered as a result of these declines. The ecology of Asian freshwater turtles is grossly understudied and research becomes more challenging as populations decline. Basic ecological information is needed to delineate important roles played by these species in ecosystems and to inform conservation efforts. We studied the diet and ecological roles of *P. megacephalum* in five streams in Hong Kong. Fruits, mainly of *Machilus* spp., made up >70% of the diet, but they also consumed crabs, molluscs, and beetles. Mean diet volume differed among sites, and may be explained by the history of illegal trapping of the sites. Digestion of fruits by *P. megacephalum* enhanced germination success of seeds by about 30% and suggests that these turtles may play important roles in cross-ecosystem resource subsidies. Understanding important ecological roles played by endangered species, such as *P. megacephalum*, can help to garner support for their conservation, and we recommend that ecological studies on Asian freshwater turtles to be carried out wherever robust populations remain.

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Yik-Hei Sung[1,2], Billy C.H. Hau[1] and Nancy E. Karraker[1,3]

[1] School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

[2] Present Address: Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China

[3] Present Address: Department of Natural Resources Science, University of Rhode Island, Kingston, RI 02881, U.S.A.

Corresponding Author:

Yik-Hei Sung[1,2]

E-mail address: heisyh@gmail.com, yhsung@hkbu.edu.hk

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Yik-Hei Sung[1,2,*], Billy C.H. Hau[1] and Nancy E. Karraker[1,3]

[1]School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China
[2]Present Address: Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China
[3]Present Address: Department of Natural Resources Science, University of Rhode Island, Kingston, RI 02881, U.S.A.

Corresponding author
Yik-Hei Sung
heisyh@gmail.com, yhsung@hkbu.edu.hk

ABSTRACT

Populations of the big-headed turtle *Platysternon megacephalum* are declining at unprecedented rates across most of its distribution in Southeast Asia owing to unsustainable harvest for pet, food, and Chinese medicine markets. It was proposed for upgrading from endangered to critically endangered as a result of these declines. The ecology of Asian freshwater turtles is grossly understudied and research becomes more challenging as populations decline. Basic ecological information is needed to delineate important roles played by these species in ecosystems and to inform conservation efforts. We studied the diet and ecological roles of *P.*

megacephalum in five streams in Hong Kong. Fruits, mainly of *Machilus* spp., made up >70% of the diet, but they also consumed crabs, molluscs, and beetles. Mean diet volume differed among sites, and may be explained by the history of illegal trapping of the sites. Digestion of fruits by *P. megacephalum* enhanced germination success of seeds by about 30% and suggests that these turtles may play important roles in cross-ecosystem resource subsidies. Understanding important ecological roles played by endangered species, such as *P. megacephalum*, can help to garner support for their conservation, and we recommend that ecological studies on Asian freshwater turtles to be carried out wherever robust populations remain.

INTRODUCTION

Populations of Asian turtles have been declining at rapid rates because of insatiable demand for pet, food and traditional medicine markets (*Cheung & Dudgeon, 2006*). Over 80% of species are threatened and more than 50% are listed as endangered or critically endangered by the International Union for the Conservation of Nature. Populations of most Asian turtles have declined to such low levels making makes basic ecological studies impossible for many species (*Shen et al., 2010*).

The ecological roles of most Asian freshwater turtles remain unknown. Studies in North America showed that freshwater turtles can considerably influence ecosystem processes (*Sterrett et al., 2014*) through movements of seeds and nutrients from aquatic to terrestrial habitats (*Moll & Jansen, 1995*) and potentially by enhancing seed germination. Understanding the ecological roles of and ecosystem services facilitated by endangered freshwater turtles can raise public awareness which is crucial for successful conservation (*Mace et al., 2012*).

Populations of *Platysternon megacephalum* are declining at unprecedented rates across its distribution (*Hendrie, 2000; Stuart & Timmins, 2000; Sung et al., 2013; Tana et al., 2000; Wan et al., 2015*), and thus it was proposed to upgraded from Endangered to Critically Endangered recently (*Horne et al., 2012*). However, there is no evidence that harvesting is abating. Captive breeding of *P. megacephalum* has been carried out by various zoos and hobbyists, yet it has achieved little success (*Shelmidine et al., 2016; Sung et al., 2014; Wei et al., 2016*), which may be due to our limited knowledge about their natural history (*Sung et al., 2014*). Only recently have researchers begun studying this species in the wild including distribution (*Pipatsawasdikul et al., 2010*), spatial ecology (*Shen et al., 2010; Sung et al., 2015a*), growth (*Sung et al., 2015b*) and reproduction (*Sung et al., 2014*). The information will benefit conservation programs on the species, but much remains unknown. For example, information as basic as the diet of wild individuals is lacking. This turtle was long regarded as strictly carnivorous, feeding on molluscs, crustaceans and fish, but that information was based solely on anecdotal observations (*Bonin et al., 2006; Ernst & Barbour, 1989*).

In order to develop conservation actions for endangered species, such as *P. megacephalum*, basic ecological information is needed. The objectives of this study were to quantify the diet of wild *P. megacephalum* and investigate ecological roles associated with diet.

MATERIALS & METHODS

Study area

We conducted this study in five streams in the Hong Kong Special Administrative Region, China (22°09’–22°37’N, 113°50’–114°30’E). Elevations of the study sites range from 300–800 m above sea level, and riparian vegetation is mainly secondary forest dominated by *Machilus* spp. Among the five study streams, four are located in national parks and are accessible by the public and one is in a private, fenced and patrolled conservation area. We cannot disclose the exact locations of study sites to ensure the security of these populations; we referred to study sites as KF (private conservation area), MS, SH, TO and TN. In Hong Kong, *Platysternon megacephalum* is protected under the Wild Animals Protection Ordinance Cap. 170, which prohibits any collection or use, but turtle populations in protected areas have been subjected to illegal harvesting (*Sung et al., 2013*)

Sample Collection

Turtles were captured as part of a mark-recapture study (*Sung et al., 2013*) carried out between September 2009 to June 2011, which included wet (April to September) and dry (October to March) seasons. We collected basic morphometric data on captured turtles, including straight-line carapace length (CL) using calipers, and body mass using a spring scale. We sexed turtles by examining secondary sexual characteristics, including distance of cloaca from the edge of the plastron and thickness of the tail base above the cloaca, and all turtles smaller than 105 mm in CL were considered to be juveniles (*Sung et al., 2013*). All procedures were approved by the Committee on the Use of Live Animals in Teaching and Research, the University of Hong Kong (CULATR 2249-10) and Agricultural Fisheries and Conservation Department of the Hong Kong Government.

We collected faecal samples from captured turtles that were kept in plastic enclosure with approximately two cm of water for 20 to 24 hours. We filtered the water and preserved the faecal samples in 70% ethanol. We collect samples from 61 individuals and no more than three samples were collected from a single individual. We attempted stomach flushing following Legler (*1977*) to obtain stomach contents, yet we were successful in only one of 30 attempts. We used only faecal samples in this study.

We sorted the samples under a dissecting microscope (MZ8, Leica Microsystems, Wetzlar, Germany) and identified diet items to order or lower taxonomic levels if possible. We determined the volume of each diet category in each sample by volumetric displacement to the nearest 0.1ml. We regarded samples of a diet item displacing > 0.1ml of volume as 0.05ml (*Lindeman, 2006*). Plant seed were assessed for damage and proportion damaged was documented.

Seed germination test

To investigate the impact of gut passage on germination success and rate of *Machilus* seeds, we collected undamaged digested seeds from faecal samples prior to preserving the rest of a sample in ethanol, and planted them in seed trays in a greenhouse. We collected *Machilus* spp. fruit from the bottoms of study streams and created one control treatment of seeds with fruit pulp and another control treatment in which we removed the pulp from the seeds. Seeds that passed through turtles represented the third treatment. One seed was planted in each unit of a tray beneath 1 cm of potting soil. Seeds of all treatments were planted in four trays in a randomized complete block design, with each tray containing eight to sixteen replicates of each treatment. Seeds were watered approximately three times per week, depending upon ambient temperature and drying of the potting soil. Seeds that did not germinate within half a year after planting were regarded as unviable.

Data Analysis

We calculated an index of relative importance (IRI), as modified by Bjorndal et al. (*1997*) from the original formula developed by Pinkas et al. (*1971*), for each diet category using the following formula: IRI $= 100(F_iV_i/\sum_{i=1}^{n}(F_iV_i))$, where F = percentage frequency of occurrence, V = percentage volume, i = type of diet item, and n = number of type of diet item, across all stomach content samples of a particular age and sex group (*Pinkas et al., 1971*).

We calculated niche breadth by abundance of diet items for female, male and juvenile turtles using Levin's niche breadth index, $N_B = 1/p_i^2$ where p_i is the proportion of diet category *i* of the total diet eaten by a particular age and sex group in a particular season. To evaluate diet diversity (*Gray, 2000*), we calculated the Shannon Index by abundance of diet items for female, male, and juvenile turtles using the formula, H $=- \sum_{i=1}^{n} p_i Inp_i$ where p_i is the proportion of diet category *i* of the total abundance of all diet items eaten by a particular sex in a particular season.

We compared mean abundance, volume, and species richness of diet items in faecal samples among seasons, sites, and between sexes using generalized linear mixed models with a negative binomial error variance. Diet samples from juveniles were excluded because of a low sample size. We included seasons, sites, and sexes as fixed factors, turtle identity as a random factor to account for individual variations, and carapace length of turtles as an offset to control the effect of body size (*Zuur et al., 2009*). Wet seasons, site KF, and females were regarded as the reference categories in the Wald *Z* test. We performed the analysis in R (*R Development Core Team, 2014*) using glmer.nb in the lme4 package (*Bates, 2010*).

We compared the diet composition among seasons, sites, and age and sex groups based on abundance of diet items with non-metric multidimensional scaling and analysis of similarity using Bray-Curtis similarity index. We used similarity percentage procedure to determine the contribution by each diet item to the differences among seasons, sites, and age and sex groups. We conducted the analyses using PRIMER 6.0 (*Clarke & Warwick, 2001*)

We compared germination success of seeds collected from faecal samples and controls seeds with and without pulp using a generalized linear mixed model with a binomial error variance. We included seed tray location as a random factor. Seeds from faecal samples were regarded as the reference category in the Wald *Z* test. We performed the analysis in R (*R Development Core Team, 2014*) using glmer in the lme4 package (*Bates, 2010*).

RESULTS

We collected 141 faecal samples from 61 individual turtles; 89 contained at least one item, 47 were from females [CL = 115.0 ± 12.7 mm (mean ± SD)], 33 were from males (CL = 144.0 ± 26.6 mm) and nine were from juveniles (CL = 72.0 ±17.6 mm). We identified 581 diet items belonging to 24 categories (Table 1). The most dominant food item was fruit of *Machilus* spp. which constituted over 80%, 80% and 73% of the volume of faecal samples of female, male and juvenile turtles, respectively. *Platysternon megacephalum* fed on a variety of animals including frogs, lizards, birds, fish, mammals, crabs, snails, and insects.

Mean abundance of diet items was significantly higher in the dry season than in the wet season (Table 2; Table 3), whereas it was similar between sexes, and among sites. Mean volume differed among sites, but it was similar between sexes and seasons (Table 3). Mean volume in KF, the protected site, was significantly higher than at other sites. Mean species richness did not differ between seasons, sexes, and sites (Table 3).

In general, the niche breadth in the wet season was broader than that in the dry season as shown by Shannon index (Table 3). Niche breadth of juvenile turtles was wider than that of adult turtles as revealed by Levin's standardized niche breadth (Table 3).

Diet composition was similar between seasons (R = -0.134, P = 0.99), and sexes and ages (R = -0.028, P = 0.82), but differed among sites (R = 0.339, P < 0.001; Figure 1). In pairwise comparisons, diet composition of turtles in KF and SH differed from that of other sites (KF: P < 0.02; SH: P <0.05), whereas the diet of turtles in MS, TN and TO was similar (P > 0.67). *Machilus* seeds and molluscs contributed the most to the dissimilarity between the diet of turtles in KF and other sites (SIMPER, contribution %: *Machilus* seeds = 30.1 - 36.8, molluscs = 8.42 - 11.8; Table 4). *Machilus* seeds and crabs contributed the most to the dissimilarity between the diet of turtles in SH and other sites (*Machilus* seeds = 21.9 - 30.1%, crabs = 6.5 - 17.0%).

Of seeds consumed by turtles 64% were damaged, either by mastication or during the digestion process. Of intact seeds that were planted, 37.5% (n = 32) germinated, compared with 3.6% (n=56) of control seeds with pulp removed (Z = -3.45, P < 0.001) and 2.9% (n = 35) with pulp intact (Z = -2.80, P < 0.005).

DISCUSSION

This is the first study to quantify the diet of the endangered *Platysternon megacephalum* in the wild and to assess its potential role in enhancing seed germination and cross-ecosystem resource subsidies (*Richardson et al., 2010*). *P. megacephalum* have long been regarded as carnivorous (*Bonin et al., 2006*), but we found that fruit constituted a major proportion of its diet, and we believe that this fruit is consumed within the stream channel. Fruit from *Machilus* trees made up >80% of the diet volume for males and females and >70% for juveniles. In Hong Kong, complete deforestation occurred before the Second World War (*Corlett, 1999*), and trees in the genus *Machilus* have become the dominant species in secondary forests (*Zhuang, 1997*). At least four species of *Machilus* occurs in the riparian habitats of the study streams. Fruiting of these *Machilus* species spans from March to August and October to December (*AFCD, 2008*), and the steep banks of hillstreams inhabited by *P. megacephalum* serve to channel large quantities of *Machilus* fruits downslope and into the streams. Thus fallen fruits provide a constant food supply to *P. megacephalum* through most of the year. Fruits of *Turpinia arguta* were also consumed, and consumption of *Ficus* fruits has also been reported by illegal hunters in Hainan, China (Y.H.

Sung, unpublished data). Given the broad distribution of *P. megacephalum* in Asia, they likely consume a higher diversity of fruits than observed in this study.

Germination success of *Machilus* seeds ingested by *P. megacephalum* was about 30% higher than seeds not ingested. Although about 65% of seeds in faecal samples were damaged, enhanced germination success following ingestion by this turtle compared with the very low germination success (<4%) of uningested seeds probably outweighs the damage to some seeds. Notably, *M. wangchiana* is rare in Hong Kong and its limited distribution in Hong Kong overlaps with that of *P. megacephalum* (*Herbarium, 2016*). Enhanced germination success of seeds ingested by turtles has been documented in other species (*Braun & Brooks Jr, 1987; Cobo & Andreu, 1988; Rust & Roth, 1981*), but most studies have focused on tortoises that both ingest and defecate seeds in terrestrial habitats.

To our knowledge, only two studies (*Kimmons & Moll, 2010; Moll & Jansen, 1995*) have examined the effects of ingestion by aquatic turtles on seed germination. Given life histories of the focal species of those studies, each would be capable of ingesting seeds in an aquatic habitat and defecating them in a terrestrial habitat, thereby transporting seeds from aquatic habitats where germination is unlikely to terrestrial habitats where it is possible. However, of three species examined, ingestion of plant seeds by *Rhinoclemmys funerea* in Costa Rica (*Moll & Jansen, 1995*), and *Trachemys scripta* and *Chelydra serpentina* (*Kimmons & Moll, 2010*) in the US did not enhance germination. Ingestion of seeds by *P. megacephalum* increases germination success but this is only beneficial if the turtle periodically leaves the aquatic habitat.

In previous research on this species' spatial ecology (*Sung et al., 2015a*), we found that individuals are highly aquatic and make few movements away from the stream. However, we believe that we probably underestimated terrestrial movements because turtles probably move into terrestrial habitats during extreme storm events in the monsoonal wet season, when these high velocity, torrential streams are far too dangerous to be visited by researchers. For example, on 15 days between May and September 2010, there were rainstorms of a severity level (*Hong Kong Observatory, 2016*) that would likely have driven turtles out of streams and have made the streams too dangerous for researchers. In addition to leaving streams during major storm events, females must also leave streams to nest. In mainland China, translocated turtles purchased from markets spent about 7% or their time on land (*Shen et al., 2010*), but it is not known how their habitat use differs from that of turtles in their original streams. Although probably contributing to seed dispersal, the role of *P. megacephalum* may be less important than that of other groups, such as frugivorous birds (*Corlett, 2011*).

Importance of animals in the diet of *P. megacephalum* may be more pronounced than it appeared in this study. We found that the most dominant animal prey items were crabs, molluscs and beetles, which all have hard exoskeletons. Remains of small or soft-bodied animals, such as earthworm, were underrepresented in faecal analysis and thus the relative importance of fruits may be overestimated has been suggested in some other omnivorous turtles (*Caputo & Vogt, 2008*). Stable isotope analysis may also be useful to elucidate its trophic level in the ecosystem (*Bearhop et al., 2004*).

Occurrence of bird feathers and rodent bones in faecal samples suggested that *P. megacephalum* may be opportunistic scavengers. Turtles, such as *Macrochelys temminckii*, have been reported to scavenge on mammals (*Elsey, 2006*). As densities of *P. megacephalum* can be relatively high in protected populations (*Sung et al., 2013*), the high standing biomass and an omnivorous feeding ecology may underscore their importance in nutrient recycling (*Sterrett et al., 2014*) in aquatic ecosystems and between the land-water interface.

Diet composition of *P. megacephalum* differed between study streams and this may be associated with the different demographic differences among sites. *P. megacephalum* may exhibit ontogenetic shifts in diet, for example from carnivorous to increasingly herbivorous with age, as has been documented in other freshwater turtles (*Chen & Lue, 1998; Parmenter & Avery, 1990; Spencer et al., 1998*). We found that juvenile *P. megacephalum* exhibited lower relative importance of plants and wider niche breadth than adults. Illegal trapping has depleted populations resulting in lower densities of large adults in all study streams except in KF, the private conservation area (*Sung et al., 2013*). This may explain the higher abundance and diversity of diet items in from turtles in the KF population, which exhibits the sex and age structure of a healthy population (*Sung et al., 2013*) It should be noted, however, that we do not have data on availability of diet items, so we cannot disregard this explanation.

CONCLUSIONS

Ecological roles of Asian freshwater turtles have garnered little attention as rapid population declines and low densities of most species have limited opportunities for ecological study. We found that *P. megacephalum* are important in the food chain may facilitate important ecological processes, including cycling of plant and animal matter in the aquatic ecosystem and enhancing seed germination across the aquatic-terrestrial habitat interface. Although *P. megacephalum* have disappeared across much of China (*Lau & Shi, 2000; Shi et al., 2007*), populations remain in Hong Kong. We recommend further studies to identify important roles played by this endangered species in aquatic and terrestrial habitats while populations remain. Such information on this species and other freshwater turtle species in Asia may lead to greater awareness about the need for conservation and will help to inform captive breeding programs.

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Table 1. **Volume, frequency of occurrence and indext of relative importance of food items.**

Percentage volume (V_i), percentage frequency of occurrence (F_i) and index of relative importance (IRI) of different food items in the diet of *Platysternon megacephalum* in five streams in Hong Kong between 2009 and 2011.

Food items			Adult female (n=33)			Adult male (n=47)			Juvenile (n=9)		
			V_i	F_i	IRI	V_i	F_i	IRI	V_i	F_i	IRI
Plant	Lauraceae	*Machilus* fruit	80.5	22. 6	91.3	80.2	31.7	94.1	73.2	31.6	87.2
	Staphyleaceae	*Turpinia* fruit	1.0	1.5	0.1	0.5	2.9	0.1	<0.1	<0.1	<0.1
		Other plant matter	3.4	16.5	2.8	4.3	12.5	2.0	10.6	21.1	8.4
Animal	Amphibia	Frog	0.2	0.7	<0.1	0.2	1.9	<0.1			
	Aves	Bird	0.3	1.5	<0.1	0.4	1.0	<0.1			
	Mammalia	Rodent				0.4	1.0	<0.1			
	Pisces	Fish	1.6	2.3	0.2	1.2	1.9	0.1			
	Reptilia	Lizard	<0.1	0.8	<0.1						
	Crustacea	Crab	4.6	10.5	2.4	4.1	9.6	1.4	4.1	5.3	0.8
	Mollusca	Snail	1.5	7.5	0.6	1.3	11.5	0.5	2.1	10.5	0.8
	Insecta	Coleoptera	5.0	9.0	2.3	6.9	6.7	1.7			
		Diptera	0.2	0.8	<0.1	<0.1	1.92	<0.01			
		Ephemeroptera	<0.1	0.8	<0.1						
		Homoptera	0.1	2.3	<0.1	<0.1	2.9	<0.1	3.8	10.5	1.5
		Hymenoptera	0.2	10.5	0.1	<0.1	4.8	<0.1	0.1	5.3	<0.1
		Hymenoptera	<0.1	0.8	<0.1						
		Isoptera	0.1	3.0	<0.1	0.3	2.9	<0.1	2.7	5.3	0.5
		Lepidoptera	0.3	0.7	<0.1	0.1	1.0	<0.1			
		Mantodea	<0.1	0.8	<0.1						
		Odonata	0.4	3.0	0.1	0.1	2.9	<0.1	0.7	5.3	0.1
		Orthoptera	0.1	0.7	<0.1	<0.1	1.0	<0.1			
		Trichoptera	0.2	0.7	<0.1	<0.1	1.0	<0.1			
		Unidentified insect	<0.1	2.3	<0.1	<0.1	1.0	<0.1			

Unidentified matter	<0.1	0.7	<0.1		2.7	5.3	0.5

Table 2. **Abundance, volume and species richness of diet items.** Mean (± SD) abundance, volume, and species richness of diet items, Levin's standardised niche breadth, and diversity [as Exp (*H'*)] in the diet of *Platysternon megacephalum* in five streams in Hong Kong between 2009 and 2011.

Age and sex group	Season	*n*	Abundance	Volume	Species richness	Niche breadth index	Exp (*H'*)
Female	Wet	37	4.6 ± 2.3	4.4 ± 3.4	23	0.08	5.75
	Dry	10	7.0 ± 8.1	8.0 ± 13.5	6	0.08	3.67
Male	Wet	26	5.6 ± 5.0	13.8 ± 14.4	17	0.09	5.93
	Dry	7	7.7 ± 7.4	6.9 ± 8.0	7	0.04	2.56
Juvenile	Wet	6	2.7 ± 2.1	1.4 ± 1.0	7	0.14	5.10
	Dry	3	3.7 ± 3.1	1.7± 1.6	5	0.10	4.01

Table 3. **Statistical results (GLMM) comparing abundance, volume and species richness of diet items between seasons, sexes and sites.** Estimates of parameters that significantly affect mean abundance and volume of diet items of *Platysternon megacephalum* in five streams in Hong Kong between 2009 and 2011. Significant ($P < 0.05$) variables are in bold.

Variables	Factors	Estimates	Standard error	Z	P
Abundance					
	Season (Dry)	**0.591**	**0.242**	**2.441**	**0.015**
	Sex (Male)	-0.065	0.211	-0.311	0.756
	Site (MS)	-0.714	0.454	-1.574	0.116
	Site (SH)	-0.026	0.278	-0.094	0.925
	Site (TN)	-0.483	0.390	-1.238	0.216
	Site (TO)	-0.367	0.335	-1.097	0.273
Volume					
	Season (Dry)	0.267	0.315	0.849	0.396
	Sex (Male)	-0.270	0.272	-0.996	0.319
	Site (MS)	**-1.694**	**0.592**	**-2.861**	**0.004**
	Site (SH)	**-0.615**	**0.350**	**-1.756**	**0.079**
	Site (TO)	**-1.172**	**0.433**	**-2.709**	**0.007**
	Site (TN)	**-1.792**	**0.558**	**-3.212**	**0.001**
Species richness					
	Season (Dry)	0.240	0.190	1.262	0.207

	Sex (Male)	-0.164	0.137	-1.190	0.234
	Site (MS)	-0.132	0.298	-0.443	0.658
	Site (SH)	0.065	0.174	0.375	0.707
	Site (TO)	0.042	0.211	0.201	0.841
	Site (TN)	0.176	0.260	0.677	0.498

Table 4. **Five most dominant food items in different study streams.** Index of relative importance of five most abundant food items in the diet of *Platysternon megacephalum* in five study streams in Hong Kong between 2009 and 2011.

	KF	MS	SH	TN	TO
Sample size	46	6	20	7	10
Mean carapace length (mm)	132	102	110	102	119
Diet item					
Machilus fruit	88.9	32.0	80.2	5.0	5.6
Crabs	1.5	44.7	1.3	65.4	37.4
Other plant matter	6.0	7.3	9.2	7.3	53.7
Coleoptera	2.4	14.6	7.4	2.3	20.2
Turpinia fruit	0.1	0.0	0.0	16.4	0.0

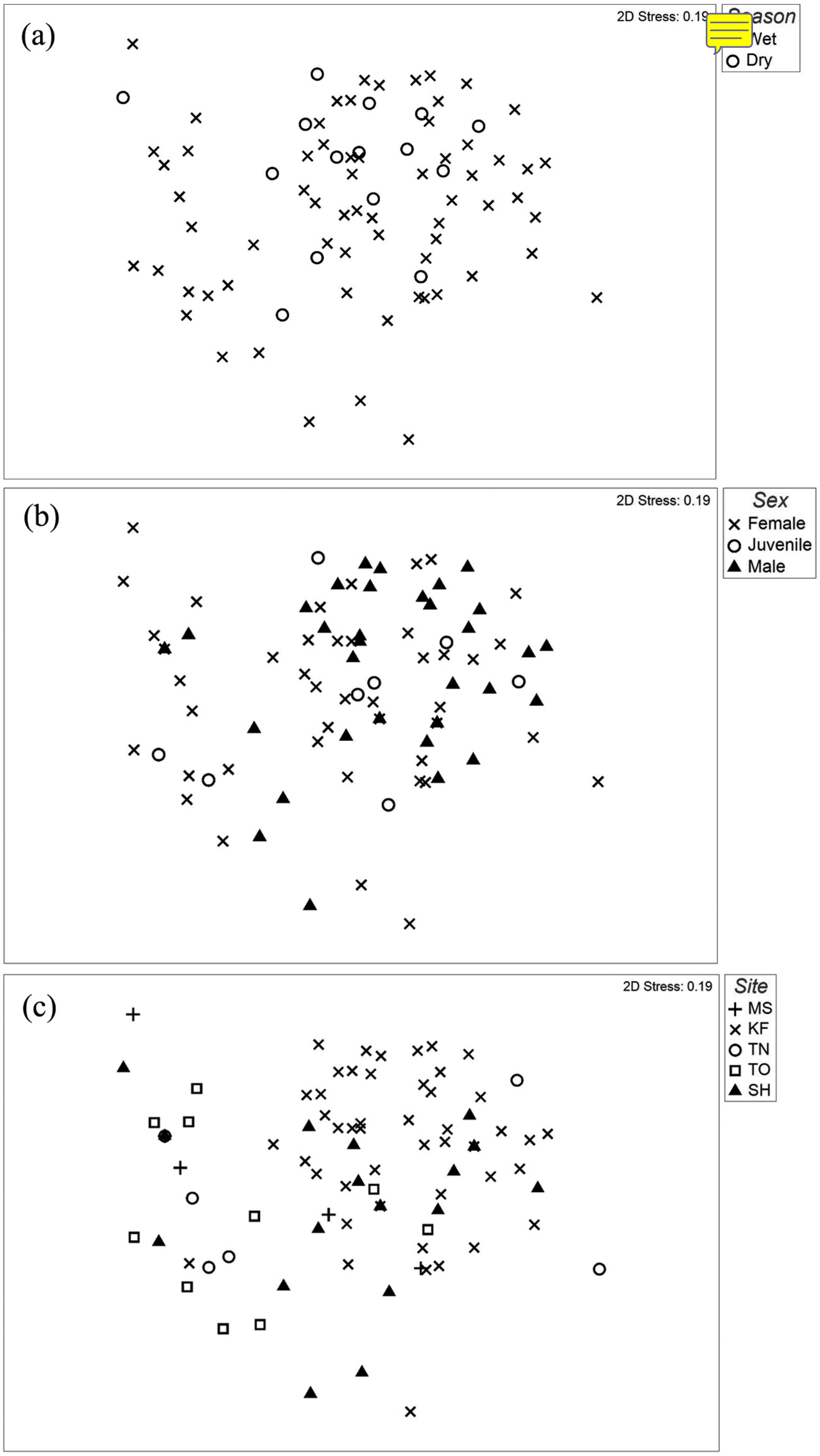

Fig. 1. **Composition of food items in different seasons, by different age and sex groups and in different sites represented on nMDS plot.** Two-dimensional non-metric multidimensional scaling representing Bray-Curtis distances among composition of food items consumed by

Platysternon megacephalum (A) in different seasons, (B) by different age and sex groups and (C) in different study sites in Hong Kong between 2009 and 2011.