RESEARCH ARTICLE

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Assessing the diet of the endangered Beale's eyed turtle (*Sacalia bealei*) using faecal content and stable isotope analyses: Implications for conservation

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Abstract

- 1. Turtles from Asia are on the brink of extinction with 53% of species considered endangered or critically endangered. Unfortunately, the ecology of many threatened species remains largely unknown.
- 2. In this study, the diet of the endangered Beale's eyed turtle (*Sacalia bealei*) was investigated using two methods, visual faecal content analysis and stable isotope analysis.
- 3. Results from both methods indicated that *S. bealei* is highly reliant on riparian resources, especially fruits and terrestrial insects. Stable isotope data indicated that terrestrial resources made up around half (47–53%) of all assimilated food resources. These findings suggest that *S. bealei* facilitates energy flow from riparian forests to stream ecosystems. Moreover, *S. bealei* is likely to be a seed disperser for riparian plants.
- 4. This study represents the first application of stable isotope methods to examine the diet of Asian freshwater turtles and their resource use. We stress the importance of similar studies to improve our understanding of remnant turtle populations before they disappear as a result of human activities.

KEYWORDS

Allochthonous resource, Asian turtle crisis, chelonian, ecological role, foraging ecology, freshwater stream, Hong Kong

1 | INTRODUCTION

More than 60% of all turtle species are threatened with extinction, making them one of the most imperilled animal groups in the world (Rhodin et al., 2018). The loss of turtle species is likely to have wider ecosystem consequences as turtles and tortoises are important for seed dispersal, nutrient cycling and soil bioturbation (Rhodin et al., 2018). Unfortunately, knowledge about the ecological role of turtles is incomplete – freshwater species in particular are

under-represented in the literature (Lovich et al., 2018; Karraker et al., 2020). This information gap is considerable in Asia, a global hotspot of freshwater turtle diversity and endemism (Buhlmann et al., 2009). The lack of basic ecological knowledge is compounded by challenges in studying rare species (Shen, Pike & Du, 2010), and these challenges undermine measures taken to mitigate rapid declines of turtle populations (Horne, Poole & Walde, 2012).

Ecological data, especially those pertaining to a species' diet, can benefit conservation efforts. For example, the reproductive success of

turtles fed with different diets differs (da Costa Araujo et al., 2013). In general, the reproductive success of captive-bred turtles may be higher when fed diets in line with the feeding habits and preferences of wild populations, boosting the success of current conservation measures (Gong et al., 2017; Rhodin et al., 2018) by providing a consistent source of captive-bred individuals to supplement wild populations (Horne, Poole & Walde, 2012).

Diet is also indicative of a species' ecological role. Many freshwater turtles are omnivores, consuming a wide variety of food items (Bonin, Devaux & Dupre, 2006). As such, turtles are a potential trophic link between terrestrial and aquatic habitats, facilitating energy flow through consumption of terrestrial resources (e.g. invertebrates or plant matter) and egestion in water (Baxter, Fausch & Saunders, 2005; Correa & Winemiller, 2018). A more comprehensive understanding of trophic positions of turtles may help clarify this resource pathway, improving our understanding of the roles of freshwater turtles in an ecosystem.

Beale's eved turtle (Sacalia bealei), one of the many threatened Asian turtle species, is endemic to south-eastern China (Anhui, Fujian, Guangdong, Guangxi, Guizhou and Jiangxi Provinces, as well as Hong Kong; Shi et al., 2008). Wild populations of S. bealei are rare across most of its native range because they have been hunted for the food and pet markets (Cheung & Dudgeon, 2006; Sung & Fong, 2018). Consequently, S. bealei is now categorized as 'Critically Endangered' on the IUCN Red List (Horne, Poole & Walde, 2012). While recent investigations revealed the species' habitat use (Hu et al., 2016) and reproductive biology (Lin et al., 2018), much about the ecology of S. bealei remains unknown. This study assessed the diet of two wild S. bealei populations in Hong Kong as part of a continuing territory-wide survey that began in 2009. Two complementary approaches were used: visual faecal content analysis and stable isotope analysis (Nielsen et al., 2017), to characterize the diet of S. bealei and identify the main source of dietary components (i.e. animals or plants, terrestrial or aquatic). The research had three objectives to help conservation: (i) to provide insight into the interdependence between S. bealei and riparian forests; (ii) to enhance our understanding of the ecological role of S. bealei, which will provide essential information for education on freshwater turtle conservation; and (iii) to inform the feeding regime of conservation breeding programmes.

2 | METHODS

2.1 | Study sites

This study was conducted in two rocky hill streams in the Hong Kong Special Administrative Region (Figure 1). The two streams are located 200 m a.s.l. in catchments comprising secondary forests. Canopy cover at both sites ranges between 70 and 95%. Although both sites are located in country parks (equivalent to protected areas or national parks) where turtle hunting is illegal, signs of poaching (e.g. baited traps) were regularly observed. Exact locations are not disclosed to SUNG ET AL.

protect these populations, and henceforth the two study sites are referred to as sites A and B.

2.2 | Sample collection and preparation

In the two study sites, 20 mark-recapture surveys were done following the methods in Sung, Karraker & Hau (2013) between 2015 and 2019. All surveys were carried out between March and October. For all captured *S. bealei*, straight-line carapace length and plastron length were measured using calipers (to the nearest 0.1 mm) and mass using a spring balance (to the nearest 10 g for turtles >100 g, and 1 g for turtles <100 g). Turtles were individually marked with microchips (Buhlmann & Tuberville, 1998) and shell-notching (Cagle, 1939). All turtles with carapace length ≥95 mm exhibited secondary sexual characteristics. Adult males exhibit darker heads with red stripes and markings, and the heads of females are brown or olive with yellow stripes (Shi et al., 2008). Although secondary sexual characteristics of turtles may appear before reproductive maturity, these individuals were categorized as adults, as there are currently no data on size or age of maturity for *S. bealei*.

Captured turtles were transported to the laboratory for collection of faecal samples. Individuals were held in the laboratory in plastic enclosures with 2–5 cm of water for 24–48 h (Sung, Hau & Karraker, 2016). Water from the enclosure was filtered and any faecal material was preserved in 70% ethanol. Faecal matter was sorted using a dissecting microscope at 20× magnification (Leica Wild M3C; Leica Microsystems, Wetzlar, Germany). All dietary items were classified to Orders, or lower taxonomic levels where possible.

For stable isotope analysis, nail tips (<5 mm) were collected from the hindlimbs of individuals using pet nail clippers (Marques et al., 2017; Marchand, Poulin & Somers, 2018). All nail samples were collected starting in mid-2016 between March and April (spring) and between August and October (summer) (Figure S1). Some individuals with faecal samples (collected in 2015) do not have nail samples for stable isotope analysis. A *t*-test showed there to be no significant difference between the two seasons in δ^{13} C (*t* = 0.37, *P* = 0.72) and δ^{15} N (*t* = 1.94, *P* = 0.09), and therefore no seasonal effect on isotopic signals of turtles was assumed.

To collect food items that turtles are likely to consume, kick netting, dip netting and hand collecting were used in the streams and riparian forests between July 2018 and May 2019. Potential dietary items collected included terrestrial plants (seeds and leaves; n = 23 (20 species)), aquatic insects (beetle larvae, beetle adults, caddisfly larvae, odonate larvae, stonefly larvae, water striders; n = 18 (10 species)), crabs and shrimps (n = 8 (3 species)), fish (n = 5 (2 species)), terrestrial insects (ants, bees, beetles, butterflies, cicadas, cockroaches, moths, orthopterans, odonates, wasps; n = 32 (23 species)) and other terrestrial anthropods (spiders and millipedes; n = 5 (2 species). The adult forms of some aquatic larvae (e.g. caddisflies, stoneflies) were not included because of their low abundance around the study streams. All samples collected for stable isotope analysis were frozen after collection and oven-dried at 60° C

FIGURE 1 Habitat of Beale's eyed turtle (*Sacalia bealei*) in Hong Kong, with the insets showing an adult male and female



for 48 h. The guts of potential invertebrate prey were removed to minimize contamination by unassimilated matter (Lau, Leung & Dudgeon, 2009). Lipids were not removed. The oven-dried samples were homogenized to a fine consistency using a mortar and pestle or a homogenizer (Bioprep-24; Hang-zhou Allsheng Instruments Co. Ltd, Hangzhou City, China). Subsequently, the samples were weighed and transferred to tin capsules for measurement of δ^{15} N and δ^{13} C using a continuous flow isotope ratio mass spectrometer at the Stable Isotope Laboratory of the University of Hong Kong.

All procedures in this study were approved by two departments of the Hong Kong government: Department of Health (DH/SHS/8/2/6 Pt 1), and the Agriculture, Fisheries and Conservation Department (AF GR CON 09/51 Pt 6).

2.3 | Data analysis

For faecal samples, the frequency of occurrence of each dietary item was calculated as the proportion of individuals that contained a particular dietary item (Bowen, 1996). Comparisons of diets were made between: (i) age groups (juveniles and adults); (ii) sexes (adult males and adult females); and (iii) populations (study sites). As multiple faecal samples were sometimes collected from the same individuals, distance-based redundancy analysis was used to visualize diet composition and assess differences between groups (Legendre & Anderson, 1999). This allowed non-independence of data collected from the same individuals to be controlled.

Unlike faecal samples, stable isotope data were collected from only one of the two wild populations surveyed (from site A). This was because *S. bealei* populations declined significantly during the survey duration as a result of illegal trapping activities. Consequently, the isotope sample size for site B was too small (n = 4) and was thus removed from subsequent analyses. The isotope data were corrected to account for isotopic fractionation between trophic levels following the values determined from claw samples of *Trachemys scripta* (Aresco, Travis & MacRae, 2015), the only reference values available for freshwater turtles. Lipid-normalization of δ^{13} C profiles of potential food items was done using equations from Post et al. (2007): for terrestrial animals with a C:N ratio ≥4, for aquatic animals with C:N ≥ 3.5 and for plants with %C ≥ 40%. Average δ^{13} C and δ^{15} N values were used if multiple data points were collected from the same individual during the surveys.

For stable isotope data, total convex hull area (*TA*), sample sizecorrected standard ellipse area (*SEAc*) and Bayesian standard ellipse area (*SEAb*) were calculated to compare isotopic niche area and niche overlap between sexes and age groups (Jackson et al., 2011). Bayesian credible intervals for *SEAb* were determined over 10^5 iterations, and the niche sizes of sexes or age groups were considered to be different if the respective credible intervals did not overlap.

The relative contribution of food items to the diet was quantified using Bayesian mixing models. To increase the robustness and accuracy of estimations, only stable isotope values from dietary items that were recovered from faecal samples were included. Food items were classified according to the following functional groups: (i) aquatic omnivorous insects (beetles and caddisfly larvae); (ii) aquatic predatory insects (odonate and stonefly larvae, and backswimmers); (iii) crabs; (iv) fishes; (v) terrestrial plants (seeds, flowers, and leaves); (vi) shrimps; (vii) terrestrial herbivores (beetles, moths and groundhoppers); (viii) terrestrial omnivores/decomposers (cockroaches and ants); and (ix) terrestrial predators (hornets, odonates and spiders). Each mixing model was run for over 10^5 iterations on four chains (thinning interval = 1).

All statistical analyses were conducted in the *R* environment (R Core Team, 2019), including the *vegan* package (Oksanen et al., 2007) for distance-based redundancy analysis, *SIBER* (Jackson

et al., 2011) for estimation of standard ellipse areas and *simmr* (Parnell, 2016) for Bayesian stable isotope mixing models.

3 | RESULTS

3.1 | Visual faecal content analysis

Faecal samples were collected from 35 individuals from two study sites (site A = 25, site B = 10) and 255 items belonging to nine categories were identified. The most dominant dietary items in faecal samples were fruits or seeds, other plant matter (leaves, fibres, flowers and woody branches) and terrestrial insects (ants, bees, beetles, cockroaches, grasshoppers and groundhoppers) (Table 1). Fruits or seeds recovered belonged to Celtis sp., Diospyros sp., Elaeocarpus sp., Garcinia oblongifolia, Ilex sp., Machilus chekiangensis and Microcos nervosa. Other items included aquatic insect larvae (beetles. caddisflies and odonates). crabs (Cryptopotamon anacoluthon), earthworms, freshwater snails (Sulcospira hainanensis), mites and spiders. In addition, parasites (nematodes) and inorganic matter (sand and stone) were found. The composition of diet items in faecal samples did not differ between ages (F = 0.799, P = 0.53; Figure 2a), sexes (F = 1.452, P = 0.24; Figure 2b) or sites (F = 1.198, P = 0.32; Figure 2c).

3.2 | Stable isotope analysis

The isotopic niche sizes (TA, SEAc and SEAb) were similar between age groups and sexes (Table 2, Figure 3). There was a 30% (SEAb) niche

overlap between adults and juveniles, with males and females overlapping by 23% (Figure 3). Bayesian mixing model outputs suggest that terrestrial omnivores (ants and cockroaches) were the most important dietary components for all individuals across sexes and age groups (Table S1; Figures 4 and 5). Overall, *S. bealei* obtains 47–53% of its dietary needs from terrestrial food sources (Table S1; Figure 5).

4 | DISCUSSION

Many Asian freshwater turtles are threatened. Little is known about their basic ecology and still less about their roles in ecosystems. Through examining the diet of the endangered *S. bealei* using two methods, visual faecal content analysis and stable isotope analysis, this study provides data that will aid in the conservation of freshwater turtles. In addition, the results offer insights into the ecological roles of freshwater turtles, shedding light on the potential consequences of the loss of turtle populations in Asia.

The results showed that *S. bealei* is highly reliant upon dietary items from riparian habitats, with more than 47% of assimilated food being attributed to terrestrial resources for all sexes and age groups (Figure 5). The stable isotope data showed that terrestrial omnivores, including cockroaches and ants, were the most dominant energy sources. From our observations, *S. bealei* were found on land occasionally (WH Lee, pers. obs.), and may consume prey on land or after the prey falls into the stream. The high reliance on terrestrial resources suggests that *S. bealei* may play a significant role in facilitating energy flow across the land-water interface and in the stream (Lovich et al., 2018). Following consumption of energy from

TABLE 1 Frequency of occurrence (mean proportion of individuals) of dietary items in faecal samples of juvenile (J), female (F) and male (M)

 Beale's eyed turtle (Sacalia bealei) at two sites in Hong Kong, collected between 2016 and 2019

	All			Site A	Site A			Site B		
	All	J	F	м	J	F	м	l	F	м
Sample size	34	15	11	8	9	10	6	6	2	2
Plants										
Fruits or seeds	0.91	0.87	1	0.75	0.78	1	0.83	1	1	0.50
Other plant matter	0.82	0.67	0.82	1	0.67	0.80	1	0.67	1	1
Animals										
Crabs	0.03	0	0	0.13	0	0	0.17	0	0	0
Earthworms	0.12	0.07	0.18	0	0.11	0.20	0	0	0.50	0
Aquatic insects	0.12	0.13	0.09	0.13	0.11	0.10	0	0.17	0	0.50
Terrestrial insects	0.44	0.47	0.36	0.50	0.56	0.40	0.67	0.33	0	0
Mites	0.03	0.07	0	0	0	0	0	0.17	0	0
Freshwater snails	0.12	0.07	0	0.38	0	0	0.17	0.17	0	1
Spiders	0.03	0.07	0	0	0.11	0	0	0	0	0
Other										
Parasites	0.06	0	0.18	0	0	0.20	0	0	0	0
Inorganic matter	0.12	0.13	0.09	0.13	0.22	0.10	0	0	0	0.50

Note: Dietary items were identified visually. Bold indicates the top three dietary items in each age and sex group.

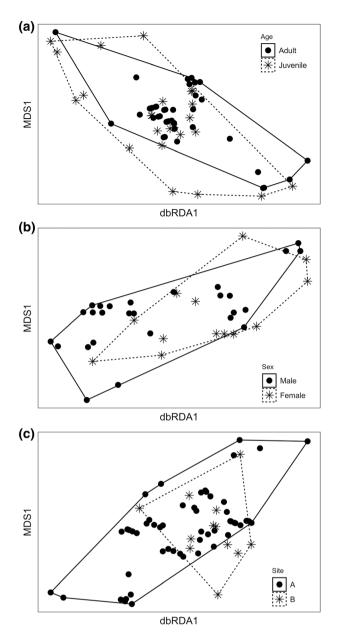


FIGURE 2 Two-dimensional non-metric multidimensional scaling representing Bray–Curtis distances among dietary items in faecal samples of individual Beale's eyed turtle (*Sacalia bealei*) for (a) age groups, (b) sexes and (c) sites

riparian forests, egestion of faeces into water might act as an energy source for smaller, instream organisms, such as macroinvertebrates. Besides the direct trophic linkages, movement of freshwater turtles can stir up the sediment, increasing nutrient availability, which has been associated with a higher abundance of invertebrates (Lindsay et al., 2013). As many freshwater turtle populations have been depleted, these results suggest that continuing depletion of freshwater turtle populations may have impacts on energy flux and biodiversity in freshwater ecosystems.

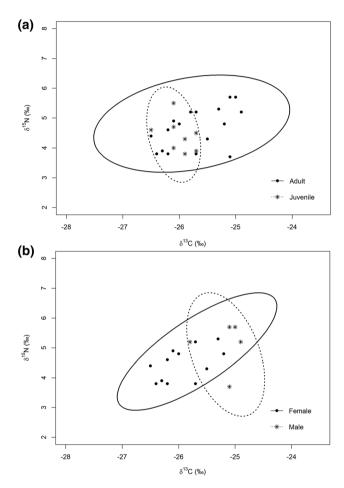


FIGURE 3 Isotope niches (SEAb, Bayesian standard ellipse area) of Beale's eyed turtle (*Sacalia bealei*) for (a) age groups and (b) sexes

TABLE 2	Stable isotope values (mean ± 1 SD (r	max, min)) and niche metrics for one po	pulation (site A) of Beale's eyed turtle (Sacalia bealei)

	Juvenile	Adult	Female	Male
Sample size	8	17	12	5
CL (mm)	64.6 ± 9.2	116.7 ± 19.7	114.8 ± 22.2	121.1 ± 12.6
δ ¹³ C (‰)	-26.0 ± 0.3 (-26.5, -25.7)	-25.7 ± 0.5 (-26.5, -24.9)	-25.9 ± 0.4 (-26.5, -25.2)	-25.2 ± 0.4 (-25.8, -24.9)
δ ¹⁵ N (‰)	4.4 ± 0.6 (3.8, 5.5)	4.7 ± 0.7 (3.7, 5.7)	4.5 ± 0.6 (3.8, 5.3)	5.1 ± 0.8 (3.7, 5.7)
TA (‰²)	0.75	2.13	1.27	0.93
SEAc (‰ ²)	0.49 (0.17, 0.78)	1.05 (0.55, 1.49)	0.73 (0.36, 1.19)	1.15 (0.33, 2.36)
SEAb (‰²)	2.99 (0.25, 5.89)	7.26 (2.20, 10.80)	4.28 (1.37, 7.22)	6.30 (1.42, 10.71)

Abbreviations: CL, carapace length; TA, total convex hull area; SEAc, sample size-corrected standard ellipse area (95% credible intervals in parentheses); and SEAb, Bayesian standard ellipse area (95% credible intervals in parentheses).

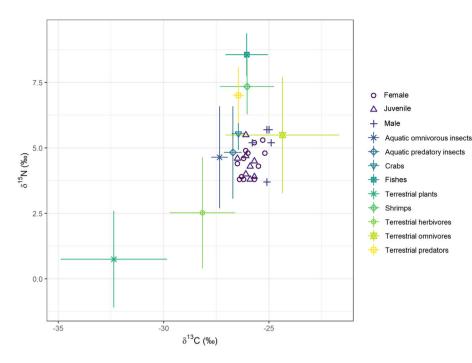


FIGURE 4 Bivariate isotope plot of δ^{13} C and δ^{15} N (mean ± SD) values for juvenile, female and male Beale's eyed turtle (*Sacalia bealei*) and potential dietary items collected from the same stream

By examining faecal samples in this study, S. bealei were shown to frequently consume fruits (≥87% occurrence for all age and size classes), indicating their potential role in dispersing the seeds of riparian plants. Several species of tortoises and box turtles of North America have been identified as important seed dispersers (Moll & Jansen, 1995; Jerozolimski, Ribeiro & Martins, 2009). However, evidence for this key ecological function for stream-dwelling species in Asia is scant (Falcon, Moll & Hansen, 2019). Higher rates of seed germination following ingestion of fruits based on data from bigheaded turtle (Platysternon megacaphalum; Sung, Hau æ Karraker, 2016) and the Southeast Asian box turtle (Cuora amboinensis) in Indonesia (Karraker et al., 2020) suggest that freshwater turtles may play important but largely unexplored functional roles. Here, S. bealei consumes a greater diversity of fruits at a higher frequency when compared with P. megacephalum (S. bealei, seven species; P. megacephalum, two species; Sung, Hau & Karraker, 2016), suggesting a role in facilitating plant propagation. To elucidate this role, further studies are needed to investigate whether S. bealei digestion enhances seed germination and how often these turtles move on land, transporting digested seeds to riparian areas.

Dietary studies on turtles have identified species whose diet does not differ between sexes and size classes (e.g. *Podocnemis unifilis*, *Trachemys scripta*, *Apalone ferox*) and some species where it does (e.g. *Podocnemis expansa*, *Mesoclemmys vanderhaegei*, *Chelydra serpentina*, *Sternotherus odoratus*; Lara et al., 2012; Aresco, Travis & MacRae, 2015; Marques et al., 2017). Diet composition of *S. bealei* was generally similar between sexes and among age groups. The overlap in dietary niches between sexes was expected for *S. bealei* because the degree of sexual size dimorphism in the species is relatively low (Lin et al., 2017). However, the stable isotope data suggest that larger adults, especially males (mean carapace length 124 mm in males vs. 116 cm in females in this study), are able to exploit a wider prey base, thus exhibiting wider isotopic niches.

In this study, the rigour of applying multiple, complementary methods of diet analysis is demonstrated (Nielsen et al., 2017). The combined approach was important for increasing the accuracy of assessment of the omnivorous diet. For example, plant matter was found to be the dominant food item in faecal samples (present in all samples), although stable isotope data suggested that plant contribution to overall turtle diet was relatively low. This is consistent with studies on other omnivorous turtles (Aresco, Travis & MacRae, 2015) and fish (Whitledge & Rabeni, 1997). This may be explained by two factors. First, small and digestible items are underrepresented in visual faecal content analysis, which may lead to an over-estimation of contribution by fruits as seeds are difficult to digest (Sung, Hau & Karraker, 2016). Second, the incongruence may be explained by nutrient routing - differential allocation of nutrients from different food items to metabolism or body growth (Ambrose & Norr, 1993). These results suggest that, while S. bealei frequently consumes plants (present in all faecal samples across seasons), animal prey, with higher protein content, contributes more significantly to turtle growth, which is reflected in their isotopic signals (Whitledge & Rabeni, 1997; Voigt et al., 2008). Nonetheless, this limitation of diet tracing methods highlights the need for using multiple methods, especially for omnivores.

5 | CONSERVATION IMPLICATIONS

The results of this study provide three implications for the conservation of *S. bealei*. First, riparian forests may be crucial for

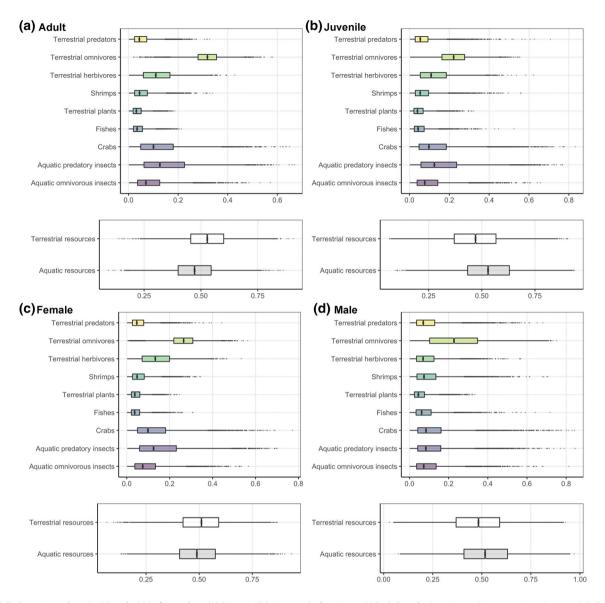


FIGURE 5 Mean (vertical lines), 50% (boxes) and 95% credible intervals (horizontal black lines) of estimated proportion of potential dietary items of (a) juvenile, (b) adult, (c) female and (d) male Beale's eyed turtle (*Sacalia bealei*)

the persistence of *S. bealei* given its reliance on terrestrial food sources. All seeds recovered from faecal samples in this study were from native shrub or tree species (AFCD, 2008), suggesting an association between *S. bealei* and riparian plant communities. This is consistent with the relatively patchy and restricted historical records of *S. bealei* populations in Hong Kong when compared with other freshwater turtle species (Karsen, Lau & Bogadek, 1998). From our observations we postulate that the present distribution of extant populations may be partly associated with the large-scale deforestation of Hong Kong before and during the Second World War (Corlett, 1999). In addition to stricter enforcement of illegal hunting, protection of riparian forests may also be crucial for the conservation of *S. bealei* and other Asian freshwater turtles. Second, *S. bealei* probably plays several significant ecological roles, including seed dispersal and the channelling of energy fluxes across the land-water interface. This suggests that past and present population declines may affect ecosystem functioning. Furthermore, given that public awareness of freshwater turtle conservation is low (Sigouin et al., 2016), enhanced understanding of their ecological roles provides essential resources for environmental education.

Third, the results suggest that an improved diet, with a greater proportion of plant matter, should be provided to captive *S. bealei* populations. As *S. bealei* populations have been harvested extensively across the species' distribution and hunting pressure remains high, *ex situ* conservation can help to restore *S. bealei* populations in the wild. Unfortunately, conservation efforts have ⁸ ↓ WILEY-

been hampered by the limited success of captive breeding programmes. This can be attributed, at least in part, to low fertilization rates in captive individuals (\sim 30%; Gong et al., 2017). Suboptimal dietary regimes are known to be one of the causes of low fertilization rates (Noble et al., 1996; Meinelt et al., 1999; Millamena & Quinitio, 2000). For example, insufficient intake of fatty acids, which are crucial for vitellogenesis, may result in reduced egg quality and therefore lower reproductive success in turtles (Craven et al., 2008; da Costa Araujo et al., 2013). It is possible that wild S. bealei frequently consume fruits because of their higher availability. However, fruit consumption may be important to turtle health. Although the nutritional benefit of fruit consumption is not clearly understood, Fong, Sung & Ding (2020) found that wild S. bealei have a more adaptable and resilient gut microbiota than captive S. bealei that are primarily fed commercial pellets. Therefore, we postulate that the efficacy of S. bealei breeding programmes would be improved by replicating the diet of wild turtles - incorporating more fruits and increasing the diversity of diet items has the potential to enhance turtle health and thereby increase reproductive success.

To our knowledge, this is the first study in Asia to examine the diet of freshwater turtles using stable isotope analysis, providing a quantitative measure of their resource use in freshwater ecosystems. Given the high levels of biomass attributable to undisturbed freshwater turtle populations (Congdon, Greene & Gibbons, 1986; Lovich et al., 2018), we believe that their possible role in ecosystem functioning is an important avenue for future research (Sterrett, Maerz & Katz, 2015). The status of many Asian turtle populations is precarious – during the course of this study, one of the two study populations was probably subjected to poaching – and similar studies on other turtle species are urgently needed to clarify their ecological roles, as well as to aid in their conservation.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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