

Diet and trophic niche of the endangered fish *Garra ghorensis* in three Jordanian populations

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Accepted for publication March 11, 2015

Abstract – *Garra ghorensis* is a small riverine cyprinid fish endemic to the southern Dead Sea that is endangered through habitat loss and invasive species. Here, their diet and trophic niche were assessed in three Jordanian populations: an allopatric population, a population sympatric with native *Capoeta damascina* and a population sympatric with invasive *Oreochromis aureus*. Stomach content analyses of samples collected between February 2011 and January 2012 revealed that detritus and algae were prominent food items in their diets, with low dietary contributions of animal material. The most frequent and abundant macro-invertebrates in intestines were Odonata nymphs and gastropod species. The calculation of trophic niche size from the stomach content data revealed that the niche of *G. ghorensis* (0.10) was generally smaller than sympatric *C. damascina* (0.24), with an overlap of 72%, whereas they had a larger trophic niche than sympatric *O. aureus* (0.20–0.13), with a niche overlap of 54%. These outputs were generally supported by stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ completed on samples collected at the end of the 2011 growth season, although these indicated a greater contribution of animal material to assimilated diet. They also indicated that the trophic niche breadth [as standard ellipse area (SEA)] of *C. damascina* ($4.18\%_{\infty}^2$) was higher than *G. ghorensis* ($2.48\%_{\infty}^2$) and overlapped by 26%. For *G. ghorensis*, their SEA was slightly larger than *O. aureus* ($4.33\text{--}4.00\%_{\infty}^2$), with an overlap of 27%. Although both methods indicated some sharing of food resources between sympatric fishes, there was no evidence suggesting detrimental outcomes for *G. ghorensis* and thus was not considered as a constraint on the status of their populations.

Key words: stomach content analysis; stable isotope analysis; trophic niche breadth; invasive species

Introduction

The escalating demands for water in arid regions have resulted in the substantial physical modification of many river systems through the construction of structures, such as dams, and activities, including abstraction (Propst et al. 2008). The resulting disturbed river environments, with losses of lateral and longitudinal connectivity, and degraded key habitats for specific fish life stages raise concern over their consequences for the sustainability of populations of native and endemic species, particularly those that are already under threat (Kingsford 2000). Moreover, disturbed environments are often more vulnerable to the invasion of non-native species, as their often more generalist traits and high capacity

for adaptation enable them to take advantage of the modified conditions (McKinney 1997; Marvier et al. 2004). Whilst this combination of habitat disturbance and invasion increases the risk of local native fish populations being extirpated and endemic fishes becoming extinct (Olden & Poff 2005), this risk varies between species according to their traits and their ability to adapt to the modified environment and coexist with invasive species (Olden et al. 2006, 2008; Hamidan & Britton 2014a,b). Consequently, understanding how endemic and threatened species respond to modified environments and interact with invaders is integral to impact the assessment and the design of conservation strategies to ameliorate and/or mitigate impact (Fausch et al. 2006).

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doi: 10.1111/eff.12226

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Water-poor countries, particularly those undergoing development of industry, agriculture and urban development, are thus increasingly modifying the physical nature of river channels to maximise the provision of freshwater to maintain and improve key services (Forslund et al. 2009). Jordan is considered the fourth water poorest country in the world (Denny et al. 2008) and its rivers are increasingly being dammed and impounded in order for water to be allocated for domestic use and ground water recharges. This has altered previously fast-flowing, shallow rivers with high longitudinal connectivity to rivers that are slower flowing, substantially deeper and often disconnected from their adjacent sections through impoundments (Hamidan & Mir 2003). Allied to this has been the release of non-native fish, usually for enhancing fish productivity in aquaculture using cichlid species such as *Oreochromis niloticus* and *Oreochromis aureus*. These introductions have also often occurred in habitats where endemic fish species are also present, such as *Garra ghorensis* (Hamidan 2004; Hamidan & Britton 2014a).

The habitat range of *G. ghorensis*, a small-bodied (<20 cm) species of the Cyprinidae family, includes the area of Jordan from south of the Mujib River through to the southern end of the Dead Sea basin (Krupp & Schneider 1989), an area subjected to major river modifications in the last two decades (Hamidan & Mir 2003). Internationally, it is Red Listed by the IUCN as 'endangered' because populations are close to extirpation in many areas of their range and there have been general population declines in others (Freyhof 2014). Where the declines have been quantified, populations have been assessed as reduced by up to 90% through habitat destruction and degradation, and invasive species such as mosquito fish *Gambusia holbrooki* (Goren & Ortolan 1999; Freyhof 2014). In Jordan, whilst the extent of decline is less clear, preliminary countrywide field surveys have suggested a range contraction to less than 10 km² (Hamidan & Mir 2003; Freyhof 2014). Where an endemic species of high ecological value is under increasing habitat impoverishment and is assessed as endangered, then research into their ecology can be justified where the studied populations are locally abundant and thus sampling will not affect population sustainability (Leunda et al. 2008). Consequently, recent work on *G. ghorensis* in its constricted range in Jordan has focused on the expression of its life-history traits in disturbed environments, revealing that even in the most disturbed environment studied, their ability to reproduce, have a relatively long life span (for the species) and grow relatively fast, was not inhibited (Hamidan & Britton 2014a,b). This study builds on this work by assessment of *G. ghorensis* diet, with a focus on assessing

feeding relationships across three contexts: in allopatry, in sympatry with a native fish and in sympatry with an invasive fish. Given their level of conservation threat, then each context could only be studied at a single site and thus replication of contexts was not possible. The objectives were to (i) assess the diet and trophic niche breadth of *G. ghorensis* and the other fishes through stomach content analysis, (ii) assess the long-term trophic niche breadth and trophic interactions of *G. ghorensis* and the other fishes through stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and (iii) assess the effect of coexisting fishes on *G. ghorensis* diet and feeding relationships, and in relation to their endangered status.

Materials and methods

Study area

The three sites were Wadi-al-Burbaita (35°69'E, 30°98'N), Ain al-Haditha (35°54'E, 31°29'N) and Wadi Ibn Hammad (35°38'E, 31°18'N). Other than the fishes used in the samples, no other fish species were recorded at the sites. Maps of the sites are available in Hamidan & Britton (2014a,b). All sites were known to contain sustainable and locally abundant populations of *G. ghorensis* following preliminary surveys completed in October 2011. Ibn Hammad (hereafter referred to as site IB) is a relatively fast-flowing (mean flow 0.9 m·s⁻¹) and shallow (<1 m) habitat in which *G. ghorensis* was the only fish present (i.e. allopatric). Wadi-al-Burbaita (hereafter referred to as site BB) has more variable flow rates and some human disturbances through water use for domestic and agricultural uses, with *G. ghorensis* present in sympatry with *Capoeta damascina*, an indigenous species of the Cyprinidae family. Ain al-Haditha (hereafter referred to site HD) was the most disturbed site, with local impoundments creating sections of slower and deeper water (to 2 m), where *G. ghorensis* was sympatric with the invasive cichlid *O. aureus*.

Fish sampling

All fish sampling was conducted under licence to the Royal Society of Nature Conservation, Jordan, once per month between February 2011 and January 2012. Electric fishing was utilised by sampling in an upstream direction for a standardised time of 30 min using handheld Samus 725 MP electro-fishing equipment. Where less than 15 fish were captured in this period, then fishing was continued to 60 min. At the conclusion of the electric fishing, all captured fish were identified to species level and a random subsample of a maximum of 30 fish per species taken to

the laboratory for further analysis. These subsampled fish were euthanised [overdose of anaesthetic (clove oil; Soto & Burhanuddin 1995)] before being preserved in 70% ethanol. In the laboratory, each fish was assigned a reference number, sexed and eviscerated, and the entire intestine preserved in 70% ethanol for further analysis. A section of dorsal muscle was also taken from fish sampled in October and November 2011 for stable isotope analysis. For these months, a macro-invertebrate sample was also collected from each site using a combination of kick-sampling and sweep netting using a hand net of 0.25-mm mesh size, with sampling in all major habitats. These were also preserved in 70% ethanol and provided samples of the putative food resources of the fish for the subsequent stable isotope analysis.

Stomach content analyses

Stomach content analyses were conducted through examining the contents of the entire intestine using dissecting microscope ($\times 7$ to $\times 45$ magnification). During the analyses, the number of empty intestines was noted. Food items were identified to the lowest taxonomic group possible, according to the remains and their ability to provide enough information for a positive identification. They were then grouped into the following categories according to the level at which they were identified: Spirogyra (algae), Gastropoda, Coleoptera, Odonata (as nymphs), Diptera, Ostracoda, Chironomid larvae, Formicidae, zooplankton (primarily *Daphnia* spp.), unidentified plant material, detritus, juvenile fish, fish eggs, unidentified invertebrate (where remains were encountered, such as wings and legs, that did not allow further identification) and digested material (tissues and structures in the stomach that could not be assigned a more specific category).

For each fish species at each site, the stomach content data were presented in two ways. Firstly, the data were combined for all months, providing a broad overview of the food items consumed by the fishes. Secondly, the data were split by season according to the mean monthly air temperatures calculated from data from a recording station close to the sampling sites (Fig. 1). Accordingly, data were combined for each species and site for December, January and February (winter: <12 °C); March, April and May (spring: 12 – 20 °C); June to September (summer, >20 °C); and October and November (autumn, 12 – 20 °C) (Fig. 1). The stomach content data were then used in the following indices and metrics. The vacuity index ($\%I_v$) was calculated as the percentage of empty stomachs to the total number of stomachs examined (Hyslop 1980). Numerical analysis of food items was applied after Windell & Bowen (1978).

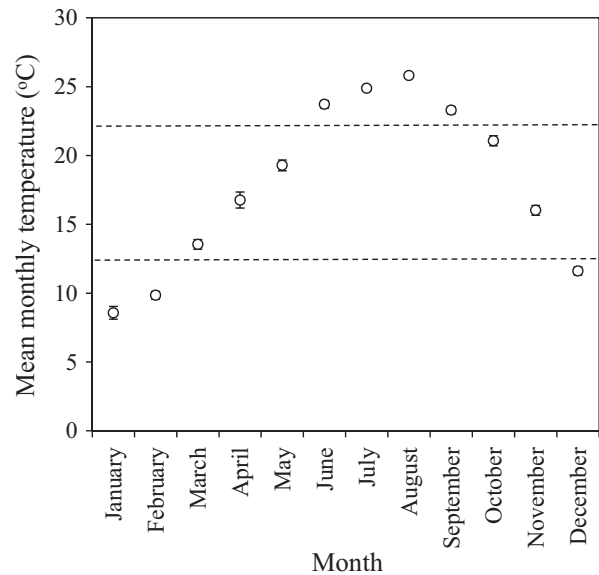


Fig. 1. Mean air temperature per month recorded from a recording station close to the study area. Error is standard error; horizontal lines mark the temperature bands used to combine monthly data by season (<12 , 12 – 20 , >20 °C).

Food items were represented as 'frequency of occurrence' ($\%F_i$) and the 'relative abundance' of a given prey item (A_i). Frequency of occurrence, defined as the percentage of stomachs in which that prey occurred, was calculated as follows: $\%F_i = (N_i/N) \times 100$, where: N_i = number of intestines containing item i and N = the total number of nonempty intestines. The relative abundance of prey items, defined as the percentage of total stomach contents in all stomachs that comprised of that prey item, was calculated as follows: $A_i = (\sum S_i / \sum S_t) \times 100$, where S_i = the stomach contents (numbers) composed of prey i and S_t = the total stomach contents (number) of all stomachs in the entire sample (total number of all stomach items). Note that for A_i calculations, detritus, spirogyra and digested material were unable to be included due to the requirement for numerical data. For analyses of F_i and A_i by season, all food items were included in calculations, but only the principal food items were selected for reporting for the sake of brevity. Trophic niche breadth was calculated from the stomach content data to test the specialisation of the diets, according to Levins (1968) as follows:

$$B = \frac{1}{\sum p_j^2}$$

where B = Levins' measure of niche breadth, P_j = proportion of diet that comprised food item j and estimated by the following: $(N_j/Y)(\sum p_j = 1.0)$, where N_j = number of individual fish using food resource j and $Y = \sum N_j$ = total number of individual

fish sampled. Niche overlap was calculated as a percentage, according to the following:

$$P_{jk} = \left[\sum_{i=1}^n (\text{minimum } p_{ij}, p_{ik}) \right] 100$$

where P_{jk} = percentage overlap between species j and species k , p_{ij} = the proportion of food item i in the total food items used by species j , p_{ik} = the proportion of item i in the total items used by species k and n = total number of food items. Percentage overlap is the simplest measure of niche overlap to interpret because it is a measure of the actual area of overlap of the resource utilisation of the two species. This overlap measure was used by Schoener (1970) and has been labelled the Schoener overlap index (Hurlbert 1978). Abrams (1980) recommends this measure as the best of the measures of niche overlap. Niche breadth and niche overlap were both calculated with EcoMethodology 7.2 software package (Exeter Software, NY, USA).

Stable isotope analysis

Stable isotope analysis provided a longer-term perspective of the diet of the fishes as it represents assimilated diet (up to 6 months where dorsal muscle is used; Grey 2006), where $\delta^{15}\text{N}$ is the indicator of trophic level and $\delta^{13}\text{C}$ is the indicator of energy source (Cucherousset et al. 2012). The dorsal muscle samples were from a random selection of up to 30 fish per species and site, taken from samples collected in October and November 2011, that is, from fish at the end of their growth season when their stable isotope data from their dorsal muscle would be representative of their assimilated diet during the preceding summer months (Perga & Gerdeaux 2005). These were complemented by analyses of replicate samples of the putative fish-food resources (benthic macro-invertebrates, back-swimmers and algae) collected during the same sampling months, as already described. Triplicate samples used where possible. All samples were dried at 50 °C for 48 h before being sent to the Cornell Isotope Laboratory for analysis (Cornell University, New York, USA). The outputs were values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each individual fish and their putative food resources. As the tissues and macro-invertebrates had been preserved in 70% ethanol, there was the possibility that some consequent shifts in stable isotope signatures had occurred. For example, Kelly et al. (2006) suggested that although ethanol did not significantly enrich $\delta^{15}\text{N}$ in tissues of Arctic charr *Salvelinus alpinus*, some significant changes were detected in $\delta^{13}\text{C}$. However, given the absence of species-specific stable isotope correction factors for *G. ghorensis*, *C. da-*

mascina and *O. aureus*, the fish data were used uncorrected, on the basis that $\delta^{15}\text{N}$ was unlikely to have altered significantly (Kelly et al. 2006), and any correction factor for shifts in $\delta^{13}\text{C}$ would have to be applied equally over the three species and thus would not alter their trophic positions (TPs) and trophic niche sizes relative to each other. Similarly, whilst the macro-invertebrate samples can also shift slightly in their stable isotope signatures following preservation in ethanol (e.g. Sarakinos et al. 2002), and given they were used to only assess relative TP, they were also used in their uncorrected form.

To assess differences in the TP of each fish per species and site (i), the $\delta^{15}\text{N}$ data were converted to TP using the formula $[(\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{baseline}})/3.4] + 2$, where N_{baseline} is the mean $\delta^{15}\text{N}$ of the putative food resources. The items used as putative resources at each site were assessed by the outputs of both the stomach content data and the extent of their differentiation with the fish isotope values (cf. Fig. 2). The TP data were then tested using a generalised linear model (GLM), as the data were not normally distributed. In the model, TP was the dependent variable, the interaction of site and species was the independent variable, and differences in TPs between species and sites were tested by pairwise comparisons with Bonferroni adjustment for multiple comparisons. The same model structure was also used to test for differences between species and sites in $\delta^{13}\text{C}$. To determine the trophic niche width of each species at each site according to assimilated diet, the metric 'SEA' was used (SEA_c) (Jackson et al. 2011, 2012). These ellipses are based on the distribution of individuals in isotopic space as an estimate of each species core trophic niche using the 'siar' package (Parnell et al. 2010; Jackson et al. 2011) in the 'R' computing programme (R Core Development Team 2012). The subscript 'c' in ' SEA_c ' indicates that a small sample size correction factor was used, as sample size tended to be below 20 per species (Jackson et al. 2011). Where *G. ghorensis* was present in sympatry, then the extent of their overlap in trophic niche with the other fish species present was quantified (%).

Results

Site IB: allopatric *G. ghorensis*

The mean length of the *G. ghorensis* at site IB was 89.5 ± 20.3 mm. Of the 165 fish analysed, 54 had empty stomachs ($I_v = 18\%$). Frequency of occurrence of diet indicated that detritus, digested material and spirogyra were the most frequently encountered food categories in their stomachs, with Odonata nymphs the only macro-invertebrate present in their diet at a frequency $>1\%$ (Table 1). In contrast to the

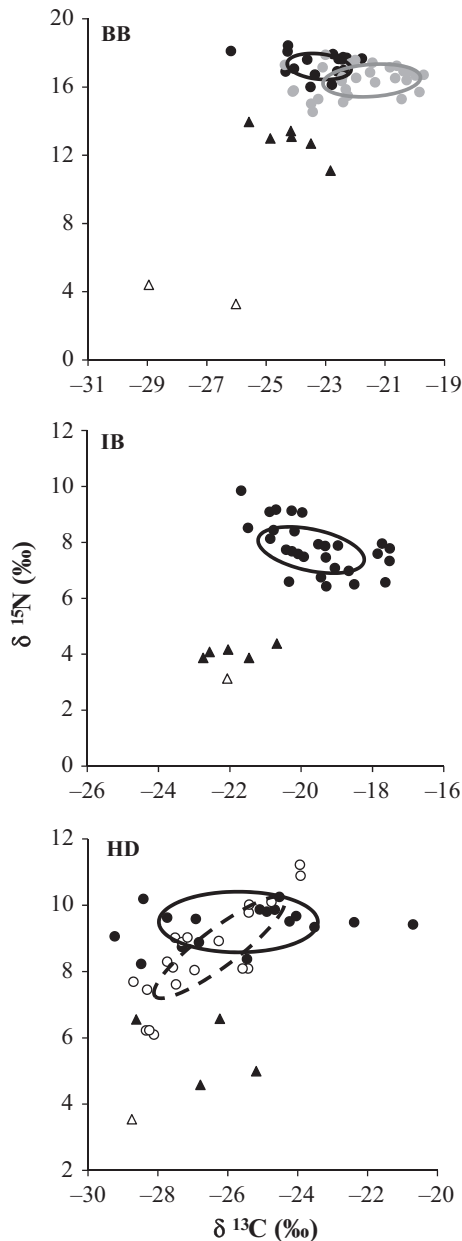


Fig. 2. Stable isotope biplots of *Garra ghorensis* (black circles), *Capoeta damascina* (grey circles) and *Oreochromis aureus* (clear circles), and their trophic niche breadth as measured by standard ellipse area where solid black line = *G. ghorensis*, grey line = *C. damascina* and dashed line = *O. aureus*. Black triangles are macro-invertebrate samples and clear triangles are algal samples. Note differences in scales on the axes.

other populations studied, no gastropods were encountered in the stomachs (Table 1). Detritus and spirogyra were the most important dietary items in all seasons, with the greatest frequency of occurrence in autumn (Table 2). Odonata and zooplankton were more prominent in diet in winter than other seasons (Table 2). The relative abundance data indicated that in the stomachs with Odonata were present there were an important species, particularly in the spring, although values were relatively high in all seasons

(Tables 3 and 4). The overall dietary niche breadth was 0.34, being the highest recorded across the three *G. ghorensis* populations, with this also generally reflected in the seasonal data (Table 5) where niche breadth was highest in winter, perhaps reflecting the increased proportion of Odonata and zooplankton in diet in that season (Tables 2 and 5). The stable isotope data of 28 allopatric *G. ghorensis* (mean length: 54.1 ± 15.8 mm) indicated that their mean TP was 3.21 ± 0.05 and their SEA (as a measure of trophic niche size) was $2.86\%_0^2$ (Fig. 2).

Site BB, *G. ghorensis* in sympatry with native *C. damascina*

The mean length of the *G. ghorensis* used in the analyses at site BB was significantly smaller than *C. damascina* (54.11 ± 20.3 and 85.03 ± 28.7 mm, respectively; ANOVA: $F_{1,325} = 125.81$, $P < 0.01$). Of 158 *G. ghorensis* stomachs analysed, 69 were empty ($I_v = 43\%$), whereas of 168 *C. damascina* stomachs analysed, 14 were empty ($I_v = 8\%$). For both species, the frequency of occurrence data indicated that detritus was the most frequently encountered food item, with Spirogyra, Gastropoda and plant material also present, albeit in differing frequencies between them (Table 1). The main contrast in these data between the species was in Odonata nymphs, which were not recorded in any *G. ghorensis* stomachs but were recorded in 26% of *C. damascina* (Table 1). Coleoptera, Diptera, juvenile fish and zooplankton were also present in *C. damascina* diet, although their frequency of occurrence was $<2\%$ (Table 1). The dominance of detritus in the diet of both fishes was also apparent in each season, with frequency of occurrence peaking in spring for *C. damascina* (88%) and in winter for *G. ghorensis* (67%) (Table 2). For *C. damascina*, Odonata nymphs were most prominent in diet in summer (50%; Table 2). The relative abundance data emphasised the difference in the diet of the sympatric fishes related mainly to the Odonata in *C. damascina* in all seasons (Tables 3 and 4). The relative abundance of Gastropoda was high in *G. ghorensis*, especially in spring, suggesting their importance as a dietary item (Tables 1, 3 and 4).

The overall niche breadth of *G. ghorensis* was relatively low when compared to *C. damascina* (0.10 and 0.24, respectively; Table 5). Their overall niche breadths overlapped by 72%. Seasonally, the smallest calculated niche breadth for *G. ghorensis* occurred in summer, when they were mainly consuming detritus, and was highest in spring, when the contribution of spirogyra and Gastropoda increased (Tables 1 and 5). A value for *G. ghorensis* in autumn was unable to be calculated due to the low diversity of items taken,

Table 1. Frequency of occurrence of the food item categories of each fish species in each sampling location (all data).

Food item	BB		IB <i>G. ghorensis</i> (N = 165)	HD	
	<i>G. ghorensis</i> (N = 158)	<i>C. damascina</i> (N = 168)		<i>G. ghorensis</i> (N = 123)	<i>O. aureus</i> (N = 141)
Detritus	67.4	73.4	37.8	21.8	50.0
Digested material	20.2	22.7	28.8	12.9	1.1
Unidentified invertebrate	3.4	7.1	8.1	4.0	3.3
Spirogyra	4.5	14.3	20.7	52.5	58.5
Gastropoda	3.4	3.9	0.0	24.8	7.4
Plant material	4.5	14.3	2.7	0.0	9.6
Odonata nymph	0.0	26.0	8.1	19.8	0.0
Zooplankton	0.0	1.3	0.9	2.0	0.0
Coleoptera	0.0	0.6	0.0	0.0	0.0
Diptera	0.0	0.6	0.0	0.0	0.0
Juvenile fish	0.0	0.6	0.0	0.0	3.2
Chironomid larvae	0.0	0.0	0.9	0.0	4.3
Formicidae	0.0	0.0	0.0	0.0	1.1
Fish eggs	0.0	0.0	0.0	0.0	5.3
Ostracoda	0.0	0.0	0.9	3.0	0.0

Table 2. Frequency of occurrence of selected food items of each species in each sampling location in spring, summer, autumn and winter (N = number of gut examined).

Site	Species	Season	N	Items				
				Detritus	Spirogyra	Gastropoda	Odonata	Zooplankton
BB	<i>G. ghorensis</i>	Spring	44	27.3	13.6	9.1	0.0	0.0
		Summer	54	42.0	0.0	1.0	0.0	3.0
		Autumn	24	20.0	0.0	0.0	0.0	0.0
		Winter	36	67.4	4.5	3.4	0.0	3.4
	<i>C. damascina</i>	Spring	45	88.1	9.5	7.1	11.9	0.0
		Summer	60	73.2	17.8	3.5	50.0	7.10
		Autumn	19	83.3	11.1	5.5	27.7	11.1
		Winter	44	52.6	15.8	7.9	7.9	15.8
IB	<i>G. ghorensis</i>	Spring	45	48.7	18.9	0.0	8.1	2.7
		Summer	69	28.8	11.5	0.0	5.7	1.9
		Autumn	30	50.0	28.0	0.0	7.1	0.0
		Winter	30	11.8	35.3	0.0	11.8	17.7
HD	<i>G. ghorensis</i>	Spring	44	18.9	40.5	24.3	35.1	2.7
		Summer	29	6.9	68.9	31.0	13.7	6.9
		Autumn	5	40.0	40.0	40.0	0.0	40.0
		Winter	45	37.1	45.7	20.0	8.6	2.9
	<i>O. aureus</i>	Spring	45	22.2	33.3	2.2	4.0	2.2
		Summer	49	36.3	69.6	3.0	0.0	0.0
		Autumn	11	100.0	50.0	16.6	0.0	0.0
		Winter	36	63.3	46.7	13.3	0.0	6.7

indicating a very small niche. The niche breadth of *C. damascina* was also considerably higher than *G. ghorensis* in all seasons except spring. The highest dietary overlap with *C. damascina* occurred in winter and varied between 41% and 72% across the seasons (Table 5).

Stable isotope analysis was completed for 15 *G. ghorensis* (mean length: 41.6 ± 15.8 mm) and 17 *C. damascina* (mean length: 69.5 ± 22.1 mm). The differentiation between the fish and their putative food resources suggested that macro-invertebrates

were important assimilated items compared with algae (mean $\delta^{15}\text{N}$: all fish: $16.75 \pm 0.13\text{‰}$; macro-invertebrates: 12.87 ± 0.40 ; algae: 3.84 ± 0.56 ; Fig. 2). There were significant differences between the species for TP, although the difference was less than one TP (GLM: Wald $\chi^2 = 76.70$, d.f. = 4, $P < 0.01$); *G. ghorensis* being higher (3.32 ± 0.07 vs. 3.03 ± 0.05 ; $P < 0.01$). Although the TP of *G. ghorensis* was slightly higher than at site IB, testing revealed that the difference was not statistically significant (0.11 ± 0.08 , $P > 0.05$). The GLM

Table 3. Relative abundance (as mean number of items per stomach) of food item categories of each fish species in each sampling location (all data).

Food item	BB		IB	HD	
	<i>G. ghorensis</i> (N = 158)	<i>C. damascina</i> (N = 168)		<i>G. ghorensis</i> (N = 165)	<i>G. ghorensis</i> (N = 123)
Unidentified invertebrate	9.7	6.6	29.0	1.2	3.7
Gastropoda	87.1	8.3	0.0	44.4	26.7
Odonata nymph	3.2	81.0	41.9	35.3	0.0
Zooplankton	0.0	1.2	3.2	1.6	0.0
Coleoptera	0.0	1.8	0.0	0.0	0.0
Diptera	0.0	0.6	0.0	0.0	0.0
Juvenile fish	0.0	0.6	0.0	0.0	3.2
Chironomid larvae	0.0	0.0	3.2	0.0	42.9
Formicidae	0.0	0.0	0.0	0.0	0.9
Fish eggs	0.0	0.0	0.0	0.0	22.6
Ostracoda	0.0	0.0	9.7	16.7	0.0

Table 4. Relative abundance (as mean number of items per stomach) of selected food items of each species in each sampling location by season.

Site	Species	Season	N	Items		
				Gastropoda	Odonata	Zooplankton
BB	<i>G. ghorensis</i>	Spring	44	100.0	0.0	0.0
		Summer	54	33.3	0.0	66.6
		Autumn	24	0.0	0.0	0.0
		Winter	36	87.1	3.2	9.7
	<i>C. damascina</i>	Spring	45	0.0	83.3	0.0
		Summer	60	1.7	93.1	3.4
		Autumn	19	4.3	86.9	4.4
IB	<i>G. ghorensis</i>	Spring	45	0.0	75.0	25.0
		Summer	69	0.0	38.4	46.2
		Autumn	30	0.0	33.3	66.6
		Winter	30	0.0	42.9	28.6
HD	<i>G. ghorensis</i>	Spring	44	23.9	72.8	1.1
		Summer	29	80.6	19.3	0.0
		Autumn	5	14.6	0.0	58.4
		Winter	45	66.0	20.0	6.0
	<i>O. aureus</i>	Spring	45	1.3	0.0	0.0
		Summer	49	9.1	0.0	81.8
		Autumn	11	100	0.0	0.0
		Winter	36	85.7	0.0	10.7

Table 5. Trophic niche breadth of each species in each site, overall and by season, according to the methods of Levins (1968) and calculated from stomach content data. Values in parentheses are the extent of the niche overlap between the fishes at that site in that season. Note at site BB in autumn, there were insufficient items in diet to enable calculation.

Site	Species	Overall	Spring	Summer	Autumn	Winter
BB	<i>G. ghorensis</i>	0.10 (71.6%)	0.58 (41.2%)	0.25 (62.6%)	–	0.20 (72.2%)
	<i>C. damascina</i>	0.24	0.16	0.42	0.38	0.48
IB	<i>G. ghorensis</i>	0.34	0.47	0.54	0.47	0.61
HD	<i>G. ghorensis</i>	0.20 (54.4%)	0.59 (41.7%)	0.423 (57.7%)	1.00 (39.7%)	0.44 (73.8%)
	<i>O. aureus</i>	0.13	0.44	0.343	0.580	0.26

testing differences in $\delta^{13}\text{C}$ between the species were significant (Wald $\chi^2 = 335.14$, d.f. = 4, $P < 0.01$), with *G. ghorensis* $\delta^{13}\text{C}$ depleted compared to *C. damascina* (-23.27 ± 0.37 vs. -21.74 ± 0.28)

(Fig. 2). The trophic niche sizes according to SEAc revealed that the niche of *C. damascina* ($4.18\%_0^2$) was considerably larger than *G. ghorensis* ($2.48\%_0^2$), with a niche overlap of 8% (Fig. 2).

Site HD, *G. ghorensis* in sympatry with invasive *O. aureus*

The mean length of analysed *G. ghorensis* at site HD was 80.32 ± 20.4 mm and *O. aureus* was 129.7 ± 27.8 mm, with these differences not being significant (ANOVA: $F_{1,254} = 0.41$, $P > 0.05$). Of the 123 *G. ghorensis* stomachs analysed at the site, 22 were empty ($I_v = 17\%$), with 47 of 141 *O. aureus* stomachs also being empty ($I_v = 33\%$). Frequency of occurrence data indicated that both species were feeding strongly on algae (as Spirogyra), with detritus also frequently occurring in their stomachs (Table 1). Gastropoda and Odonata nymphs were also taken frequently by *G. ghorensis*, with this much less apparent in *O. aureus* (Table 1). Instead, the *O. aureus* stomachs contained low frequencies of fish eggs, Formicidae, Chironomid larvae and juvenile fish (<6%), all of which were absent in *G. ghorensis* (Table 1). Seasonally, the frequency of occurrence of Gastropoda and Odonata nymphs in *G. ghorensis* diet was always considerably higher than for *O. aureus*, with these items well represented in all seasons (except autumn) for *G. ghorensis* (Table 2). In summer, both fishes had high frequencies of spirogyra in their diet (Table 2). The relative abundance of Gastropoda was high for both species (Table 3), although the effect was seasonal, as they were present less frequently in spring in both fishes (Table 4). The relative abundance of Odonata was high in *G. ghorensis*, especially in spring, but was absent in *O. aureus*, whereas chironomid larvae and fish eggs were relatively abundant in *O. aureus* but not *G. ghorensis* (Tables 3 and 4). The dietary niche breadth of *G. ghorensis* was always higher than *O. aureus*, irrespective of season, although there was overlap in their diets (42–74%) (Table 5). Niche breadth of *G. ghorensis* was lowest in summer, when items including zooplankton were absent in diet (Tables 2 and 5).

Stable isotope analysis was completed for 16 *G. ghorensis* (83.6 ± 14.6 mm) and 12 *O. aureus* (75.9 ± 13.5 mm). Mean $\delta^{15}\text{N}$ of all fish was $7.82 \pm 0.17\text{‰}$, macro-invertebrates $5.67 \pm 0.52\text{‰}$ and algae 3.54‰ , suggesting both macro-invertebrates and algae contributed to assimilated diet (Fig. 2). The outputs of the GLMs revealed that the difference in mean TP between *G. ghorensis* and *O. aureus* was significant (TP: *G. ghorensis* 3.72 ± 0.07 , *O. aureus* 3.45 ± 0.06 , $P = 0.03$), but was not for $\delta^{13}\text{C}$ (1.15 ± 0.50 , $P > 0.05$; Fig. 2). The TP of *G. ghorensis* at this site was significantly higher than at sites BB and IB ($P < 0.01$ in both cases). Trophic niche size according to SEAc revealed *G. ghorensis* had a slightly larger trophic niche than *O. aureus* (4.33‰^2 and 4.00‰^2 , respectively), with the niches overlapping by 27% (Fig. 2).

Discussion

Garra ghorensis is endangered throughout its range, restricting the number of populations that could be used to study aspects of their ecology that would then inform conservation strategies. Here, three populations were used, each representing a different context (allopatric, sympatric with a native fish and sympatric with an invasive fish) without replication. Correspondingly, whilst the data outputs indicate the extent to which the *G. ghorensis* populations were responding to the different contexts, the lack of replication might inhibit the identification of more general dietary patterns in relation to aspects such as invasions. Nevertheless, the outputs did suggest that in general, the populations of *G. ghorensis* were heavily reliant on feeding on algae (spirogyra) and detritus that whilst are of low nutritive value, are rarely limiting (Persson 1983). In stomachs, contributions of animal material to diet appeared low, although the stable isotope data suggested they made more important contributions to assimilated diet than the stomach content data suggested.

The reliance of the fishes on detritus and spirogyra in the stomach contents might represent a dietary preference over animal material or might be reflective of low availability of animal material at each site, especially given the stable isotope outputs. Also, given the variation in site characteristics, then food availability might also have differed between sites and influenced diet choice. However, this could not be investigated further using the data set. Nevertheless, the relatively high contribution to diet of algae and detritus in all sites is important, given the niche overlaps evident between *G. ghorensis* and invasive *O. aureus* (stomach content analysis: 54%; stable isotope analysis: 27%), as it can be speculated that these resources were unlikely to be limiting in the sites (Persson 1983). Moreover, when invasive populations establish following an introduction, niche-based competition theory predicts that where there is interspecific niche overlap between species that results in competition, then the competitors will shift to alternative food resources, reducing their trophic niche but with partitioning promoting their coexistence. That this was not evident here suggests there was no requirement to do so, that is, despite the resource sharing, competition was not evident and so the fishes did not alter their diet (Guo et al. 2014). Indeed, the *G. ghorensis* population that was sympatric with *O. aureus* comprised of relatively fast-growing, highly fecund individuals (Hamidan & Britton 2014a,b), providing no supporting evidence that there were detrimental ecological consequences arising from the sharing of food resources.

The complementary use of stomach contents and stable isotope data to study the diet and feeding relationships of fishes is now routine, including for investigating feeding interactions of invasive and native fishes (e. g. Leunda et al. 2008; Cucherousset et al. 2012). They can, however, often provide contrasting outcomes. For example, Locke et al. (2013) used stomach contents, stable isotopes and trophically transmitted parasites to analyse the diet of pumpkin-seed *Lepomis gibbosus* and revealed each method provided virtually unrelated information, with no significant correlation in prey importance across the methods. In our study, however, there were some consistent outcomes in the combined use of stomach contents and stable isotopes. For example, they both indicated that *C. damascina* had a larger trophic niche than *G. ghorensis* at site BB and these niches overlapped, although stomach content data did suggest that *G. ghorensis* niche breadth was higher than *O. aureus* in all seasons, with this was not apparent in the stable isotope data. This difference might relate to the stomach content data being used seasonally, whereas the stable isotopes analysed assimilated diet at the end of the growth season. In addition, the extent of assimilation into muscle tissue of items such as detritus, plant material and spirogyra, all common items in the stomachs, might be relatively low due to their difficulty of digestion, resulting in their prolonged presence in the intestine (and so potentially a high contribution to stomach content data) but low assimilation (and so potentially a low contribution to stable isotope data) (McCutchan et al. 2003). This low assimilation of plant material was supported by the differentiation in stable isotope values between fish and their putative resources that suggested macro-invertebrates might have been more important in terms of assimilated diet than suggested by the stomach content data. Thus, these animal resources might have been making relatively important energetic contributions to the fishes and so been important for maintaining their population stabilities.

In summary, the outputs of the two dietary analytical techniques suggested that whilst there were some differences in the diet breadths of *G. ghorensis*, they were primarily consuming detritus and algae in each site, with this also being the case for the sympatric fishes, with the low proportion of animal material consumed being important for their assimilated diet. Whilst this resulted in some overlap in the diet of *G. ghorensis* and the sympatric fishes, it was cautiously concluded that the fishes were unlikely to have been competing for food resources as their main dietary items were not limiting and so the endangered status of *G. ghorensis* was unlikely to be resulting from negative feeding interactions with an invasive fish.

Acknowledgements

The authors would like to thank the Royal Society for the Conservation of Nature that facilitated this work and provided all logistics. We thank Mr Anas Abu Yehya and Eiz aldeen al Aqeel for their considerable assistance in the laboratory work. At the field level, the help of Mujib Reserve staff was appreciated, especially Hisham Dhaisat, Amer Al-Oqbi, Habes Ammarin and Ali Laimoun for their assistance in the fieldwork.

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