Electronic Supplementary Material/Online Resource A, B & C

Trophic ecology and persistence of invasive silver carp *Hypophthalmichthys molitrix* in an oligotrophic South African impoundment

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Online Resource A

Body condition

To determine the condition factor (CF) (Beckman 1948), the Fulton's condition factor, and the length-weight relationship, specimens were weighed (g), and both the total length (TL) and standard length (SL) measured. The indices were used to enable comparison with other studies (Spatura & Gophen 1985, Liss et al. 2013) (Table 1S). The CF was only compared intraspecifically due to different body forms and growth patterns between the two species (Blackwell et al. 2000), and also only between non-reproducing individuals using an Analysis of variance (ANOVA).

Table 1S: Indices used to assess the body condition of *H. molitrix* and *O.mossambicus* in Flag Boshielo Dam

Index	Equation	Application	Reference
Condition factor	**CF = *(W*10⁵SL³)*** *SL* = standard length (cm); *W* = weight (g)	Index of body condition	Beckman 1948
	K = *(W/TL³)*100 000* *TL* = total length (cm)	Fulton's condition factor	Carlander 1950
Length-weight relationship (LWR)	**W = *a*SLᵇ*** *a* = geometric mean (= 0.0098); *b* = body shape constant (= 3.14)	Estimating weight for a given length with parameters obtained from Bayesian meta-analysis	Froese & Pauley 2012

A total of 168 specimens, comprising of 87 *H. molitrix* and 81 *O. mossambicus*, were collected across four seasons in FBD during 2011. The CF of *H. molitrix* varied significantly with season (ANOVA: $F_{2,\ 84} = 3.80$; $n = 87$; $p < 0.001$) with a minimum in spring and maximum in autumn, which was significantly higher than all the other seasons ($p < 0.001$) (main manuscript Table 2). No significant difference in the CF between the different sexes was observed for *H. molitrix* (Welch two sample t-test (*df*) = 58.472; $t = -0.3$; $p = 0.731$). *Hypophthalmichthys molitrix* specimens were considered sub-adults with no observed active gonads during any of the sampling periods. The mean Fulton's condition factor for *H.*

molitrix was K = 0.72 ± 0.2 with only 3.4% with K ≥ 1. The mean measured weight of *H. molitrix* caught in FBS was 14.6% lower than mean predicted LWR calculated (Froese & Pauley 2012). Measured weights during autumn were 11.7% higher; 26.4% lower in winter; 45.5% (146.4 g per specimen) lower in spring; and 6.0% lower in summer, than the expected weight. The CF of *O. mossambicus* was significantly higher during summer (CF = 3.9), compared to the minimum during winter (CF = 3.4) and spring (ANOVA: $F_{3, 77} = 4.90$; $n = 81$; $p < 0.01$).

The impact of the food limitation (nutritional stress) might be evident through the lower CF of *H. molitrix* in FBS as compared to other systems. The spring CF of *H. molitrix* was the lowest (CF = 0.88), being much lower than in a comparable study where reproduction does not occur (minimum CF = 1.78) (Spatura and Gophen 1985). The Fulton's condition factor (K = 0.72) was also lower than the North American *H. molitrix* population measured under a range of environmental conditions (K = 1.19; water temperature 19. 6 - 26.6 ^{0}C; summer – autumn) (Liss et al. 2013), although individuals of the North American population were reproducing (Liss et al. 2013). Variation in the CF is partially related to energy expenditure on reproduction (Liss et al. 2013), however, the *H. molitrix* specimens sampled in the current study were considered as sub-adults/juveniles with no observed active gonads during the sampling period. Sexual maturity is reached at 2 to 7 years (weight *c*. 900 – 1200 g) depending on water temperature and resource availability (Kolar et al. 2007). The high CF of *H. molitrix* during autumn is likely attributed to increased algal consumption during the preceding summer when chlorophyll-*a* levels were higher.

Online Resource B

Stomach contents

A total of 168 specimens comprising 87 *H. molitrix* and 81 *O. mossambicus* were collected in FBD between April and December 2011. The prey-specific abundance and frequency of occurrence of both are graphically displayed in figure 2, detailed in Table 2S).

Table 2S: Stomach contents of silver carp *Hypophthalmichthys molitrix*, and Mozambique tilapia *Oreochromis mossambicus* sampled on a seasonal basis in Flag Boshielo Dam, South Africa, during 2011

Prey	Autumn		Winter		Spring		Summer	
H. molitrix	$\%F_i$	P_i	$\%F_i$	P_i	$\%F_i$	P_i	$\%F_i$	P_i
Bacillariophyceae	0.65	34.00	0.00	0.00	0.00	0.00	0.29	2.20
Dinophyceae	0.76	28.23	0.42	3.00	0.00	0.00	0.00	0.00
Chlorophyceae	0.35	4.17	0.00	0.00	0.62	2.00	0.53	12.56
Cyanophyceae	0.12	3.50	0.00	0.00	0.00	0.00	0.00	0.00
Euglenoids	0.12	1.50	0.00	0.00	0.00	0.00	0.00	0.00
Sediment	1.00	20.82	0.83	21.90	1.00	66.67	1.00	80.24
Vegetative detritus	0.88	31.73	0.50	3.33	1.00	11.24	0.12	2.00
Unidentified	0.24	23.50	1.00	78.83	1.00	20.86	1.00	12.24
O. mossambicus								
Bacillariophyceae	0.06	4.00	0.25	7.75	0.50	3.63	0.50	3.40
Dinophyceae	0.29	5.00	0.00	0.00	0.00	0.00	0.10	1.00
Chlorophyceae	0.00	0.00	0.06	1.00	0.88	2.14	0.20	15.50
Cyanophyceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Euglenoids	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sediment	1.00	87.35	1.00	87.25	1.00	71.56	1.00	58.30
Vegetative detritus	0.94	8.19	0.56	7.56	0.69	11.27	1.00	14.10
Unidentified	0.18	18.33	0.19	34.67	0.88	19.43	1.00	22.70

$\%F_i$ = Frequency of occurrence of a dietary item; P_i = prey-specific abundance of a dietary item to the diet of the species

Modified Costello method (Amundsen et al. 1996, modified from Costello 1990)

The original Costello method (Costello 1990) defined the frequency of occurrence ($\%F_i$) of a given prey type N_i as the number of stomachs in which that prey type occurs, divided by the total number of stomachs that contained prey (N), expressed as a percentage with a sum to 100 % requirement (Equation 1). The proportional contribution ($\%A_i$) of each prey type consumed by all the individuals (ΣS_i) was expressed as the percentage of total stomach contents (ΣS_t) consumed by the predators. The proportional contribution of the various prey items to the diet of the consumer should also sum to 100 %.

$$\%F_i = \frac{Ni}{N} * 100$$

$$\%A_i = \frac{\sum Si}{\sum St} * 100$$

(1)

This two-dimensional expression of prey contribution ($\%F_i$ and $\%A_i$) to the diet of the predator allowed the assessment of prey importance to the diet of the population, i.e. dominant (100% contribution and occurrence) to rare, but also provided insight in to the feeding strategy of the predator population. However, Amundsen et al. (1996) noted the importance of distinguishing between the dietary preferences of different individuals, instead of merely examining the diet of the population as a whole. Further shortcomings of the Costello method are discussed in Amundsen et al. (1996).

Amundsen et al. (1996) modified the Costello method to enable the expression of the within- and between individual phenotypic contribution to the population's niche width on a similar two-dimensional plot (Costello 1990). The calculation of the frequency of occurrence remained the same (Equation 1), but instead of calculating the proportional ($\%A_i$) contribution of prey types based on the total stomach contents (Equation 1), Amundsen et al. (1996) suggested that the contribution of each prey group should be expressed i.e. the prey-specific abundance (P_i) (Equation 2; Amundsen et al. 1996, *modified from* Costello, 1990).

$$\%F_i = \frac{Ni}{N} * 100$$

$$P_i = \frac{\sum Si}{\sum S_{t_i}} * 100$$

(2)

The P_i of prey type i equals the sum of the stomach proportions (ΣS_i) containing prey type i, divided by the total number of stomachs (S_{ti}) that actually contained prey type i. This differs from the original Costello method as the contribution of the specific prey is expressed as a prey-specific abundance (expressed in fraction rather than in percent), instead of as a percentage abundance (A_i) in the entire stomach contents of all the individuals (Amundsen et al. 1996). The sum of P_i*'s* of each prey type i does not sum to 100 %, instead the product of P_i and $\%F_i$ equals the standard prey abundance (A_i) (Amundsen et al. 1996) (Equation 3).

$$A_i = P_i * \%F_i \tag{3}$$

In other words, the sum of area enclosed by the complementary two points (P_i and $\%F_i$) on the graph for each prey type (Figure 2 of manuscript) would equal the total area of the diagram (= 100% or A_i) (Amundsen et al. 1996). A low $\%F_i$ with a high P_i indicates a high within population phenotypic variance with some individuals specializing on particular prey items (Amundsen et al. 1996).

Stable isotopes

The statistical software package, Stable Isotope Analysis in R (SIAR v. 4.2) (Parnell et al. 2010) was used to construct a Bayesian isotopic mixing model to examine the proportional contribution of dietary sources to the isotopic signature of each species. The 25, 75, and 95% Bayesian credibility intervals (analogue of confidence intervals) of the contribution of the different food sources were obtained using a Markov Chain Carlo simulation, using Dirichlet distribution, in the SIAR package (Parnell et al. 2010). The trophic discrimination factors used were 3.4 ± 1.0‰ (Mean ±SD) for δ^{15}N and 0.4 ± 1.0‰ for δ^{13}C (Post 2002), and the model was parameterized based on the main ingested dietary sources.

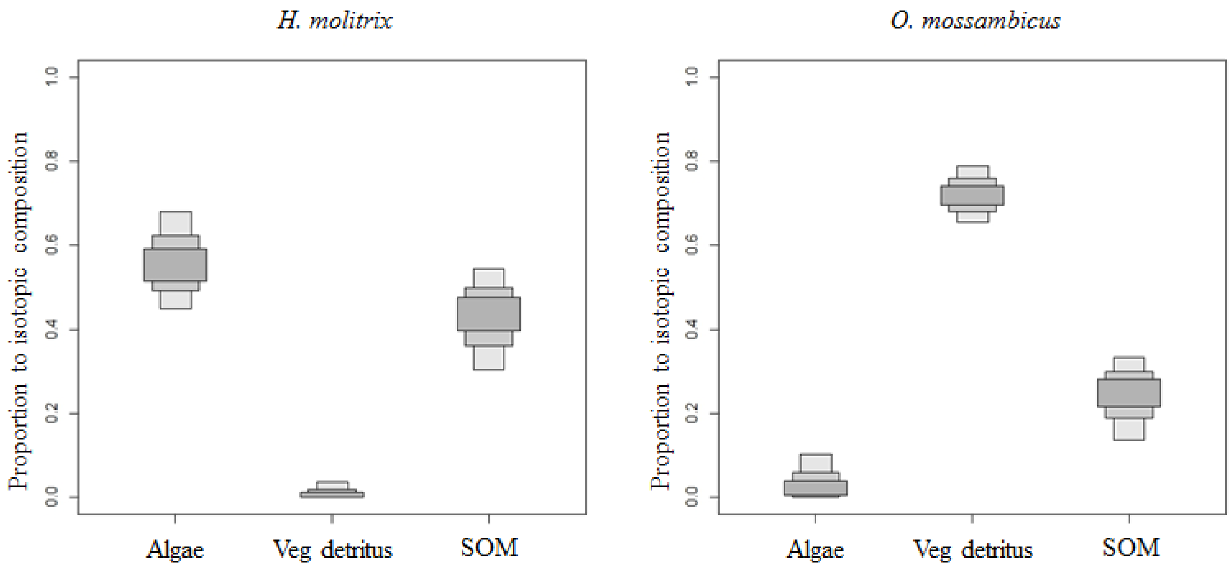

Figure 1S: Proportional contribution (Median with 25, 75, 95% Bayesian credibility intervals) of dietary sources to the diet of *H.* molitrix (left), and *O. mossambicus* (right), in Flag Boshielo Dam during 2011. The dietary sources included algae, vegetative detritus (veg detritus), and sediment organic matter (SOM). This Bayesian model solution, generated by the package, Stable Isotope Analysis in R (SIAR), required high (*c.* 10 ‰) trophic enrichment factors and the model may be considered undetermined

Online Resource C

Community matrix analysis

To reconstruct the community of consumers in the oligotrophic FBD, we included: a) invertebrates (Chironomidae, Gomphidae, and Naucoridae); b) 11 fish species (Table 3S); c) fish fry of various species; d) Pleuroceridae (operculate snails); e) Potamonautidae (crabs); and f) Atyidae (freshwater shrimp) (Table 3S). With the exception of Atyidae that occupied a T.L of 2.7, the assemblage of invertebrates occupies the T.L range 1.2 to 2.2. The fishes in the analysis occupied a T.L ranging from 2.2 (*Labeo rosae*) to 3.1 (*Tilapia sparrmanii*).

Table 3S: Summary of the stable isotope analysis results and population metrics used (Mean ± SD). Seasonal variation in the isotopic niche utilization of *H. molitrix*, and *O. mossambicus* in Flag Boshielo Dam are presented. The isotopic niche width, as described by the small samples size corrected Standard Ellipse Area (SEA_c), trophic level (T.L.) and sample size (*n*) are displayed, as well as the isotopic niche overlap between *H. molitrix* and other indigenous species sampled during summer 2011

- = not measured/not calculated

	Mean ± SD		Range					SEA_c	
H. molitrix	‰δ^{15}N	‰δ^{13}C	‰δ^{15}N	‰δ^{13}C	T.L.	SEA_c	*n*	overlap	
Autumn	13.4 ± 0.6	-20.8 ± 1.8	12.5 – 14.2	-17.8 – -24.8	2.68	2.24	20	-	
Winter	14.0 ± 0.4	-20.2 ± 1.9	13.2 – 14.8	-17.2 – -23.6	2.85	1.69	23	-	
Spring	14.2 ± 0.3	- 22.7 ± 1.7	13.7 – 14.7	-18.8 – -24.9	2.92	1.59	22	-	
Summer	14.2 ± 0.7	- 21.6 ± 2.0	12.5 – 15.3	-18.5 – -26.3	2.92	3.86	22	-	
Mean	14.0 ± 0.6	-21.3 ± 2.1	12.5 – 15.3	-17.2 – -26.3	2.84	3.68	87	-	
O. mossambicus									
Mean	13.7 ± 1.1	- 25.5 ± 2.8	9.9 – 14.9	-14.2 – -29.0	2.78	1.14	89	0	
Fish									Weight (g)
Enteromius trimaculates	13.9 ± 0.9	-22.7 ± 1.5	12.9 – 17.1	-20.6 – -24.8	2.83	4.40	11	0.46	4.2 ± 1.2
Barbus unitaeniatus	13.75 ± 1.9	-21.4 ± 2.0	10.8 – 15.9	-18.0 – -24.8	2.78	4.44	15	0.33	1.7 ±1.1
Clarias gariepinus	14.8 ± 0.8	-24.2 ± 1.2	13.8 – 15.8	-22.7 – -26.6	3.09	2.82	9	<0.01	318.2 ± 848.4
Labeo cylindricus	13.9 ± 1.3	-21.7 ± 2.0	10.3 – 16.1	-16.2 – -24.7	2.81	5.71	25	0.60	8.8 ± 8.1
Labeo rosae	11.9 ± 1.2	-21.05 ± 3.7	10.9 – 14.9	-16.5 – -25.8	2.23	14.98	14	<0.01	211.6 ± 175.0
Labeobarbus marequensis	13.5 ± 0.57	-22.18 ± 1.4	13.1 – 16.5	-17.8 – -23.6	2.73	2.72	7	0.32	20.3 ± 39.2
Micralestes acutidens	14.5 ± 0.9	-23.1 ± 1.5	12.4 – 16.5	-20.4 – -25.6	3.01	3.40	30	0.42	2.8 ± 1.8
Pseudocrenilabrus philander	14.8 ± 0.6	-23.9 ± 0.8	13.3 – 15.6	-21.9 – -24.8	3.11	1.70	14	<0.01	2.8 ± 1.0
Schilbe intermedius	13.8 ± 1.4	-21.6 ± 0.2	12.9 – 14.8	-21.4 – -21.7	2.81	-	2	-	259.8 ± 25.1
Tilapia rendalli	12.8 ± 1.1	-22.3 ± 2.8	10.7 – 14.3	-17.0 – -25.1	2.50	10.2	14	0.17	56.1 ± 127.7
Tilapia sparrmanii	14.9 ± 1.1	-23.0 ± 1.5	12.7 – 15.8	-20.2 – -24.9	3.11	5.70	7	0.46	9.9 ± 3.1
Fry Unknown spp.	13.5 ± 0.9	-25.9 ± 2.2	11.9 – 14.7	-19.3 – -27.7		6.11	21	0.27	-
Other						-	-	-	
Pleuroceridae (snail)	10.9 ± 0.7	-21.6 ± 4.0	8.9 – 12.2	-10.9 – -25.2	1.95	-	20	-	
Potamonautidae (crab)	13.7 ± 0.5	-20.5 ± 1.2	13.3 – 14.4	-19.2 – -22.0	2.74	-	4	-	
Atyidae (shrimp)	13.4 ± 1.4	-21.4 ± 0.6	12.0 – 15.1	-20.6 – -22.1	2.66	-	5	-	
Invertebrates									
Chironomidae	8.4 ± 1.1	-26.5 ± 1.2	6.3 – 10.1	-24.7 – -29.4	1.24	-	40	-	
Gomphidae	10.6 ± 1.2	-24.5 ± 1.3	8.9 – 12.5	-22.3 – -26.8	1.87	-	8	-	
Naucoridae	10.1 ± 0.9	-24.4 ± 1.9	8.0 – 12.3	-20.3 – -26.6	1.73	-	27	-	
Sources									
Filamentous algae	0.49	-25.4	-	-	-1.10	-	1	-	
Periphyton	7.4 ± 0.54	-21.5 ± 4.1			0.91		4		
Vegetative detritus	3.2 ± 1.1	-27.0 ± 2.0	2.1 – 4.3	-24.5 – -29.0	-0.30	-	4	-	
Sediment	6.1 ± 1.1	-22.9 ± 1.0	4.3 – 7.9	-21.8 – -24.0	0.53	-	7	-	

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