

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

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Abstract

The alien invasive silver carp *Hypophthalmichthys molitrix* established a self-sustaining feral population in an oligotrophic impoundment, Flag Boshielo Dam, in South Africa. The ability of this population to persist in a dam with low algal biomass (median annual suspended chlorophyll-*a* = 0.08 µg. L⁻¹) and limited access to rivers considered large enough for successful spawning, has implications for their invasive potential in other systems. This study used stomach content and stable isotope analysis to assess the trophic ecology of *H. molitrix* which, was then compared with indigenous Mozambique tilapia *Oreochromis mossambicus*, on a seasonal basis during 2011. *Hypophthalmichthys molitrix* were generalist filter feeders, with a diet consisting primarily of sediment, vegetative detritus, dinoflagellates, and diatoms. The dominance of sediments in their stomachs suggests occasional benthic scavenging.

However, *H. molitrix* occupied a higher trophic level (T.L. = 2.8) than expected, suggesting that this population subsidized their diet with an unidentified dietary constituent, characterised by enriched nitrogen values. Although the stomach contents indicated dietary overlap between *H. molitrix* and *O. mossambicus*, stable isotopes revealed fine-scale resource partitioning, despite that both species occupied the same trophic level. Irrespectively, the persistence of this feral *H. molitrix* population in an oligotrophic impoundment highlights their phenotypic plasticity.

Keywords: Asian carp, diet, dietary overlap, stable isotope analysis, stomach content analysis

Introduction

Bioenergetic and predictive species distribution modeling approaches are extensively relied upon in non-indigenous species risk assessments (Chen et al. 2007, Herborg et al. 2007, Lohmeyer and Garvey 2009, Cooke and Hill 2010). Yet, a thorough understanding of the physiological and ecological parameters that define a species fundamental niche (Hutchinson 1978) is required for accurate risk assessments (Soberón and Peterson 2005, Kulhanek et al. 2011, Cooke 2015). Species that are released from abiotic and biotic factors that define their realized native range niche (Grinnellian niche, Grinnell 1917) often display phenotypic plasticity that enables them to establish in novel environments (Soberón and Peterson 2005). Combining information on dietary plasticity with measures of physiological tolerances, in both the native and invaded range of the species, can increase the accuracy of predictive modeling approaches (Cooke 2015).

Silver carp *Hypophthalmichthys molitrix* (Valenciennes 1844), are native to eastern Asia, but have become an invasive species of global importance after being introduced worldwide for aquaculture (Kolar et al. 2007, Gozlan et al. 2010). Although *H. molitrix* can persist in lotic environments (Kolar et al. 2007), they are incapable of maintaining self-sustained feral populations in isolated reservoirs without continuous stocking, as observed in other impoundments (Spatura and Gophen 1985, Kamilov 2014, Gophen and Snovsky 2015). Their specialist planktivorous diet further suggests that they are incapable of persisting in environments with low algal biomass, such as the Laurentian Great Lakes of North America

(Kolar et al. 2007, Cooke and Hill 2010). Nevertheless, the possibility that they may invade the meso-oligotrophic Great Lakes region (chlorophyll-*a* *c.* 3 $\mu\text{g. L}^{-1}$, Carrick 2005) (Calkins et al. 2011, Kocovsky et al. 2012), led to the implementation of a range of expensive control measures to prevent them spreading from adjacent river catchments (Rasmussen et al. 2011). However, more information regarding their life-history traits in other invaded ranges is required before their invasive potential can be ascertained (Coulter et al. 2013, reviewed in Cooke 2015).

Hypophthalmichthys molitrix was imported into South Africa in 1975 for experimental control of phytoplankton, and subsequently released into the oligotrophic Flag Boshielo Dam (hereafter, FBD) in the 1990s (Prinsloo and Schoonbee 1987, Brits 2009). Eradication attempts removed *c.* 15 000 individuals from FBD in 1990 (Brits 2009), but, the population became feral and their distribution range has since expanded downstream to include river systems in the Kruger National Park (Brits 2009, Lübcker et al. 2014). The invasion into FBD did not follow a typical lag phase before establishing (Brits 2009, Gozlan et al. 2010), highlighting that oligotrophic impoundments with limited access to large rivers are potentially suitable for their establishment.

The recruitment success of *H. molitrix* is associated with access to long (> 80 – 100 km) free-flowing rivers with a suitable hydrology (Kolar et al. 2007, Kocovsky et al. 2012), although differences in spawning requirements (phenotypic plasticity) between their native and invaded ranges have recently been observed in several studies (Kocovsky et al. 2012, Coulter et al. 2013, Deters et al. 2013, reviewed in Cooke 2015). Irrespectively, *H. molitrix* developed an atypical, persistent and reproducing population in an oligotrophic impoundment in South Africa (Brits 2009, Lübcker et al. 2014) with limited access to rivers considered suitable for spawning (Figure 1). The latter may indicate a level of phenotypic plasticity that is not considered in previous modeling approaches (Cooke 2015). *Hypophthalmichthys molitrix* have been observed to spawn at the confluence of the Elands and Olifants River in FBD (Brits 2009) (Figure 1), and this represents one of the few cases that *H. molitrix* has been observed to spawn in small and short rivers (see also Aliev 1976 and Coulter et al. (2013)). The Olifants River is the main river flowing into FBD (*c.* 83 km long), and access for *H. molitrix* from the dam to the river is limited by a weir located *c.* 6.6 km upstream of the dam mouth (Brits 2009). Similarly, on the Elands River, another inflow river of FBD, *H. molitrix*

can only access about 5.6 km of the river channel. It is possible that *H. molitrix* might not spawn annually in FBD, as also noted in the Illonios River, USA (Irons et al. 2007, Hayer et al. 2014), but their persistence for over 24 years demonstrates their resilience.

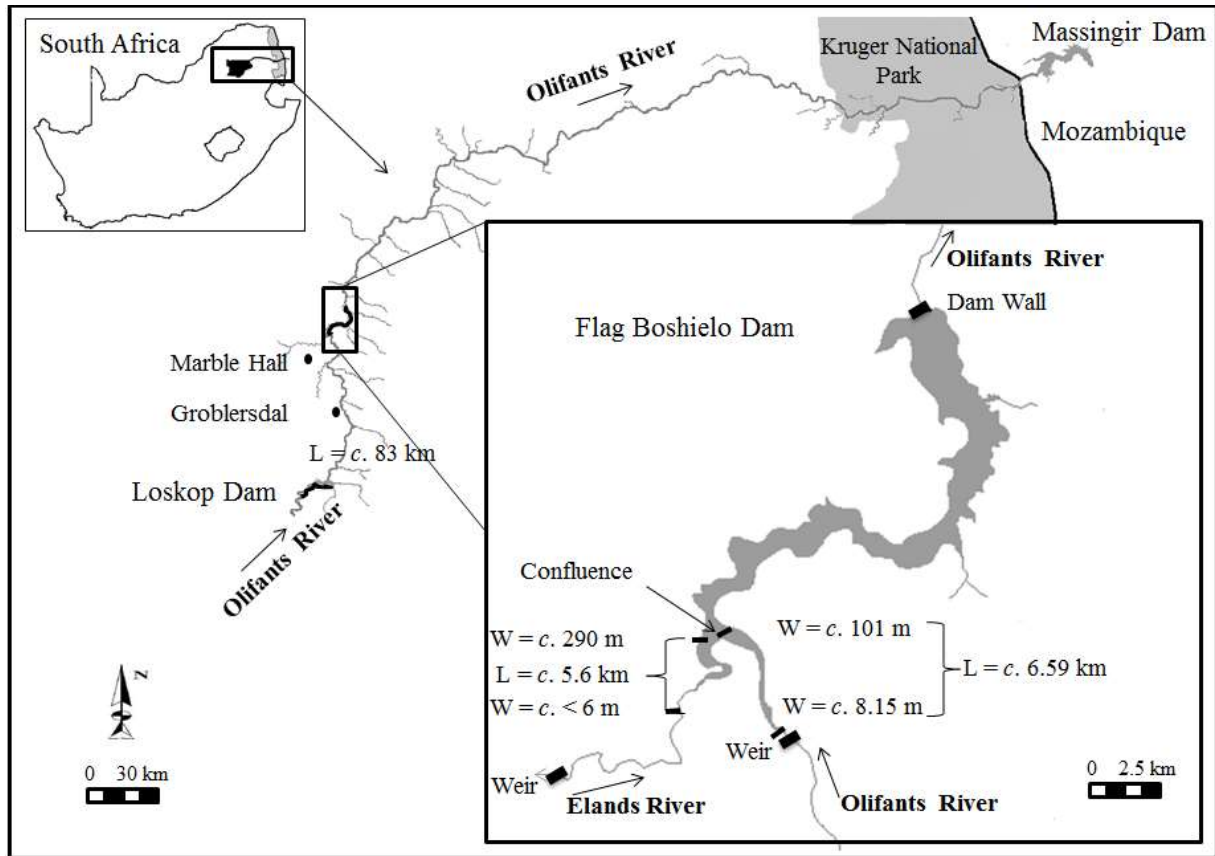


Figure 1: Location of Flag Boshielo Dam (24°47' S, 29°25' E), a man-made impoundment in the Olifants River system in South Africa. L = river length in kilometer (km), W = channel width in meter (m)

Hypophthalmichthys molitrix are well suited for biological control of phytoplankton but have been implicated as drivers of adverse ecological impacts in recipient ecosystems (Spatara and Gophen 1985, Irons et al. 2007, Sampson et al. 2009). Although initially described as a specialist phytoplankton filter feeder in their native range (Kolar et al. 2007), studies conducted in invaded ranges described *H. molitrix* as an opportunistic, generalist filter feeder with a diet consisting of phytoplankton, zooplankton, bacteria, and detritus (Bitterlich 1985, Spataru and Gophen 1985, Lu et al. 2002, Kolar et al. 2007, Sampson et al. 2009, Calkins et al. 2011). High-density *H. molitrix* populations reportedly mediate changes in the zooplankton, phytoplankton, and fish community structure, initiating a decrease in the abundance of indigenous fish species despite having limited dietary overlap (e.g., Spataru and

Gophlen 1985, Irons et al. 2007). The economic and ecological impacts associated with *H. molitrix* invasions rank them as one of the worst aquatic pests in the world (Fowler et al. 2007). Most studies of *H. molitrix*, however, focused on their trophic ecology and persistence in large eutrophic rivers (Kolar et al. 2007). To our knowledge, no studies have documented their diet and self-sustaining persistence in an oligotrophic impoundment. The effects of a *H. molitrix* invasion are potentially more detrimental for impoundments with a low standing algal biomass, due to their efficiency of straining large amounts of phytoplankton (Cooke and Hill 2010), potential dietary plasticity, and potential to obtain high population densities (Kolar et al. 2007, Sass et al. 2010).

The primary aim of this study was to describe the trophic ecology of *H. molitrix* within the food web of an oligotrophic impoundment, using stomach content and stable isotope analysis. We compared the diet of *H. molitrix* to that of an indigenous fish species, Mozambique tilapia *Oreochromis mossambicus* (Peters 1985), on a seasonal basis. The approach adopted was similar to Bitterlich (1985), and we assessed the dietary overlap between a fish species without a true stomach (*H. molitrix*) and a species with a true stomach (*O. mossambicus*). *Oreochromis mossambicus* are a major component of the fish fauna of FBD, and their dietary niche is likely to overlap with *H. molitrix* (Bitterlich 1985, Dabrowski et al. 2014a). In addition, we compared the trophic niche of *H. molitrix* with other indigenous species sampled once-off during summer to identify potential species that *H. molitrix* might compete with. This study is the first to describe the dietary niche, persistence and trophic interrelationships of *H. molitrix* in an oligotrophic impoundment.

Materials and methods

Study site

Flag Boshielo Dam is a man-made impoundment situated at the confluence of the Olifants and Elands rivers, Mpumalanga Province, South Africa (Figure 1), with a catchment area of 23,555 km² (Department of Water Affairs and Forestry 2005 (DWAFF)). The mean depth of Flag Boshielo Dam is 8.6 m (maximum 33 m) with a surface area of 1 288 ha, stretching 26 km from the dam mouth to the dam wall (DWAFF 2005). The mean annual water inflow (2006 – 2012) from the Elands River is 28.51 x 10⁶ m³ (average discharge 0.9 m³.s⁻¹) and 218.46 x

10^6 m^3 ($7.0 \text{ m}^3 \cdot \text{s}^{-1}$) from the Olifants River, and a mean water retention time of 4.9 months (Dabrowski et al. 2014b). The Olifants River is considered perennial, but often recedes to a series of fragmented pools because of over-abstraction of water for agriculture, and periodic droughts experienced in the catchment due to the arid to semi-arid regional climate (Botha 2010). A detailed description of the study site is given in Dabrowski et al. (2014b) and Brits (2009).

Physical and chemical water quality analysis

Water quality samples were collected every two months at four sites between April and December 2011, detailed in Dabrowski et al. (2014b). We provide a summary of water quality parameters that are relevant to the persistence of *H. molitrix* which, includes pH, dissolved oxygen, electrical conductivity, suspended chlorophyll-*a*, secchi disc depth, and water temperature.

Table 1: Summarised water quality parameters measured during 2011 in Flag Boshielo Dam across four seasons ($n = 16$ samples) (Dabrowski et al. 2014b)

Parameter	Median	Min	Max
pH	8.96	7.38	9.42
Dissolved Oxygen ($\text{mg} \cdot \text{L}^{-1}$)	10.02	7.04	14.2
Electrical Conductivity ($\mu\text{S} \cdot \text{cm}^{-1}$)	403	177	576
Temperature ($^{\circ}\text{C}$)	27.2	18.1	31.8
Secchi Depth (cm)	62	41	85
Inorganic Nitrogen ($\text{mg} \cdot \text{L}^{-1}$)	<u>0.11</u>	<u>0.11</u>	0.52
Inorganic Phosphorus ($\text{mg} \cdot \text{L}^{-1}$)	<u>0.06</u>	0.01	0.08
Suspended Chlorophyll- <i>a</i> ($\mu\text{g} \cdot \text{L}^{-1}$)	0.08	0.01	0.6

Underlined, censored values replaced at 0.55 times instrument detection limit

Flag Boshielo Dam had an alkaline pH ranging from 7.4 to 9.4, with low chlorophyll-*a* concentrations (Table 1). The median suspended chlorophyll-*a* concentration measured during the year across all four sites was $0.08 \mu\text{g} \cdot \text{L}^{-1}$. Autumn had the lowest measured suspended chlorophyll-*a* (median = $0.04 \mu\text{g} \cdot \text{L}^{-1}$) with winter suspended chlorophyll-*a* (median = $0.16 \mu\text{g} \cdot \text{L}^{-1}$) levels being marginally higher (in absolute terms) than spring (median = $0.08 \mu\text{g} \cdot \text{L}^{-1}$). The maximum suspended chlorophyll-*a* ($0.60 \mu\text{g} \cdot \text{L}^{-1}$) was measured

at the inflow of the Olifants River during summer (median = 0.44 $\mu\text{g. L}^{-1}$, range = 0.11–0.60 $\mu\text{g. L}^{-1}$). The median inorganic nitrogen and phosphorus concentrations were close to instrument detection limit, classifying FBD as oligotrophic according to guidelines published by DWAF (2002). No incidents of algal blooms were observed or reported during the study period. The relatively low median secchi disc value of 62 cm measured, in combination with the low suspended chlorophyll-*a* values, indicates non-algal water turbidity with the water clarity being reduced by other factors. The fine suspended particles could not be filtered out with 3 μm filters, and the total suspended solids (median 5.68 mg. L^{-1} from 2006 to 2011) suggested that the turbidity was not due to minerals or organic material. This was supported by the low levels of dissolved organic carbon (median 8 mg. L^{-1} , Dabrowski et al. 2014b).

Fish sample collection

Sampling took place once every two months between April and December 2011 ($n = 4$ sampling occasions). We collected a minimum of 20 individual *H. molitrix* and *O. mossambicus* specimens per sampling trip (Table 2). Three, 25 m long multi-filament gill nets with stretched-mesh sizes of 70, 90, and 130 mm were set during daylight, as described in Dabrowski et al. (2014a). A hook and line were predominantly used to collect *H. molitrix*, as they occur in high densities in FBD. *Hypophthalmichthys molitrix* occur throughout FBD and were collected opportunistically, but the highest concentrations occurred at the confluence and mouth of the Olifants River (Figure 1).

Only specimens with a total length of > 200 mm were collected to reduce variation associated with ontogenetic dietary shifts. After collection, fish were weighed (W , in g) and their standard length (SL , in cm) was recorded. Fish condition factor (CF) was calculated according to Beckman (1948) using the equation $CF = W * 10^5 SL^3$, following Spatura and Gophen (1985). The calculated Fulton's condition factor (Carlander 1950) and length-weight relationship (Froese & Pauley 2012) is detailed elsewhere (Online Resource A), provided to enable comparison with other studies that used different body condition indices.

Table 2: Sample summary, measurements, and condition factor (CF) of *H. molitrix*, and *O. mossambicus*, collected in Flag Boshielo Dam during 2011 (mean \pm SD). SL = standard length in centimeter (cm); n = sample size; % Empty = percentage of empty stomachs

Species	Season	n	% Empty	Weight (g)	SL (cm)	CF
<i>H. molitrix</i>	Autumn	20	15	441.7 \pm 130.4	29.3 \pm 4.3	1.80 \pm 0.51
	Winter	23	49	466.3 \pm 178.8	34.0 \pm 3.6	1.14 \pm 0.15
	Spring	22	5	175.5 \pm 18.6	27.4 \pm 3.1	0.88 \pm 0.20
	Summer	22	5	500.5 \pm 217.9	32.2 \pm 4.2	1.46 \pm 0.15
	Total/Mean	87	23	395.8 \pm 201.6	30.8 \pm 4.6	1.30 \pm 0.44
<i>O. mossambicus</i>	Autumn	21	19	845.1 \pm 420.4	27.5 \pm 5.5	3.74 \pm 0.50
	Winter	20	20	906.6 \pm 312.8	29.8 \pm 4.3	3.37 \pm 0.48
	Spring	20	20	662.8 \pm 252.3	26.6 \pm 4.6	3.46 \pm 0.57
	Summer	20	50	342.3 \pm 283.5	22.6 \pm 9.8	3.88 \pm 0.34
	Total/Mean	81	27	659.8 \pm 392.2	30.1 \pm 8.9	3.61 \pm 0.51

Stomach content analysis

The foregut of *H. molitrix*, extending from the proximal end of the intestine to the middle of the first loop, was preserved in 5% formaldehyde for 24 h prior to storage in 75% ethanol. This portion of the gut is approximately one twelfth of the total length of the intestine and dietary items recovered here are relatively undigested (Xie 1999). Each foregut sample was divided into three sub-samples and the proportional volumetric contribution of each dietary item was determined within each of the three sub-samples. This was achieved by evenly spreading each sub-sample of the stomach contents in a Sedgwick–Rafter counting cell chamber after dilution with 100 ml distilled water per gram of sample (Hyslop 1980). Samples were examined under a compound microscope (Carl Zeiss, Germany) at 1250 x magnification and the dietary items classified to class according to Thorp and Covich (2001) (Van Vuuren et al. 2006). Herein, we report the mean proportional contribution of each dietary item averaged for the three sub-samples to ensure that the total contribution of each dietary item to the entire foregut was accurately represented. The identified dietary items were divided into eight categories: vegetative detritus; diatoms (Bacillariophyceae); benthic dinoflagellates (Dinophyceae); cyanobacteria (Cyanophyceae); green algae (Chlorophyceae); Euglenoids; sediment; and unidentifiable material. Unidentifiable material and sediment were

included to prevent overestimations of the proportional contribution of other dietary items present in the stomachs. The proportion of empty stomachs was also recorded. Benthic diatoms, such as *Fragilaria ulna*, form part of the class Bacillariophyceae, but were noted separately if detected in the stomach contents. The ingestion of benthic diatoms by pelagic, filter feeding *H. molitrix* can be considered indicative of sporadic benthic niche exploitation, likely to occur when food resources are limited.

A modified Costello method (Amundsen et al. 1996, *modified from* Costello 1990) was used to calculate the contribution of each dietary item identified in the stomach content analysis, for each season. This index uses the frequency of occurrence and prey-specific abundance of each prey type to give a two-dimensional representation of prey importance (dominant to rare) and feeding strategy (specialist to generalist) (Amundsen et al. 1996). The prey-specific abundance was calculated based on the sum of the stomach proportions that contained a particular prey type, divided by the total number of stomachs that contained that specific prey item (Amundsen et al. 1996). This differs from the standard proportional contribution calculation, which divides the sum of the contribution of a particular prey type by the entire stomach contents (sum to 100% requirement). The prey-specific abundances are expressed as a fraction, rather than as a percentage, and the product of the frequency of occurrence and prey-specific abundance equals the proportional contribution of that prey type (Amundsen et al. 1996) (detailed in Online Resource B). Proportional data were arcsine transformed prior to statistical analysis using statistical software package R (R Core Team, 2013). A Bray–Curtis similarity matrix with a 4th root transformation was constructed using PRIMER statistical package version 6 (Clarke & Gorley 2006) and a one-way analysis of similarity (ANOSIM) was performed to test for dietary differences between species and seasons (Global-R). An R statistic close to 1 indicates strong differences in the diet, while a value close to 0 indicates diet similarity.

Stable isotope analysis

Approximately 5 g of white caudal muscle was dissected from each specimen and frozen before analysis. Samples were degreased with 1:2 chloroform: ethanol solution to remove lipids (Logan et al. 2008), oven-dried overnight at 70 °C and homogenized. The carbon:nitrogen (C:N) mass ratios met the requirements for successful lipid extraction (C:N

mass ratio of *c.* 3.5, Post et al. 2007), and further lipid corrections were unnecessary. Aliquots of each sample were weighed (0.8–0.9 mg) into tin capsules, pre-cleaned in Toluene, prior to combustion at 1020 °C in an elemental analyzer (Flash EA, 1112 Series, Thermo™, Thermo Fisher Scientific, Bremen, Germany). The carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios (expressed in delta (δ) notation) were determined using a continuous-flow isotope ratio mass spectrometer (CFIRMS, Delta V Plus, Thermo Fisher, Bremen, Germany). Results are reported relative to the standards Vienna Pee Dee Belemnite (VPDB) for carbon, and atmospheric air (AIR) for nitrogen, and isotopic ratios were expressed in percent per thousand (‰) (Coplen 1994). Duplicate samples, with an in-house standard and blank interspersed after every 10 samples, were analysed to ensure reproducibility. The reproducibility of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were <0.2‰.

The samples required to delineate the food web structure in FBD were collected once-off during the austral summer, from 6 – 8 December 2011. Samples were collected inshore at the mouth of the Olifants River, the confluence (Figure 1), as well as the main basin close to the dam wall on rock, sand, and plant substrates, depending on availability of dietary items which included a) invertebrates (Chironomidae ($n = 40$), Gomphidae ($n = 8$), and Naucoridae ($n = 27$)); b) 11 fish species ($n = 148$ samples) (Summarised in Online Resource C, Table 3S), c) fish fry of various species ($n = 21$), d) Pleuroceridae (operculate snails) ($n = 20$), e) Potamonautidae (crabs) ($n = 4$), and f) Atyidae (freshwater shrimp) ($n = 5$). Vegetative detritus, sediment organic matter (i.e., organic components present in the sediment sample), filamentous algae, and invertebrates were collected by hand. The vegetative detritus consisted of dead organic material originating predominantly from plant origins. Smaller, or rare specimens of various fish species were collected using a 1 mm mesh seine net and an electrofisher (SAMUS 725MP), in addition to sampling with gill nets (all species listed in Online Resource C, Table 3S). Attempts to integrate water column phytoplankton and zooplankton samples by vertically dragging a phytoplankton net with a 20 μm mesh from the bottom-up and straining 10 ml sub-samples at the confluence, mouth of the Olifants River, main basin, and close to the dam wall (Figure 1), yielded insufficient material to obtain an isotopic measurement. The inability to secure phytoplankton and zooplankton during the sampling for this research is not the result of insufficient effort. Subsequent to this there have been two additional efforts to sample phytoplankton by towing a phytoplankton net behind a boat. One of these efforts involved a 1.2 m diameter phytoplankton net with a 20 μm mesh

towed for roughly 1 kilometer, but it still yielded insufficient material to measure (< 0.1 mg wet weight). Failure to obtain any phytoplankton samples reaffirms the low algal productivity of FBD, and subsequent, intensified attempts to sample phytoplankton in FBD were also unsuccessful. The sample pretreatment protocol for baseline samples was the same as that described for the fish muscle samples, except that carbonates were removed from all prey samples using 1% HCl solution and rinsed in distilled water (Jacob et al. 2005).

There were no apparent site-related differences in the isotopic signature of the sampled baseline items, or between *H. molitrix* collected from various sites. The data were therefore not analysed per site, but pooled for the analyses. Furthermore, *H. molitrix* can travel up to 64 km.d⁻¹, depending on river flow and other variables (DeGrandchamp et al. 2008), but they usually travel around 0.2–10.6 km.d⁻¹ when feeding (DeGrandchamp et al. 2008). We, therefore, held the assumption that the *H. molitrix* would integrate any variation in the isotopic signature of the same prey, at different locations during the 2-3 month period represented by the sampled muscle tissue.

For all analyses, we assessed normality using a Shapiro-Walks normality test while an *F*-test was used to compare the variances between variables, before applying an appropriate parametric (Analyses of Variance; ANOVA, followed by a Tukey's *post hoc* comparison) or non-parametric Kruskal-Wallis χ^2 test followed by a *post hoc* pairwise Wilcoxon rank sum test. Values are presented as means \pm one standard deviation (SD) where applicable, and significance was assumed at $p < 0.05$. A Welch Two Sample *t*-test was used to assess the overall inter-specific differences in the isotopic ratios of the two species. 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 $3.4 \pm 1.0\text{‰}$ (Mean \pm SD) for $\delta^{15}\text{N}$ and $0.4 \pm 1.0\text{‰}$ for $\delta^{13}\text{C}$ (Post 2002), and the model was parameterized based on the main ingested dietary sources. The standard Ellipse Area (SEA_c), which represents the core isotopic niche (40% of the data) of each fish population, was used to estimate the degree of isotopic overlap among fish species (Jackson

et al. 2011). The size of the isotopic niche widths was determined using the *Stable Isotope Bayesian Ellipses* package (SIBER) in R (Jackson et al. 2011), and statistical comparisons were performed in the Bayesian modeling framework after performing 10^6 posterior draws, detailed in Jackson et al. (2011).

The relative trophic level (hereafter T.L.) occupied by consumers was determined relative to the baseline of FBD, following the method of Rogowski et al. (2009) which, is required to enable comparison between *H. molitrix* sampled from different sites and between similar systems (Rogowski et al. 2009). Isotrophic lines in isotopic space have been defined for the Olifants River system (Woodborne et al. 2012), and similar to Rogowski et al. (2009), we utilized a primary consumer (T.L. = 2) (Pleuroceridae, operculate snails) in this study to guide the interpretation of the FBD trophic structure based on a 3.4‰ $\delta^{15}\text{N}$ trophic discrimination factor. Statistical analyses were performed using the statistical software package R (R Core Team, 2013).

Results

Stomach content analysis

A total of 168 specimens comprising 87 *H. molitrix* and 81 *O. mossambicus* were collected in FBD between April and December 2011 (Table 2). The stomach contents of *H. molitrix* consisted primarily of sediment (mean = $49.8 \pm 30.4\%$), unidentifiable material (mean = $25.2 \pm 29.5\%$), vegetative detritus (mean = $11.0 \pm 21.4\%$), diatoms (Bacillariophyceae) (mean = $5.7 \pm 13.8\%$), and benthic dinoflagellates (Dinophyceae) (mean = $5.7 \pm 13.0\%$), based on the proportional contribution ($n = 67$ stomachs containing prey). Green algae (Chlorophyceae) (mean = $2.4 \pm 5.5\%$), cyanobacteria (Cyanophyceae) (mean = $0.1 \pm 0.6\%$), and other flagellates (Euglenoids) (mean = $0.04 \pm 0.3\%$) were also detected, albeit in low quantities (detailed in Online Resource B, Table 2S). The observed prey-specific abundance and frequency of food items differed seasonally (Global $R = 0.7$, $p < 0.001$). In autumn, large proportions of diatoms (mean = 34.0%), vegetative detritus (mean = 31.7%), and sediments (mean = 20.8%) occurred in most stomachs. Green algae (mean = 4.2%), Euglenoids and cyanobacteria occurred in smaller proportions and lower frequencies during autumn (Figure 2). Autumn had the largest prey richness (eight prey categories), while winter and spring had

the lowest number of prey categories (four prey categories) observed in the stomachs of *H. molitrix*. In winter, unidentified material contributed the largest proportion of ingested dietary items (mean = 78.8%), relative to sediments (mean = 21.9%) and vegetative detritus (mean = 3.3%). In spring, sediments occurred in most stomachs at higher proportions (mean = 66.7%) than unidentified material (mean = 20.9%), vegetative detritus (mean = 11.2%), and green algae which occurred in 62% (mean) of stomachs, although mostly in small quantities (mean = 2.0%). In summer, the number of identified prey categories increased to five, which included Bacillariophyceae, sediment, unidentified material, and Chlorophyceae. The prey-specific contribution of Bacillariophyceae was 3.4 % (Figure 2), and the presence of the benthic diatom *F. ulna* was noted. . Sediments, however, still made up the main component of ingested food items (mean = 80.2%). The CF of *H. molitrix* differed significantly with season (ANOVA: $F_{2, 84} = 3.80$, $n = 87$, $p < 0.001$) with a minimum in spring (CF = 0.9) and maximum in autumn (CF = 1.8) (Table 2) which, was significantly higher than all the other seasons ($p < 0.001$) (detailed in Online Resource A).

The stomach contents of *O. mossambicus* consisted primarily of sediments in terms of prey-specific abundance (mean = 76.1%) and frequency (100% of stomachs) throughout the year (Figure 2, $n = 59$ stomachs containing prey). The prey-specific proportion and frequency of occurrence of the other food items, however, differed seasonally (Global $R = 0.53$, $p < 0.001$). In autumn, vegetative detritus had a high frequency of occurrence (94% of stomachs), but occurred in low proportions (mean = 8.2%). In winter, sediment occurred in all the analysed stomachs, contributing 87.3 % (mean) of the stomach contents.. Vegetative detritus occurred in most of the stomachs (mean = 56%) in low proportions (mean = 7.6%), in contrast to unidentified material that occurred at lower frequency (19% of stomachs), but higher proportions (mean = 34.7%) during winter. In spring, there was an increase in the frequency of occurrence of vegetative detritus, green algae, diatoms, and unidentified materials, but they each occurred in small (<20%) proportions (Figure 2). The proportional contribution of sediment to their diets was 72% (mean) during spring. In summer, the proportion of sediment (mean = 58.3%), unidentified materials (mean = 22.7%), vegetative detritus (mean = 14.1%), and diatoms (mean = 3.4%) remained relatively similar to the other seasons, but green algae occurred (frequency) in fewer stomachs (mean = 20%) relative to spring (mean = 88%). Diatoms occurred in 50% (mean) of the stomachs, but in low proportions (mean = 3.4%) during summer, consisting predominantly of *F. ulna*.

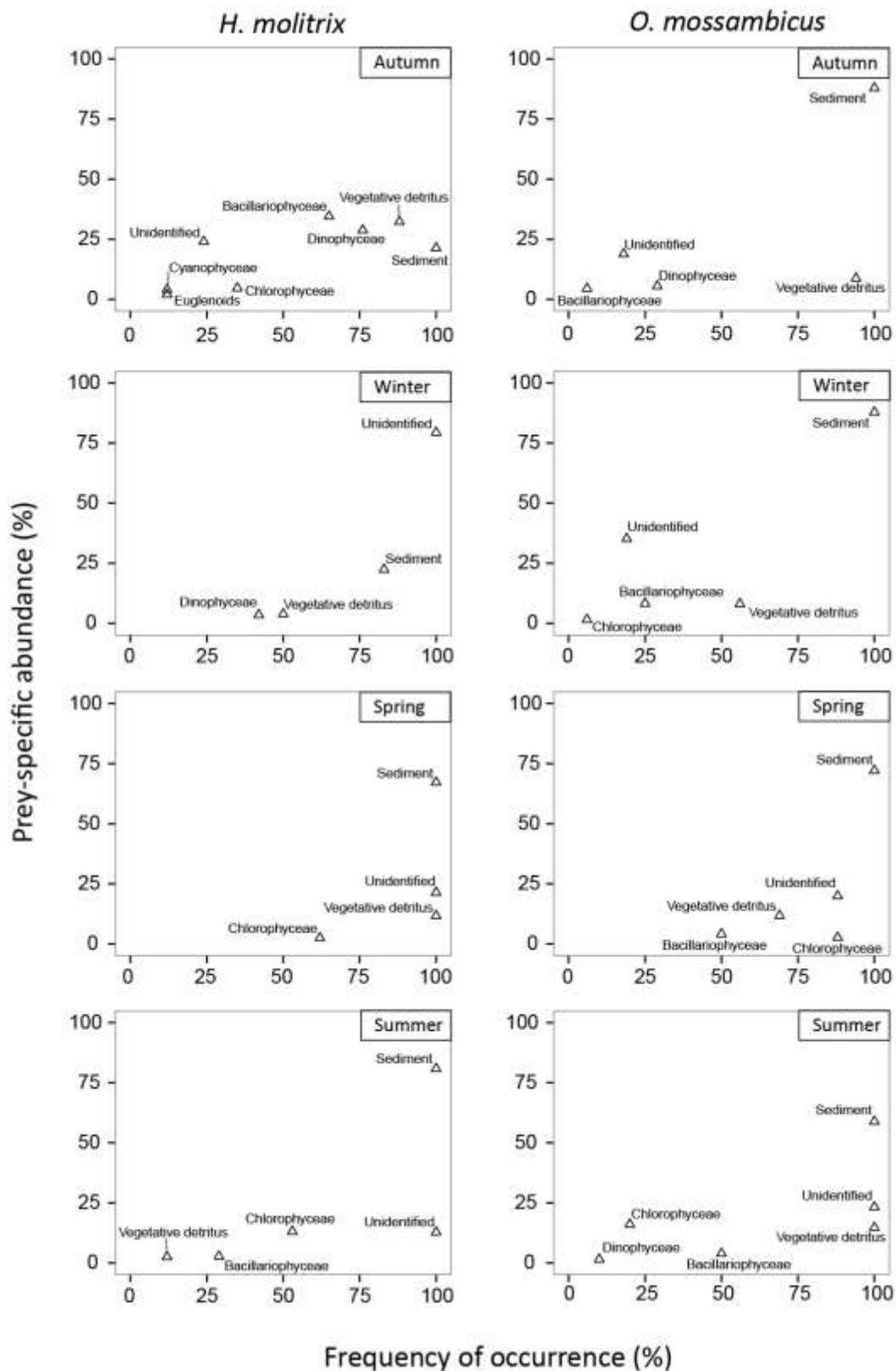


Figure 2: Seasonal differences in the diet of *H. molitrix* (left), and *O. mossambicus* (right), sampled in Flag Boshielo Dam during 2011. The stomach contents analysis, expressed as prey-specific abundance and frequency of occurrence, demonstrates the contribution of each dietary source to their feeding strategies

Stable isotope analysis

Significant seasonal shifts in $\delta^{15}\text{N}$ (Kruskal-Wallis $\chi^2 = 25.2$, $df = 3$, $p < 0.001$) and $\delta^{13}\text{C}$ (Kruskal-Wallis $\chi^2 = 17.6$, $df = 3$, $p < 0.001$) were observed for *H. molitrix* (Figure 3). A pairwise Wilcoxon rank sum *post hoc* test indicated that $\delta^{15}\text{N}$ in autumn was significantly lower than in spring, summer, and winter ($p < 0.01$) (Table 3). The mean $\delta^{13}\text{C}$ in spring was significantly depleted when compared to autumn and winter ($p < 0.01$), but it was not significantly different from summer ($p = 0.15$). The isotopic niche width (SEA_c) of *H. molitrix* was significantly larger during summer and autumn than in winter and spring ($p < 0.05$), compared in the Bayesian framework (Table 3).

Table 3: Seasonal variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Mean \pm SD), as well as the isotopic niche width utilized [Standard Ellipse Area (SEA_c)] by *H. molitrix* and *O. mossambicus* in Flag Boshielo Dam, sampled during 2011

Species	Season	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	SEA_c
<i>H. molitrix</i>	Autumn	13.4 \pm 0.6	-20.8 \pm 1.8	2.24
	Winter	14.0 \pm 0.4	-20.2 \pm 1.9	1.69
	Spring	14.2 \pm 0.3	-22.7 \pm 1.7	1.59
	Summer	14.2 \pm 0.7	-21.6 \pm 2.0	3.86
	Mean	14.0 \pm 0.6	-21.3 \pm 2.1	3.68
<i>O. mossambicus</i>	Autumn	13.6 \pm 0.8	-26.3 \pm 0.8	1.77
	Winter	14.1 \pm 0.4	-26.7 \pm 0.7	0.86
	Spring	14.3 \pm 0.4	-26.2 \pm 0.4	0.44
	Summer	13.8 \pm 0.4	-25.8 \pm 0.4	0.54
	Mean	13.7 \pm 1.1	-25.5 \pm 2.8	1.14

Significant seasonal shifts in $\delta^{15}\text{N}$ (Kruskal-Wallis $\chi^2 = 16.7$, $df = 3$, $p < 0.001$) and $\delta^{13}\text{C}$ (Kruskal-Wallis $\chi^2 = 15.8$, $df = 3$, $p < 0.01$) values were also observed for *O. mossambicus*. The $\delta^{15}\text{N}$ values were significantly lower in autumn than all the other seasons ($p < 0.01$), except summer ($p = 0.55$), while $\delta^{13}\text{C}$ was significantly higher in summer relative to the other seasons ($p < 0.05$) (Table 3). The SEA_c of *O. mossambicus* also differed across seasons, it was significantly larger in autumn than in the other three seasons (Figure 3).

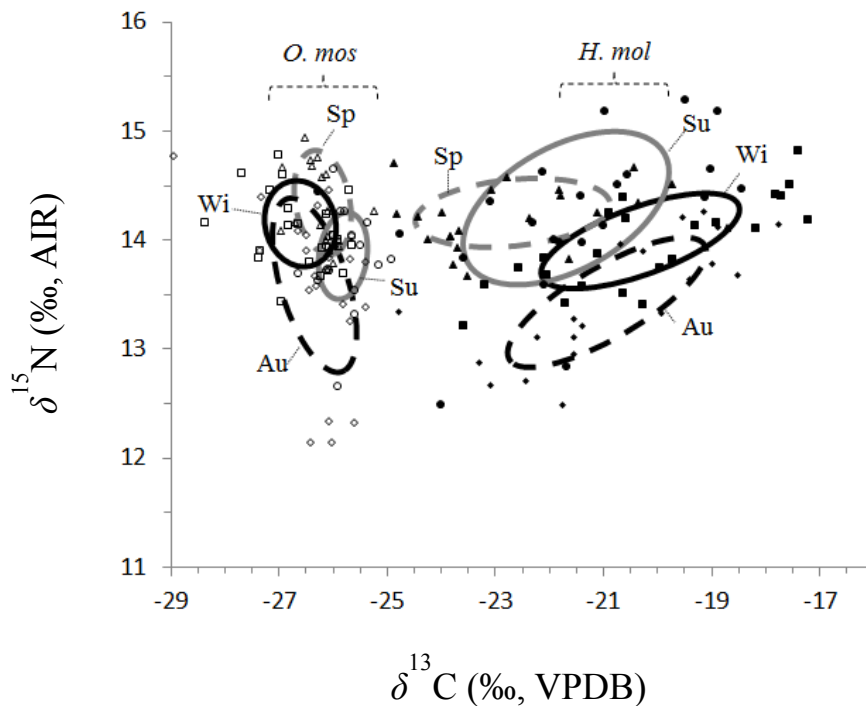


Figure 3: Isotope biplot with the Standard Ellipse Area (SEA_c) indicating the isotopic niche width of *H. molitrix* (*H. mol*, filled symbols), and *O. mossambicus* (*O. mos*, open symbols) sampled in Flag Boshielo Dam during 2011. Seasons: Au = autumn (\diamond), Wi = winter (\square), Sp = spring (Δ), Su = summer (\circ)

Hypophthalmichthys molitrix and *O. mossambicus* had similar mean $\delta^{15}\text{N}$ values ($14.0 \pm 0.6\text{‰}$ and $13.7 \pm 1.1\text{‰}$, respectively) (Welch two sample t -test (df) = 165.031, t = 0.1, p = 0.919), but the mean $\delta^{13}\text{C}$ values were significantly (Welch two sample t -test (df) = 143.3, t = 16.0, p < 0.001) more depleted in *O. mossambicus* ($-25.5 \pm 2.8\text{‰}$) than in *H. molitrix* ($-21.3 \pm 2.1\text{‰}$) (Table 3). No isotopic niche overlap occurred between the two species across all four seasons (Figure 3). The isotopic niche width (SEA_c) of *H. molitrix* was significantly larger than that of *O. mossambicus* across all seasons (p < 0.001). The Bayesian mixing model (SIAR) yielded significantly enriched $\delta^{15}\text{N}$ values relative to the food sources for both species. The enrichment factor for algae was +6.5‰, +7.9‰ for sediment organic matter (SOM), and +10.8‰ for vegetative detritus relative to $\delta^{15}\text{N}$ values of *H. molitrix*. Similarly, the enrichment factor for algae (+ 7.6‰), SOM (+11.8‰) and vegetative detritus +8.9‰ was also high relative to $\delta^{15}\text{N}$ values of *O. mossambicus*. This is indicative of underdetermined models, but proceeding with this caveat, it indicated that the diet of *H. molitrix* was largely

composed of green algae (55.6%) and sediment organic matter (43.0%), with minimal contribution of vegetative detritus (1.4%) (Online Resource B, Figure 1S). The diet of *O. mossambicus* consisted of vegetative detritus (71.6%) and sediment organic material (25.2%), with minimal contribution of algae (9.8%).

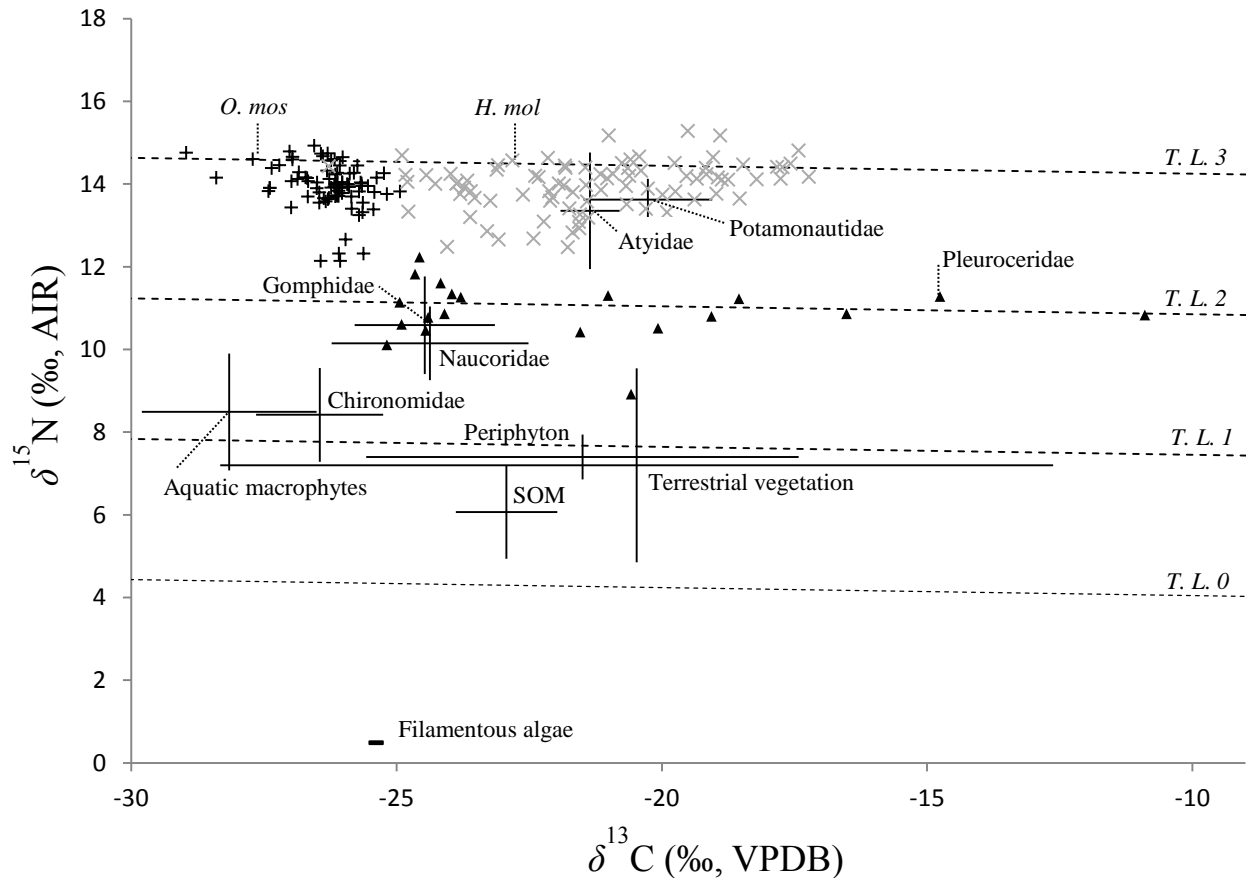


Figure 4: Isotope biplot indicating the prey and aquatic community trophic structure in Flag Boshielo Dam, sampled during summer 2011 (Mean \pm SD). Individual data points for all the sampled *H. molitrix* (*H. mol*) and *O. mossambicus* (*O. mos*) are displayed. The $\delta^{15}\text{N}$ of both *H. molitrix* and *O. mossambicus* was enriched relative to the potential prey identified by the stomach content analysis. T. L = Trophic level

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*). The SEA_c of species overlapping >40% with *H.*

molitrix during summer included: redeye labeo *Labeo cylindricus* (60% overlap), banded tilapia *T. sparrmanii* (46% overlap), threespot barb *Enteromius trimaculatus* (46% overlap), and silver robber *Micralestes acutidens* (42% overlap), (Figure 5) (Online Resource C, Table 3S). The mean C:N mass of the lipid extracted fish was 3.4 ± 0.16 .

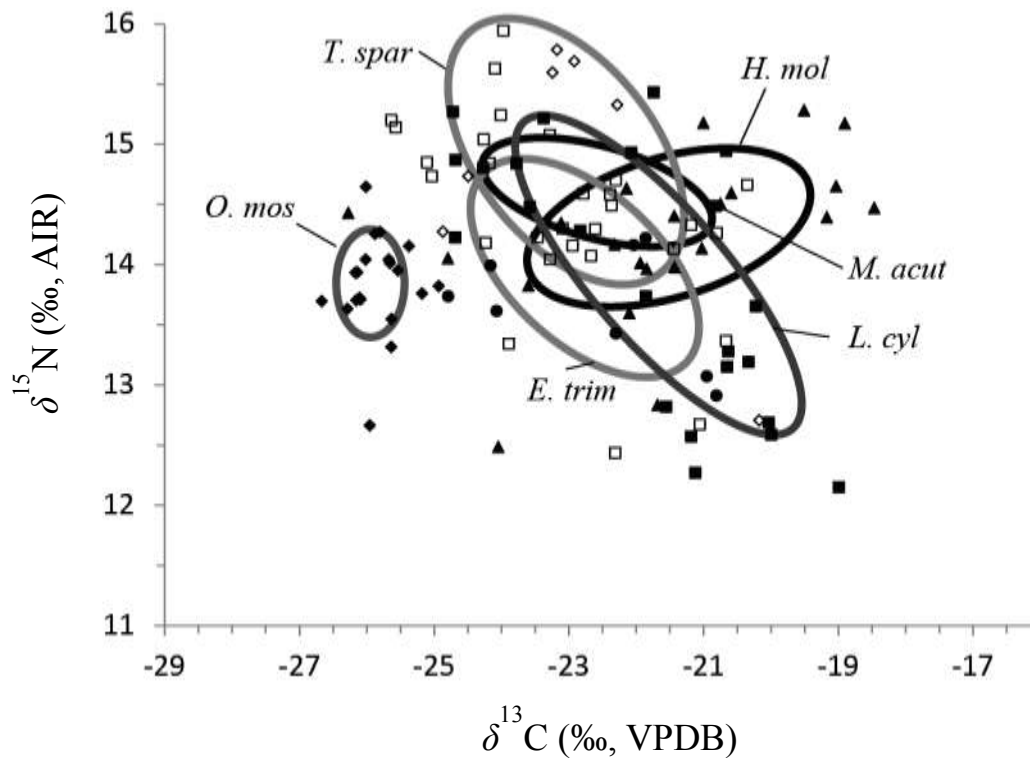


Figure 5: Isotope biplot with the Standard Ellipse Area (SEA_c) indicating the isotopic niche width of indigenous fish species overlapping > 40% with *H. molitrix* (*H. mol*), sampled in Flag Boshielo Dam during summer 2011. Fish species: *T. spar* = *Tilapia sparrmanii*, *O. mos* = *O. mossambicus*, *E. trim* = *Enteromius trimaculatus*, *M. acut* = *Micralestes acutidens*, *L. cyl* = *Labeo cylindricus*

Discussion

Sediment organic matter and detritus were the most abundant food items identified in the stomach contents of *H. molitrix*, while algae and sediment organic matter were identified as the main dietary components by the Bayesian mixing model. *Hypophthalmichthys molitrix* in FBD utilized a broad dietary niche that differed seasonally, indicative of a generalist filter

feeder. This supports the notion that they are indiscriminate filter feeders (Spataru and Gophen 1985, Kolar et al. 2007, Sampson et al. 2009, Calkins et al. 2011). Nevertheless, defining their diet in FBD proved challenging, the ingested (stomach contents) and time-averaged assimilated portion of their diet (stable isotopes) did not concur. The difference between the mean $\delta^{15}\text{N}$ of *H. molitrix* and the measured food resources (algae, SOM and vegetative detritus), which were directly observed in the stomachs of both fish species, albeit at low frequency, was more than one trophic level ($>3.4\text{‰}$), and more than the expected 1‰ enrichment if nutritionally stressed (Gaye-Siessegger et al. 2004). The $\delta^{15}\text{N}$ discrimination varied according to species, diet, and nutritional stress (Hobson et al. 1993), but generally ranges between 2 to 4‰ (Hobson et al. 1993, Vander Zanden and Rasmussen 2001, Post 2002). The stable isotope analysis therefore indicated that *H. molitrix* occupied a higher trophic level (T.L. = 2.8) than that expected from the diet identified by the stomach contents. In a comparable study, *H. molitrix* occupied a trophic level of *c.* 2.0 (Rogowski et al. 2009). The high $\delta^{15}\text{N}$ discrimination relative to any of these food sources suggested that *H. molitrix* fed on: a) other food items with higher $\delta^{15}\text{N}$ values than the items used to train the model (i.e., missing prey), or b) food items that originated from systems other than FBD. This limited our ability to completely define the trophic ecology of *H. molitrix* in FBD. Similar results and limitations were also evident in the dietary study of *O. mossambicus* in the same impoundment (Dabrowski et al. 2014a).

The preferred food items of *H. molitrix* are zooplankton and phytoplankton (Kolar et al. 2007) which, were not obtained in significant amounts to obtain an adequate isotope signature despite an extensive sampling effort. Other food items whose isotopic signature was not determined include unidentified material, and benthic diatoms, such as *F. ulna* which, was directly observed in the stomachs of both fish species (albeit at a low frequency). The unidentifiable portion of the diet represented a white, mucus-like substance often surrounding a fragment of plant material that is common in the stomach contents of a filter feeding fish. *Hypophthalmichthys molitrix* are known to strain bacteria by using mucus produced by large supra-branchial organ (Opuszyński 1981, Kolar et al. 2007) which could explain their higher $\delta^{15}\text{N}$ values, although the contribution of the photosynthetic bacteria (Cyanophyceae) was low. Nevertheless, the high proportion of unidentifiable material more likely represents a variety of lysed dietary items identified in the stomach contents, as opposed to some unknown, unidentified consumed prey group. (i.e., they are not ingesting some unidentifiable

food component). Yet, we acknowledge that the presence of an unidentifiable dietary component might have contributed to the discrepancy between the stomach content and stable isotope results – although unlikely. The other suggestion that the food items could have originated from systems other than FBD is partly supported by the occasional presence of *Microcystis aeruginosa*, an algal species found upstream in the eutrophic Loskop dam (Dabrowski et al. 2013) which, was observed in several *H. molitrix* stomachs (< 1% proportional contribution of stomachs in which it occurred), but was never sampled in FBD (Dabrowski et al. 2013, 2014a, b). The cyanobacteria from Loskop dam (Dabrowski et al. 2013) have an enriched $\delta^{15}\text{N}$ signature ($\delta^{15}\text{N} = 16.2 \pm 0.1\text{‰}$) compared to primary producers in FBD (this study), and if it is occasionally present in inflow water into FBD, *H. molitrix* might be feeding on it and this could partly explain the elevated *H. molitrix* $\delta^{15}\text{N}$ values relative to other food sources in FBD.

Previous bioenergetics (Cooke and Hill 2010) and species distribution models (Chen et al. 2007, Herborg et al. 2007, Lohmeyer and Garvey 2009) have indicated that sufficient algal biomass is a prerequisite for establishment and persistence of *H. molitrix*. For instance, a 2400 g resting, non-reproducing *H. molitrix* requires 91 kJ. d⁻¹ to maintain its body mass at 20 °C (Cooke and Hill 2010). This is equivalent to feeding in a system with minimum chlorophyll-*a* load of 15.5 $\mu\text{g. L}^{-1}$ (Cooke and Hill 2010). The low standing phytoplankton biomass (maximum summer suspended chlorophyll-*a* = 0.60 $\mu\text{g. L}^{-1}$) and warmer tropical environments in FBD (annual median water temperatures = 27 °C), imply that *H. molitrix* might be unable to meet their energetic requirements (Cooke and Hill 2010). Continuous filtering of large volumes of water to strain the sparse phytoplankton (or other food items) will be energetically costly and unsustainable. It is therefore of interest how *H. molitrix* are able to meet their energetic demands in the presence of such restricted algal biomass and limited suspended organic material in FBD. The low condition factors (minimum 0.88 in spring) (Table 2) reported in this study, relative to *H. molitrix* from other lentic waterbodies (minimum 1.78, Spatara and Gophen (1985)), suggest that they might be nutritionally stressed (Online Resource A).

Both SOM and detritus were collected from the benthic zone, and attempts to collect sufficient suspended particulate matter were not successful due to very low abundance. However, the presence of the Dinophyceae and the benthic diatom, *F. ulna*, in combination

with the high proportion of sediments in the stomach contents, suggests periodic feeding in the benthic zone. Although benthic feeding is considered uncommon in *H. molitrix*, they have been reported to stir up bottom sediments under conditions of low algal biomass (Costa-Pierce 1992, Cooke 2015). We recommend that detritus and other benthic dietary sources should be considered as an important dietary component of *H. molitrix* (also see Bitterlich 1985). Vegetative detritus is often an abundant food resource in tropical aquatic systems (Zengeya et al. 2011), and it appears that *H. molitrix* might have supplemented their phytoplanktivorous diet with detritus in FBD, as observed in previous studies (Opuszyński 1981, Bitterlich 1985, Burke et al. 1986, Kolar et al. 2007). The observed dietary plasticity highlights the oversight of excluding alternative dietary sources, such as detritus, in the bioenergetics model used to predict their invasive potential in North America (Cooke and Hill 2010). Nevertheless, more research is required to ascertain the source of the enriched $\delta^{15}\text{N}$ food source, and other dietary sources such as e.g., dreissenid pseudofeces, should also be considered.

Stable isotope analysis revealed that niche partitioning occurred between *H. molitrix* and *O. mossambicus*, despite having a similar diet. Although both species had a high proportion of sediment and detritus in their stomach contents, the stable isotope analysis revealed fine-scale dietary niche partitioning. *Oreochromis mossambicus* also occupied a higher than expected trophic position of 2.8, and $\delta^{15}\text{N}$ values were also highly enriched (> double) relative to measured food sources. They are known generalist bottom feeders that consume detritus, comprised of diatoms and plant material (Bowen 1982). Differences in the $\delta^{13}\text{C}$ and large niche size (SEA_c) of *H. molitrix* indicated a euryphagous diet, consisting of a wide range of food sources. In contrast, the smaller niche size of *O. mossambicus* suggests a stenophagous diet, predominantly comprised of detritus (Dabrowski et al. 2014a), facilitating the niche partitioning between the two species.

We detected isotopic niche overlap between *H. molitrix* and other indigenous species, such as *Labeo cylindricus* (60% overlap), sampled during summer (Figure 5). The isotopic overlap ranges from 0 to 1, with an overlap of >0.60 considered biologically significant, following Guzzo et al. (2013). The observed overlap is likely as a result of general omnivory and the exploitation of common food resources among the displayed species (Figure 5). Yet, inferring direct interspecific competition between co-occurring species in nature is difficult, and more

research on the long-term trophic interactions between *H. molitrix* and the identified species are required including sampling during different seasons.

Conclusion

This study highlights the dietary plasticity of *H. molitrix* under environmental conditions considered sub-optimal for their continued persistence. The opportunistic, non-selective filter-feeding behaviour, and potential benthic scavenging observed in this study, might enable establishment in other areas with low algal biomass. Several gaps still remain in our understanding of the diet of *H. molitrix*, as well as their impact on the aquatic food web of FBD. Future studies will need to intensively sample additional food sources at a seasonal resolution to determine the source of the elevated $\delta^{15}\text{N}$ in both *H. molitrix* and *O. mossambicus* in this impoundment.

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