

Trophic interrelationships between the exotic Nile tilapia, *Oreochromis niloticus* and indigenous tilapiine cichlids in a subtropical African river system (Limpopo River, South Africa)

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Abstract

The stable isotope ratio and seasonal changes in diet of two indigenous (*Oreochromis mossambicus*, *Tilapia rendalli*) and one exotic (*Oreochromis niloticus*) tilapiine cichlids in the subtropical Limpopo River, South Africa were investigated to determine patterns of resource partitioning. Stomach contents of *O. niloticus* and *O. mossambicus* indicated high dietary overlap across size class, habitat and season, with both species primarily feeding on vegetative detritus. However, stable isotope analysis revealed that the two *Oreochromis* species had different stable isotope ratios derived from different food sources. The relatively $\delta^{13}\text{C}$ -depleted *O. niloticus* indicates a phytoplankton-based diet, while the $\delta^{13}\text{C}$ -rich *O. mossambicus* indicates a macrophagous diet dominated by vegetative detritus and periphyton. The high similarity in stomach contents and the interspecific differences in isotopic composition reveal fine-scale patterns of food resource partitioning that could be achieved through selective feeding. *Tilapia rendalli* was largely macrophagous and fed mainly on aquatic macrophytes and had a low dietary overlap with both *O. niloticus* and *O. mossambicus*. In the Limpopo River, detritus and algae are probable the most abundant food resources and the causal factors responsible for the observed patterns of resources partitioning among the tilapiines are usually difficult to ascertain. Fish may be able to perceive food resources in terms of the dynamics that determine their availability. Detailed studies of variation in food resource availability and fish habitat use within the system are needed to evaluate this hypothesis.

Running head: Trophic interrelationships of African tilapiines

Key words: Tilapiines,, ontogeny, invasive/indigenous fishes, stomach content analysis, isotope analysis

Introduction

The introduction of invasive species and habitat destruction are considered to be among the leading causes of extirpations and extinctions of species in fresh water systems (Sala et al. 2000). The adverse ecological impacts of the Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) on recipient river systems worldwide has drawn attention to the problems associated with fish introductions (de Vos et al. 1990; Ogutu-Ohwayo and Hecky 1991; Twongo 1995; Canonico et al. 2005). Native to the Nile River basin, Lake Chad, south-western Middle East and the Niger, Benue, Volta and Senegal Rivers (Daget et al. 1991), *O. niloticus* has been widely introduced in southern Africa for aquaculture (van Schoor 1966; Welcomme 1988; de Moor and Bruton 1988; Schwank 1995). It is well-suited for aquaculture because it is extremely hardy, has a wide range of trophic and ecological adaptations, and it possesses adaptive life history characteristics such as high fecundity, fast growth rate and parental care (Welcomme 1988; Getabu 1994; Balirwa 1998; Njiru et al. 2004). These adaptive attributes predispose *O. niloticus* to be a highly successful invader. Feral populations are now established in most river catchments in the southern African sub-region where it has been cultured and/or deliberately introduced for aquaculture and recreational fishing (Schwank 1995; Chifamba 1998; Skelton 2001; Marshall 2006; Weyl 2007; Zengeya and Marshall 2007). These feral populations have recently been implicated in causing adverse effects on the recipient river systems such as a decreased indigenous fish abundance and extirpation of indigenous congeners through competitive exclusion and hybridisation (Chifamba 1998; Moralee et al. 2000; van der Waal and Bills 2000; D' Amato et al. 2007).

In South Africa, *O. niloticus* was initially introduced in the Cape Flats area (Cape Town, Western Cape Province) and in KwaZulu-Natal Province in the 1950s for aquaculture (van

Schoor 1966). Its range has since expanded to include the Limpopo and other eastern rivers in South Africa and Mozambique where it is now established and spreading (van der Waal and Bills 1997, 2000; Weyl 2007). The advent of *O. niloticus* in the Limpopo river system is a cause for concern for the conservation of indigenous congeneric species, especially for the Mozambique tilapia, *O. mossambicus* (Peters 1852), which is likely to become extirpated from the river system through hybridization and competition arising from its habitat and trophic overlaps with that of *O. niloticus* (Cambray and Swartz 2007). Other indigenous tilapiines in the Limpopo river system include the macrophagous redbreast tilapia, *Tilapia rendalli* Boulenger, 1896 and the banded tilapia, *T. sparrmanii* A. Smith, 1840.

The ecology of seasonal rivers within the Limpopo river system is poorly understood (van der Waal 1997) and the effect that *O. niloticus* may have on these indigenous species is largely unknown. It is therefore critical to establish whether these species are sharing resources because adverse impacts of *O. niloticus* invasions on indigenous species due to habitat and trophic overlaps are well-documented elsewhere (Getabu 1994; Njiru et al. 2004; Canonico et al. 2005; Zengeya and Marshall 2007). This study investigated the trophic inter-relationships between the exotic *O. niloticus* and two other indigenous tilapiine species in the central sub-catchment of the Limpopo River Basin, Limpopo Province, South Africa using two complementary methods: stomach content and isotope analysis. Specific objectives of the study were: (1) to determine whether tilapiine fishes in the Limpopo river system partition food resources, (2) to examine the influence of ontogeny and seasonal changes in resource availability on food resource partitioning among the tilapiines species and (3) to establish whether the introduced *O. niloticus* adversely affects co-occurring indigenous fish populations through predation and/or competition.

Methods

The study area is located in the central sub-catchment of the Limpopo River basin in northern Limpopo Province, South Africa. This area comprises of the Limpopo River, from the confluence of the Crocodile and Marico rivers to Crooks corner on the western boarder of the Kruger National Park and their associated tributaries (*ca.* 22°S - 24°S; 26°E –31° E; Fig. 1). The region has an arid to semi-arid climate with a mean annual rainfall of less than 400 mm in the Limpopo valley to over 1 500 mm along the Drakensberg escarpment in the east (WSM/DWAF 1995). The rainy season (October-April) is short, erratic and unreliable and this often results in frequent droughts (FAO 2004).

The geology of the sub-catchment comprises of granitic gneiss and migmatite, which are not permeable water-bearing strata, therefore, rivers and streams in the drainage system have seasonal and episodic surface water flows (Busari 2008). Peak discharge is between January and March, and minimum water flows usually occur in either July or August but in drier years the river flow may cease after 40 days or less and remain dry for periods of up to 36 months (FAO 2004). The Limpopo River is a seasonal sand-bed river which recedes into long stretches of dry sand, interspersed by a staggered series of residual pools during the dry season (van der Waal 1997; Minshull 2008). These seasonal pools provide dry season refuge for fish and have been shown to support diverse fish communities in relatively high densities comparable to more stable and productive ecosystems elsewhere (Minshull 2008).

Fish were collected between September 2008 and December 2009 from 20 sites (Fig 1; Table 1) using a variety of sampling gears. Small tributary streams and shallow residual pools were sampled using a seine net (35 m × 2 m with a 12 mm stretched mesh) and a SAMUS725MP

electrofisher (Samus Special Electronics, Warsaw, Poland). Large and deep river channels, farm dams and weirs were sampled using three fleets of gill nets (set overnight), each consisting of four panels (10 m long and 2 m deep) with a stretched mesh sizes of 44 mm, 60 mm, 75 mm and 144 mm. Catch per unit effort by species was taken as an index of abundance. Specimens were sorted according to species, weighed (g), measured to total length (TL, mm) and placed into 50 mm body size classes with a minimum of 20 fish in each size class wherever possible. For scarce species, all available specimens were analysed. Immediately upon capture, stomachs and *ca* 5 g of white caudal muscle tissue from the left flank were dissected out. Stomachs were preserved in 5% formaldehyde for 24 hrs prior to storage in 75% ethanol. Stomach contents were analysed using the methods of Zengeya and Marshall (2007); the contents of each stomach were suspended in 100 ml of water per gram of stomach contents and examined under an inverted microscope. Each item in the diet was then identified to the lowest possible taxonomic level after which the food items were then combined into broader taxonomic categories for quantitative comparisons (*ca.* cyanophyta, green algae, diatoms, periphyton, fine detritus, plant detritus, macrophytes, zooplankton and macrofauna). The contribution of each food category in each gut was estimated using a modification of the methods used in Platell and Potter (2001) by evenly spreading all contents from each gut in the counting cell chamber and examining under microscope. The area occupied by each food category in the chamber was later converted to the percentage of each food category.

In order to compare the isotopic values of the different tilapiine species: 1) fish specimens were collected within the same season (September –November, 2010), since isotopic value can vary quite significantly throughout the year and 2) analysis was restricted to samples collected in locations where the three species coexist. Fish muscle samples were stored frozen

prior to been oven-dried at 60° C for 48 hrs, ground into a powder and analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope ratios at the Stable Light Isotope Laboratory, University of Cape Town, South Africa. Isotope ratios for carbon and nitrogen were quantified as deviations relative to isotopic standards as follows:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standards were Vienna Pee Dee Belemnite limestone for $\delta^{13}\text{C}$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}\text{N}$ (Ehleringer & Rundel, 1989).

Diet diversity was estimated by index of niche breadth (Levin 1968) and dietary overlap between body size classes within a single species or among species was calculated from a niche overlap coefficient (Pianka 1974). Indices may range from zero (indicating a specialised diet or no overlap) to 1.0 (indicating an even use of food resources or complete overlap) with values > 0.6 being considered to represent a biologically significant overlap (Langton 1982). Fish diet (5 food categories) was analysed by a fixed factor 3-way MANOVA (season, habitat and size class), and Scheffe method of multiple comparisons of treatment effects. Angular transformation was used for dietary proportions of ingested prey items and the relationship between ontogeny and fish isotopic composition was analysed by regression analysis. Stable isotope ratios were analysed by a one factor ANOVA with species as a fixed factor. All statistical analyses were run using STATISTICA (Statsoft, 2000). The effect of site on diet within a species was tested, and found to vary according to habitats in fish were sampled, therefore for this study fish from different locations were grouped into three categorical habitat types for quantitative comparisons (main river channel, residual pools and farm dams/weirs).

Results

Four of the five tilapiine species occurring in the Limpopo river system were recorded. *Oreochromis mossambicus* was the most abundant and widespread (80% of the total catch and > 70% of all sampling stations) while *T. rendalli* was widespread but not abundant (15% of the total catch and > 70% of all sampling stations). *Oreochromis niloticus* was only encountered in the main channel of the Limpopo River and the immediate reaches of its associated tributaries east of the Limpopo/Shashe rivers confluence (< 15% of all sampling sites) where it constituted 40% of the landed catch. *Tilapia sparrmanni* was rare (< 1% of the total catch and >10% of all sampling stations). *Oreochromis macrochir*, which first appeared in the river system in 1992 (Kleynhans and Hoffman 1992), was not recorded.

Muscle tissue from all tilapiine species was $\delta^{15}\text{N}$ enriched (range = 9.3–16.5 ‰) and $\delta^{13}\text{C}$ -depleted (range = –23.2 – –30.5 ‰). *Oreochromis niloticus* exhibited the most $\delta^{13}\text{C}$ -depleted and was significantly different from both *O. mossambicus* and *T. rendalli* (Figure 2) (ANOVA: $F_{2, 74} = 22.36$; $n = 77$; $P < 0.001$). Stomach content analysis (5 food categories, 3 species) revealed significant differences in diet among the three tilapiine species [repeated MANOVA: $F_{8, 868} = 24.24$; $n = 230$; $P < 0.001$). *Tilapia rendalli* was a macrophage which fed primarily on living aquatic macrophytes while *O. mossambicus* and *O. niloticus* were both herbivorous/detritivorous feeding mainly on vegetative detritus (Table 2). In contrast, there were no significant differences (ANOVA: $F_{2, 73} = 1.44$; $n = 77$; $P > 0.198$) among the three tilapiine species for $\delta^{15}\text{N}$.

The diet of *O. niloticus* based on stomach content analysis indicates a greater niche breadth for smaller size classes which fed on greater proportions of diatoms, zooplankton and insects relative to larger size classes which were mainly detritivores (Table 2). In contrast, *O. niloticus* exhibited

significant relationship for $\delta^{15}\text{N}$ with TL ($P < 0.01$; Fig. 2) and the associated low r^2 value indicates an increase in diet breadth with size. Smaller size classes of *T. rendalli* fed on relatively greater proportions of diatoms, zooplankton and insects as compared to larger body size classes which fed primarily on aquatic macrophytes (Table 2).

In contrast, the $\delta^{15}\text{N}$ values for *O. mossambicus* showed no ontogenic changes in diet for $\delta^{15}\text{N}$ ($r^2 = 0.20$; $P > 0.20$) (Fig. 2). The diet of *O. mossambicus* was opportunistic and consisted of a wide range of prey items across all size classes (Table 2). Based on $\delta^{13}\text{C}$ all the 3 species showed no ontogenetic dietary shifts (Fig. 2).

Seasonal shifts in diet (5 food categories & 2 seasons) were observed for *O. mossambicus* (repeated MANOVA: $F_{4, 420} = 5.25$; $n = 88$; $P < 0.001$) and *O. niloticus* (repeated MANOVA: $F_{4, 385} = 7.60$; $n = 79$ $P < 0.001$), but not for *T. rendalli* (repeated MANOVA: $F_{4, 255} = 2.03$; $n = 53$; $P > 0.09$) (Fig. 3).

Significant interactions between diet and habitat were observed for *O. mossambicus* [5 food categories & 3 habitats (repeated MANOVA: $F_{8, 425} = 3.82$; $n = 88$; $P < 0.001$)] and *O. niloticus* [5 food categories & 2 habitats (repeated MANOVA: $F_{4, 385} = 7.60$; $n = 79$ $P < 0.001$)] but not for *T. rendalli* [5 food categories & 3 habitats (repeated MANOVA: $F_{8, 250} = 1.62$; $n = 53$; $P > 0.12$)].

Two major trophic groups can be distinguished amongst the Tilapiine cichlids in Limpopo River Basin (Table 3). The detritivore /microphages (*O. niloticus*; *O. mossambicus*) fed primarily on vegetative detritus and had high niche overlap across all size classes and seasons (Table 3.). *Tilapia rendalli* was macrophagous and fed mainly on macrophytes across all size classes and seasons.

Discussion

The similarity in stomach contents combined with the difference in isotopic composition between *O. mossambicus* and *O. niloticus* reveal fine-scale patterns of food resource partitioning that could be achieved through selective feeding. The depleted carbon isotopic composition of *O. niloticus* indicates a phytoplankton-based diet while the enriched composition in *O. mossambicus* suggests a diet dominated by vegetative detritus and periphyton. Phytoplankton communities in tropical rivers typically have $\delta^{13}\text{C}$ values of between *ca.* -40 and -25‰ while detrital aggregate of macrophytes, benthic algae and soil organic matter usually range between *ca.* -35 and -10‰ (Finlay and Kendall 2007). Although the isotopic values of carbon sources broadly show overlaps among different river systems, localised comparisons in a given river system often reveal distinct compositions of carbon sources (Finlay and Kendall 2007). Muscle tissue from organisms with a long life span such as tilapiine fish integrates dietary isotope ratios over periods of months to years (Vander Zanden and Rasmussen 1999; Hesslein et al. 1993); therefore, the observed differences may reflect dietary differences that may persist over similar time scales or longer.

Oreochromis niloticus is known to selectively feed on phytoplankton (Moriarty and Moriarty 1973; Getabu 1994; Bwanika et al. 2004; Zengeya and Marshall 2007) whereas *O. mossambicus* consumes mostly detritus comprised of diatoms and plant material (Bowen 1979, 1981; Trewavas 1983). Trophic distinctions among tilapiine species are however, not always clearly defined and species often exhibit opportunistic and versatile feeding strategies that reflect the abundance and composition of food sources in different environments, seasons and the presence or absence of competing fish species and predators (Bowen and Allanson 1982; Gophen et al. 1993; Balirwa 1998; Njiru et al. 2004; Zengeya and Marshall 2007).

In the present study, *O. mossambicus* and *O. niloticus* fed primarily on vegetative detritus but their diet spectrum was wide and varied according to season and habitat. Food resource scarcity often occurs during the dry season, when the river recedes into long stretches of dry sand interspaced by a series of residual pools that offer dry season refuge to fish. Food resource bottlenecks are likely to occur among the confined fish populations. The high dietary overlap between *O. niloticus* and the *O. mossambicus* may reflect the importance of vegetative detritus in the species diet during this time; vegetative detritus is often the most abundant food resource in most tropical rivers and it is usually available throughout the year (Winemiller & Winemiller, 2003). *Tilapia rendalli* in the present study was found to be macrophagous and fed mainly on living aquatic macrophytes resulting in low overlap with the other tilapiine species studied.

The relative proportions of algae, diatoms and zooplankton and insects in the fishes' diet increased during the wet season probably as a result of nutrient-rich flood waters that stimulated greater primary and secondary productivity and also provided new habitats and alternate food resources from sub-merged adjacent terrestrial habitats. Similarly, patterns of detritivory have been observed from fishes in the Congo and Rumpinuni Rivers, where a switch from an omnivorous to detritus diets occurs during the dry season when food resources become scarce (Lowe –McConnell, 1987).

Clear ontogenetic diet shifts were for evident for *O. niloticus* and *T. rendalli*, where small fish fed on greater proportions of animal material (zooplankton, insects and protozoa) relative to adult fish which were typically herbivorous/detritivorous. In contrast, *O. mossambicus* showed no diet changes throughout ontogeny. Its diet was opportunistic and consisted of a wide range of prey items across all size classes. This difference in diet probably reflects an adaptation by the

latter to the seasonal nature of the Limpopo River which continuously exposes fish to changing availability of habitats and food resources. Generalist feeders like *O. mossambicus*, likely use omnivory, among other adaptive life history traits, to survive these seasonal perturbations by feeding on whatever is available (Bowen 1982).

Contrary to our findings, other comparative studies on the feeding patterns of tilapias in the region (e.g., Upper Zambezi, Zambia; Kafue floodplain, Zambia and Lake Chivero, Zimbabwe) using only stomach content analyses have shown little or no evidence of seasonal and/or inter-specific variation in diet (Kelly 1968; Chapman et al. 1971; Winemiller and Winemiller 2003; Zengeya and Marshall 2007). This disparity in dietary patterns may reflect the practical limitations of conventional stomach content analysis which often may lead to equivocal conclusions (Hyslop 1980; Bootsma et al. 1996). Similarly, earlier stomach content studies on the food habits of rock-dwelling cichlids in Lake Malawi revealed little or no evidence of inter-specific variation in diet (Fryer 1959; Ribbink et al. 1983). However, subsequent studies using stable isotopes revealed strong patterns of niche complementarity in terms of habitat and dietary overlap (Bootsma et al. 1996).

Stomach content analysis represents a point-in-time snapshot of the food ingested by a fish, and may not present an accurate picture of which prey items are actually assimilated into the animal tissue. Furthermore, identifying partially digested prey can be difficult, and the patchy nature of most food resources means that a large number of samples must be analysed before the entire niche breadth of a species can be identified. These caveats are especially true when dealing with herbivorous/detritivorous species where stable isotope analyses should be the preferred

method of dietary analyses as it represents a spatial and temporal integration of assimilated rather than ingested food over a prolonged period (Peterson and Fry 1987; Fry 2006).

The use of stable isotopes in dietary analysis should however be applied with caution due to some limitations in their application (Vanderklift and Ponsard 2003; Marshall et al. 2007; Finlay and Kendall 2007). In brief these major limitations are 1) nitrogen isotope fractionation varies according to species, food source and dietary nitrogen content. 2) The isotope ratio of carbon varies with species composition, metabolic pathway of photosynthesis, season, and geographical region. 3) It is also, often difficult to discern food sources in ecosystems with multiple organic inputs and consumers. Despite these caveats, stable isotopes have been successfully applied to aquatic ecosystem research (Peterson and Fry 1987; Jepsen and Winemiller 2002; Campbell et al. 2005; Fry 2006). The dual use of stable isotopes and stomach contents analysis in this study, aids in our understanding of trophic dynamics in the system, as the two methods will give a point in time and time averaged estimate of a given species diet.

Previous studies that have documented extensive habitat and trophic overlaps between *O. niloticus* and indigenous *Oreochromis* species have subsequently implied that inter-specific competition could partly explain, among other factors, the successful establishment and spread of *O. niloticus* into a novel river system where it is rapidly replacing indigenous *Oreochromis* species (de Vos et al. 1990; Chifamba 1998; van der Waal and Bills 2000; Goudswaard et al. 2002; Zengeya and Marshall 2007). We recognize the fact that although descriptive studies cannot demonstrate competition directly, the well-documented success of *O. niloticus* in invading novel tropical hydrological systems worldwide does, however, provides strong circumstantial evidence to support the hypothesis of inter-specific competition. Owing to its

hardy nature and wide range of trophic and ecological adaptations, *O. niloticus* is currently among the most widely distributed exotic fish worldwide, having established feral populations in most tropical and sub-tropical environments to which they have gained access (Welcomme 1988; Canonico *et al.* 2005). *Oreochromis niloticus* is an aggressive competitor and large males often out-compete other species for limited spawning and nursing grounds (Lowe-McConnell, 2000). The resultant spatial partitioning is also thought to restrict the feeding patterns of the indigenous species (JL Minshull, former Curator of Ichthyology, Natural History Museum of Zimbabwe, Bulawayo, personal communication).

In most tropical rivers, the actual impact of introduced fish species is generally difficult to ascertain because data on community structure and functioning before the introductions is often lacking. This lack of knowledge is true for the Limpopo River Basin where the ecology of seasonal rivers in the river system is poorly understood (van der Waal 1997). *Oreochromis niloticus* is now established and spreading within the Limpopo River and other eastern rivers in South Africa and Mozambique (van der Waal and Bills 1997, 2000; Weyl 2007). However, whether *O. niloticus* eventually becomes a significant element of the fauna and the impact it will have on other fishes remains to be seen. Its spread will largely depend on its continued introduction by farmers/anglers and its ability to adapt to the extreme environmental conditions associated with the seasonal and semi arid Limpopo River system.

Patterns of resource partitioning among the tilapiines in the Limpopo River system seem to be established early in life (*ca* >50 mm SL) and the causal factors responsible for such differences in resource use by sympatric species are usually difficult to ascertain. The high similarity in stomach contents and the interspecific differences in isotopic composition among

Limpopo River tilapiines suggest patterns of resource partitioning that could be achieved by the ability of fish to selectively feed on what is available and the ability to perceive the dynamics that determine food resource availability. Detailed studies on seasonal variation in food resource availability and fish habitat use within the system are needed to evaluate this hypothesis.

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Figure captions

Fig. 1 The Limpopo River in South Africa with its major tributaries and the location of sampling sites (1-20; see Table 1 for specific sites) in the present study.

Fig. 2 The relationship between isotopic carbon 13 ($\delta^{13}\text{C}$) (●) and nitrogen 15 ($\delta^{15}\text{N}$) (○) against total length (TL; mm) for tilapiine fish species (*O. mossambicus*, *O. niloticus* and *T. rendalli*) from the Limpopo River and associated tributaries, Limpopo Province, South Africa.

Fig. 3 The proportion (by volume) of food items in the diet of three cichlid species (*O. mossambicus*, *O. niloticus*, and *T. rendalli*) in relation to season in the Limpopo River Basin, South Africa.

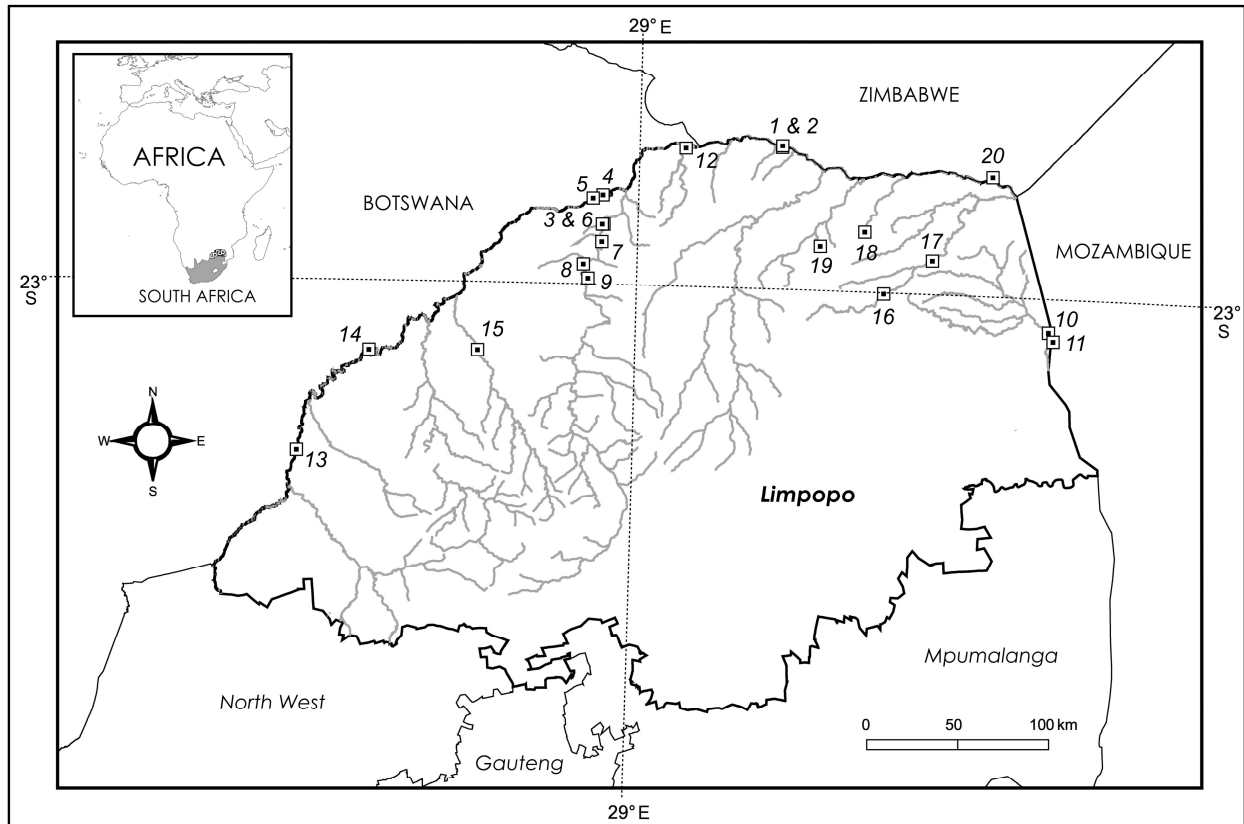


Fig. 1 Zengeya et al.

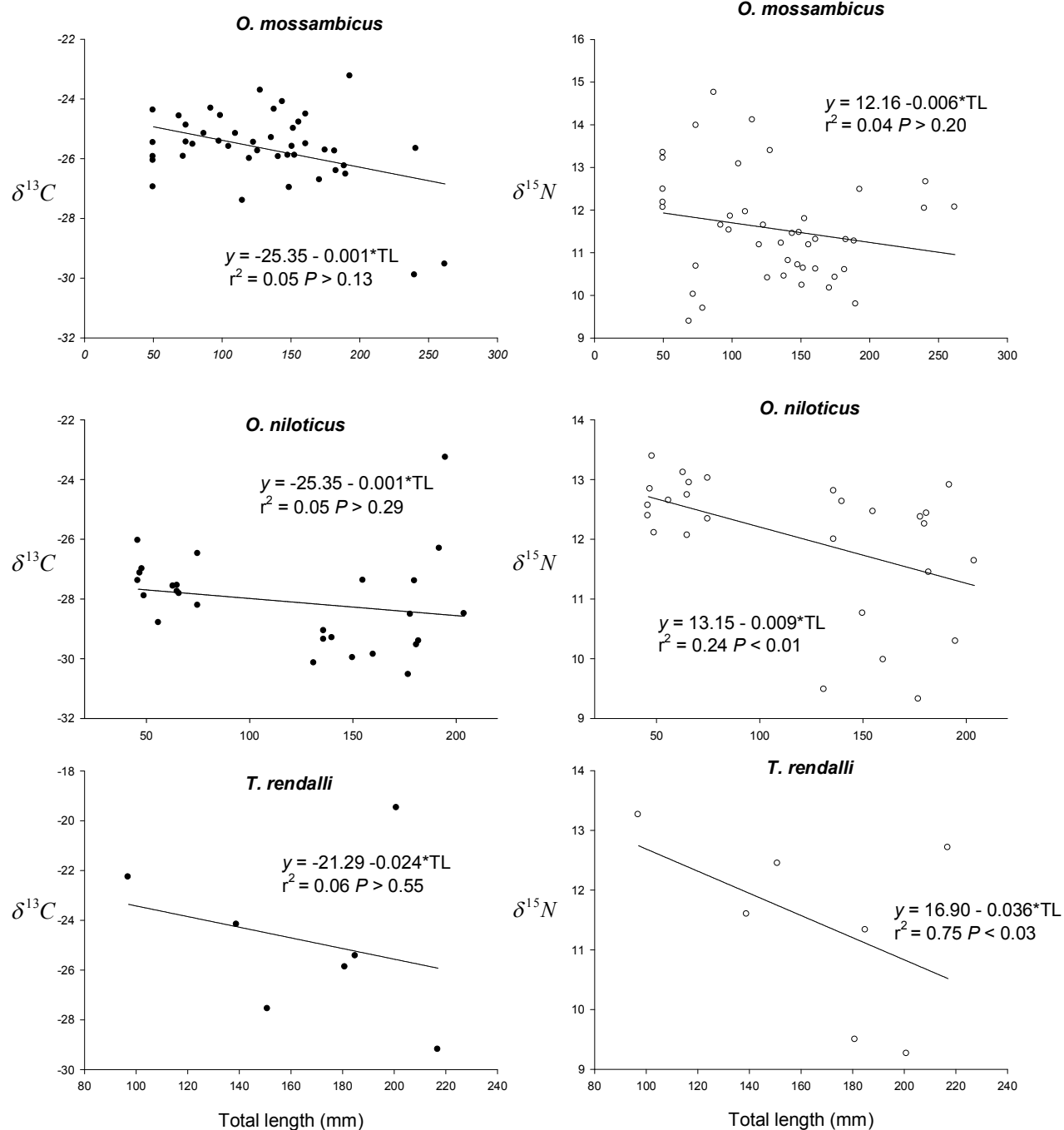


Fig. 2 Zengeya et al.

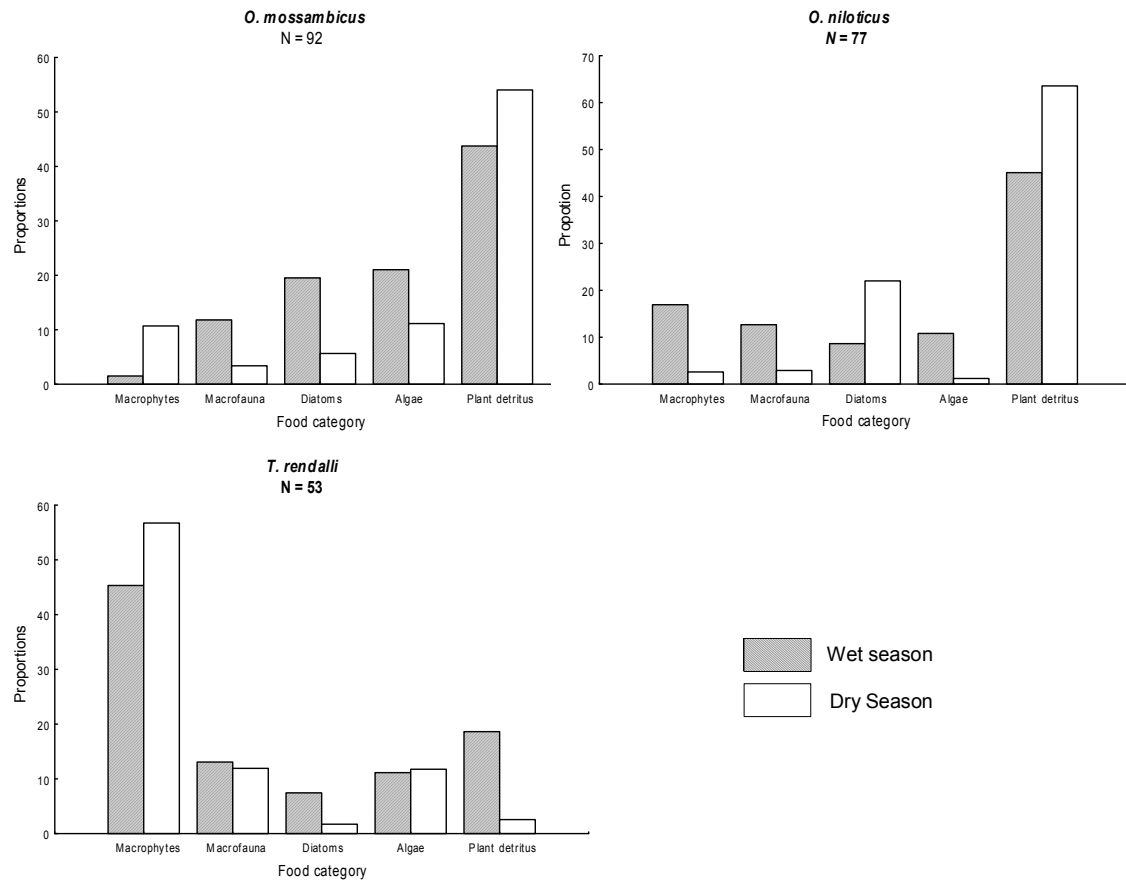


Fig. 3 Zengeya et al.

Table 1 The locality and specific geographic coordinates of fish collection sites (1-20) used in the present study along the Limpopo River and its associated tributaries in South Africa.

Site number	Location	GPS coordinates
1	Musina Exp. Farm adjacent to the Limpopo River	22°19'S-29°87'E
2	Opperanger Farm adjacent to the Limpopo River	22°20'S-29°89'E
3	Mokkopa nest, Mogalakwena River	22°38'S-28°46'E
4	Platjan Border Post, Limpopo River	22°27'S-28°50'E
5	Platjan Border Post, Limpopo River	22°27'S-28°50'E
6	Mogalakwena River	22°39'S-28°46'E
7	Mogalakwena River	22°45'S-28°46'E
8	Mogalakwena River	22°53'S-28°40'E
9	Mogalakwena River	22°58'S-28°42'E
10	Shingwedzi River, Kruger National Park	23°11'S-31°32'E
11	Dipeni point, Shingwedzi River, Kruger National Park	22°13'S-31°33'E
12	Den Staat farm, Limpopo River	22°12'S-29°16'E
13	Cumberland, Limpopo River	23°58'S-26°54'E
14	Storkpoort, Limpopo River	23°24'S-27°21'E
15	Mmaletswai, Palala River	23°22'S-28°01'E
16	Nandoni Dam, Luvuvhu River	22°59'S-30°30'E
17	Xikundu Weir, Luvuvhu River	22°48'S-30°47'E
18	Namwedi Dam	22°38'S-30°23'E
19	Nzhelele Dam, Nzhelele River	22°45'S-30°07'E
20	Limpopo River, Kruger National Park	22°02'S-31°08'E

Table 2 The proportion (% by volume) of food items in the diet of three tilapiine species (*Oreochromis mossambicus*, *O. niloticus*, and *Tilapia rendalli*) from Limpopo River, Limpopo Province, South Africa in relation to length (TL; mm). The dominant food items are shown in bold font, *n* = sample size, INB = index of niche breadth, ‘macrofauna’ is a functional prey category = insects and molluscs.

Species	Length TL (mm)	<i>n</i>	Cyanophyta	Green algae	Diatoms	Periphyton	Fine detritus	Plant detritus	Macrophytes	Zooplankton	Macrofauna	INB
<i>O. mossambicus</i>	< 50	31	2.9	0.5	18.3		0.7	65.4	2.8	7.1	1.8	0.16
	51-100	27		4.2	6.3	7.5	4.3	60.0	15.0		1.7	0.25
	101-150	10	5.2	16.1	10.9	6.2		42.5		0.7	17.0	0.42
	> 150	24	8.2	10.4	8.2	15.2	0.1	37.8	12.2	0.2	5.1	0.48
<i>O. niloticus</i>	< 50	18	2.6	1.1	17.3		3.2	47.8	0.2	27.1		0.33
	51-100	45	6.0	3.1	16.3	0.5	0.9	63.0	6.0	4.8		0.19
	101-150	6	4.9	1.6	5.3	8.9		76.4	2.7	0.6		0.11
	> 150	8	2.8	3.0	5.3	0.1	5.0	74.5	7.0	0.9		0.11
<i>T. rendalli</i>	< 50	11			11.2	27.4		4.3	35.5	1.7	20.0	0.58
	51-100	33	0.3	1.5	3.3	8.0	1.3	11.9	59.6	13.9	0.3	0.19
	101-150	5		0.3	6.5		11.3		78.3	8.5		0.14
	> 150	4	1.6	1.8	2.5	10.0		21.3	62.0			0.25

Table 3 Mean dietary overlap coefficients among three tilapiine species (*Oreochromis mossambicus*, *O. niloticus*, and *Tilapia rendalli*) from the Limpopo River Basin, South Africa in relation to size (TL; mm) and season (wet season = October – April; Dry season = May – September). The most significant overlaps are shown in bold (> 0.80) are shown in bold font.

Size class	Species	<i>T. rendalli</i>		<i>O. niloticus</i>	
		Wet	Dry	Wet	Dry
<10mm TL	<i>O. mossambicus</i>	0.52	0.17	0.91	0.95
	<i>T. rendalli</i>			0.46	0.15
>10 mm TL	<i>O. mossambicus</i>		0.04	0.88	0.94
	<i>T. rendalli</i>			0.29	0.06