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Abstract

In most tropical river systems there has been a lack of integrated ecological research to investigate the dynamics and impacts of invasive species on recipient river systems. This is in sharp contrast to temperate river systems. This thesis investigated the nature, extent, and impact of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), on indigenous congenerics within the Limpopo River basin in northern South Africa. An integrated approach was adopted to gain a better understanding of factors that allow Nile tilapia to be a successful invader and also to gain an insight into its invasion rate and conservation implications within South Africa.

Morphometric and genetic variation between Nile tilapia, indigenous congenerics and their associated hybrids were determined. Intermediate meristic characters obscured the identification of hybrid specimens from pure morpho-specimens and species identity was only confirmed through mtDNA analysis. Preliminary evidence points to unidirectional hybridization among *Oreochromis* congeners in the Limpopo River system. The hypothesis that bigger Nile tilapia males may have a competitive advantage over spawning grounds and in female mate choice is proposed.

The trophic ecology of Nile tilapia was investigated using both stomach contents and stable isotope analysis. A high similarity in stomach contents was observed but interspecific differences were revealed in the isotopic composition of diets that suggest fine scale patterns of resource partitioning that could be achieved by the ability of fish to selectively feed on what is immediately available and the ability to perceive the dynamics that determine food resource availability.

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Ecological niche models were used to determine the potential invasive range of Nile tilapia and revealed broad invasive potential over most river systems in southern Africa that overlapped the natural range of endemic congenerics. It was noted that model performance and the degree of niche conservatism varied significantly with variable selection and spatial extent of study area. This implied that the spatial distribution of suitable and unsuitable environmental variables varied between the native and introduced ranges of Nile tilapia and also indicated the ability of Nile tilapia to survive in conditions incongruent with its native range. The extreme hardiness and adaptive life history characteristics of Nile tilapia have probably predisposed it to be a successful invader in novel systems within southern Africa.

Lastly, a qualitative risk assessment method was developed as a potential application to determine the risk of establishment and spread of the invasive Nile tilapia. Results showed that in the absence of quantitative data on ecosystem structure and functioning, habitat suitability analysis in terms of known physiological tolerance limits to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern could be adequate for identifying vulnerable river systems**.** The model developed also provides an objective method that is easy to implement, modify and improve on as new data become available. Furthermore, the model can be applied to highlight areas of uncertainty where future research should be directed.

Key words: Hybridisation, Morphometrics, mitochondrial DNA (mtDNA), Trophic ecology, Ecological niche modelling, Risk assessment, Indigenous *Oreochromis* spp., Invasive Nile tilapia, southern Africa

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Disclaimer

This thesis consists of chapters prepared as stand-alone chapters that have been submitted or are intended for submission for publication purposes to different journals. As a result unavoidable repetitions and/or overlaps may occur between chapters.

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Publications

This thesis is based on the following papers, which will be referred to by their chapter numbers:

Chapter 2 Zengeya TA, Booth AJ, Bastos ADS, Chimimba CT. 2012. Morphometric and hybridization of invasive and endemic tilapia in a sub-tropical African river system (Limpopo River, South Africa) obscures the identification of the three *Oreochromis* species. In preparation for submission to *Aquatic Conservation: Marine and Freshwater Ecosystems.*

Chapter 3 Zengeya TA, Booth AJ, Bastos ADS, Chimimba CT. 2011. Trophic interrelationships between the exotic Nile tilapia, *Oreochromis niloticus* and indigenous tilapiine cichlids in a subtropical African river system (Limpopo River, South Africa). *Environmental Biology of Fishes* **92**:479–489.

Chapter 4 Zengeya TA, Robertson MP, Booth AJ, Bastos ADS, Chimimba CT. 2012. Ecological niche modeling of the invasive potential of Nile tilapia, *Oreochromis niloticus* in African river systems: concerns and implications for the conservation of *Oreochromis* species. Provisionally accepted in - *Biological Invasions.*

Chapter 5 Zengeya TA, Robertson MP, Booth AJ, Bastos ADS, Chimimba CT. 2012. A qualitative ecological risk assessment of the invasive Nile tilapia, *Oreochromis niloticus* in a sub-tropical African river system (Limpopo River, South Africa). *Aquatic Conservation: Marine and Freshwater Ecosystems* **DOI: 10.1002/aqc.2258.**

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Chapter 1

General introduction

Globally, fish are among the most introduced vertebrates because of their long association with human movements (Pillay 1977). The primary reason for fish introductions has been to increase food production through aquaculture and the augmentation of inland fisheries. As a consequence, commercially important species such as the tilapias, carps and catfishes have been widely distributed worldwide for aquaculture (De Silva et al. 2006). Food security has also been achieved through augmentation of existing fisheries by: 1) introducing new fish species such as the introduction of the Nile perch *Lates niloticus* into Lake Victoria (Witte et al. 1992; Gouswaard et al. 2002); 2) enhancing existing fisheries such as the introduction of various tilapiine species into Lakes Kariba and Victoria to improve local subsistence fisheries (Balon 1974; Oguthu-Ohwayo and Hecky 1991); and 3) filling vacant ecological niches such as the introduction of the freshwater sardine *Limnothrissa miodon* into Lakes Kariba and Kivu to fill an empty pelagic niche (Marshall 1993). Recreational and sport fishing have also developed rapidly to become a multi-billion dollar industry. As a result, the global introduction of sport fishes such as largemouth bass *Micropterus salmoides*, rainbow trout *Oncorhynchus mykiss*, and brown trout *Salmo trutta* has also been eagerly promoted (Cambrary 2003). Several fish species have also been introduced for the biological control of aquatic weeds and pests. These include mosquito fishes *Gambusia affinis* and *G. holbrooki* for mosquito control, silver carp *Hypophthalmichthys molotrix* and grass carp *Ctenopharyngodon idella* for the control of aquatic macrophytes and phytoplankton, and lastly, the mulloscivorous purpleface largemouth *Serranochromis macrocephalus* for schistosomiasis control (Schleier et al. 2008; Gozlan 2010).

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Of concern is that adverse secondary ecological impacts have been associated with fish introduced into novel river systems. As a result, invasive species are now considered to be among the leading causes of extirpations and extinctions of indigenous congenerics in recipient systems (Sala et al. 2000). Invasive species affect biodiversity in recipient systems through predation (Witte et al. 1992; Gouswaard et al. 2002; Weyl and Lewis 2006), competition (Zengeya and Marshall 2007), hybridisation (Scribner et al. 2001; D`Amato et al. 2007), habitat modification (Starling et al. 2002; Figueredo and Giani 2005; McCrary et al. 2007), and the transmission of diseases (Gozlan 2005).

Despite these well-documented adverse ecological effects, introduced species are also known to afford several socio-economic benefits in recipient systems (Da Silva et al. 2006). For example, aquaculture is perceived as a means of protein security, poverty alleviation and economic development in many developing countries (NEPAD 2005). In most invaded systems, introduced species have had a pronounced impact on fisheries in terms of increased food production and poverty alleviation by creating alternative aquaculture and fisheries livelihoods (Wise et al. 2007). As such, in most developing countries the decision to introduce exotic fish is usually based on the trade-off between socio-economic benefits and its potential adverse ecological effects (Cowx 1999).

Risk assessment and management of invasive species

The adverse ecological impacts associated with fish introductions on recipient freshwater ecosystems worldwide has drawn attention to the need to control and manage the movement of invasive species (Sala et al. 2000; Cambray 2003; Njiru et al. 2005; Pimentel et al. 2005). This has become especially important with the advent of increased global trade, transport, tourism and

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recreational activities that have provided for opportunities for organisms to spread beyond their natural ranges (Copp et al. 2005; Gozlan et al. 2010). In response to this threat, most countries have implemented legislation prohibiting new introductions and some have developed adaptive management strategies to identify and minimise the impact of invasive species (Kolar 2004; Vander Zanden 2008). Prevention is the major tenet behind most invasive species management protocols as it is often much easier and significantly less costly, especially for invasive aquatic species that are practically impossible to eradicate once established (Simberloff 2003; Lockwood et al. 2007). Ecological risk assessments have therefore been widely used as a screening tool to identify potential invasive species and to assess the risk of adverse ecological impacts associated with a given species establishment and spread to ecosystem structure and functioning (NRC 2002).

An ecological risk assessment for invasive species consists of two main components - risk identification and risk management (Anderson et al. 2004; Webb 2006). Risk identification is a process that evaluates the likelihood that adverse ecological effects may either occur or are occurring to indigenous congenerics as a result of exposure to introduced species. Risk of invasion is identified by either deductive and/or correlative methods. Deductive approaches utilise life history traits and environmental tolerances of an organism to evaluate the likelihood that a species will transit all the invasion stages (initial dispersal, establishment, spread and impact) (Lockwood et al. 2007). For example, Kolar and Lodge (2002) developed a risk assessment for introduced species in the North American Great Lakes based on the life history and environmental tolerances of past invaders (invasive species that were already established in the Great Lakes) to identify potential future invaders with similar traits. The major advantages of using such an approach to screen invasive species is that it is applicable to a variety of

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ecosystems and is easy to implement, modify and improve on as new data become available. It also highlights areas for future research by identifying areas of uncertainty within the model. The disadvantages associated with these deductive methods are that model development is data-intensive, there is limited transferability of model predictions (i.e., predictions limited to study area), and there are limited data available on failed introductions (Kolar and Lodge 2002; Kolar 2004).

Ecological niche modeling is a correlative method that utilizes associations between environmental variables and known species' occurrence localities to predict potential areas where a given species is likely to establish (e.g., Guisan and Thuiller 2005; Elith et al. 2006; Elith and Leathwick 2009). It has been successfully applied to a varied array of ecological disciplines that include ecology and evolutionary biology, impacts of climatic change, invasion biology and conservation biology (see Guisan and Thuiller 2005 for a review on the development and applications of ecological niche models). Ecological niche models have been successfully applied to predict the potential distribution of invasive fish species in novel systems (e.g., Igushi et al. 2004; McNyset 2005; Zambrano et al. 2006; Chen et al. 2007) but like deductive methods, they also have limitations to their application (Elith et al. 2006; Fitzpatrick et al. 2007). In particular, several studies have shown that niche models developed using native range occurrences may fail to predict the full extent of an invasion. This failure has often been attributed to changes in the niche of the invading species (Fitzpatrick and Hargrove 2008), biotic interactions and dispersal limitations that inhibit species to occupy potential suitable habitats (Anderson et al. 2002) and the choice of environmental variables used to train the models (Peterson and Nakazawa 2008; Rödder et al. 2009; Rödder and Lötters 2009, 2010).

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Despite these caveats, deductive approaches and ecological niche models are widely applied as a screening tool to identify potential invasive species and prevent their transmission into novel river systems (e.g., Pheloung et al. 1999; Kolar and Lodge 2002; NRC 2002; Kolar 2004; Marchetti et al. 2004; Copp et al. 2005; Schleier et al. 2008). Risk management involves the use of decision-support systems to estimate the risk of adverse ecological impacts associated with a given species establishment and spread to ecosystem structure and functioning in relation to environmental, social, and economic values of a given region (Copp et al. 2005). Risk management also enables concerned stake-holders to prioritise resource allocation for effective preventative and remediation efforts (Anderson et al. 2004; Copp et al. 2005).

Rationale

Cichlid fishes, belonging to the order Perciformes, is one of the largest and most widely distributed families of fresh and brackish water fishes and occur in tropical areas of North and South America, Asia, the Levant and Africa (Fryer and Iles 1972; Skelton 2001). Over 70% of known cichlids are found in Africa, especially in the ancient lakes of East and Central Africa (Tanganyika, Victoria and Malawi), where about 2000 endemic species are known to occur and represent the most spectacular radiations of vertebrates in the last 10 million years (Fryer and Iles 1972; Kocher 2004). The family also represents the most species-rich vertebrate family with about 3000 species exhibiting variability in body shape, colour pattern, behavioural diversity and a high degree of trophic and ecological specialisation (Fryer and Iles 1972; Meyer 1993; Kornfield and Smith 2000; Kocher 2004; Salzburger and Meyer 2004).

African cichlids are broadly represented by two major lineages - the haplochromines and the tilapiines. These two lineages are distinguished from each other by the structure of the

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apophysis on the base of the skull, which is used for articulation of the upper pharyngeal bones (Regan 1920). In tilapiines, the apophysis is formed entirely by the parasphenoid bone, while in haplochromines it consists of both the parasphenoid and basiocciptal bones (Trewavas 1983). The phylogenetic relationships of haplochromines have been studied extensively, primarily because of their radiation within the ancient lakes where 80% of known African cichlids are haplochromines (Fryer and Iles 1972; Meyer 1993; Stiassny and Meyer 1999; Salzburger et al. 2005).

Research interest in tilapiines has grown in recent decades primarily because of their growing economic importance in capture fisheries and aquaculture (Trewavas 1983; Beveridge and McAndrew 2000; Canonico 2005). Tilapiines have been divided into 10 genera according to breeding habit, trophic relationships, morphology and biogeography (Trewavas 1983; Stiassny 1991). Three major genera in the group are conveniently referred to as ‘tilapias’ based on parental care patterns. These are *Oreochromis* (arena-spawning maternal mouth-brooders), *Tilapia* (substrate-spawners) and *Sarotherodon* (paternal or biparental mouth- brooders).

Tilapias display a wide range of trophic and ecological adaptations, enabling them to occupy many different freshwater niches (Lowe-McConnell 2000), which Ross (2000) attributes to their robustness, tolerance and flexibility, and overall plasticity. They have a high reproductive rate and a remarkable physiological hardiness, adaptability and general level of tolerance to most potentially limiting environmental variables (Chervinski 1982; Philippart and Ruwet 1982). These traits have predisposed tilapias to be among the most widely distributed group of exotic fish worldwide (Costa-Pierce 2003). The primary purposes of their introduction have been to increase food production through aquaculture and augmentation of capture fisheries, to fill

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ecological niches, aquarium trade and the biological control of aquatic weeds (Welcomme 1988; Canonico et al. 2005).

Tilapias are the third most farmed fish in the world after carps and salmonids, accounting for 4% of global aquaculture production (FAO 2004). The most important tilapias in aquaculture are the mouth-brooding Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and Mozambique tilapia, *O. mossambicus* (Peters, 1852) and account for 99.5% of global tilapia production (FAO 2010). These two species are among 10 of the most introduced fish species in the world. Since the mid-1980s, there has been a shift in producer preferences away from the Mozambique tilapia towards culturing Nile tilapia that has higher growth rate and reduced tendency to stunt. Nile tilapia now dominates global tilapia aquaculture production accounting for 72% or 474 000 tons in 1995 (FAO 2010).

Nile tilapia introductions: Species description

Nile tilapia is native to the Nile River basin, south-western Middle East and the Niger, Benue, Volta and Senegal Rivers, Lakes Chad, Tanganyika, Albert, Edward, and Kivu (Trewavas 1983; Daget et al. 1991). Nile tilapia has been widely introduced for aquaculture, augmentation of capture fisheries, and sport fishing (Trewavas 1983; Welcomme 1988). It is well-suited for aquaculture because of its wide range of trophic and ecological adaptations, and its adaptive life history characteristics enable it to occupy many different tropical and sub-tropical freshwater niches (Trewavas 1983). Nile tilapia exhibits an opportunistic and versatile feeding strategy that reflect the abundance and composition of food sources in different environments, seasons and either the presence or absence of competing fish species and predators (Gophen et al. 1993; Balirwa 1998; Njiru et al. 2004; Njiru et al. 2007; Zengeya and Marshall 2007; Zengeya et al.

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2011). Its reproductive biology is characterised by fast growth rate, early sexual maturity (5-6 months), a high degree of parental care, ability to spawn multiple broods within a season, and a high fecundity associated with its large body size (Trewavas 1983; Ojuok et al. 2007). Nile tilapia is known to attain a standard length of approximately 60 cm (SL) and large males are often aggressive competitors that out-compete other species for limited spawning and mouth-brooding grounds (Lowe-McConnell 2000).

Nile tilapia introductions: Concerns and implications

The adverse ecological impacts of the Nile tilapia on recipient river systems worldwide has drawn attention to the general problems associated with fish introductions (de Vos et al. 1990; Ogutu-Ohwayo and Hecky 1991; Twongo 1995; Canonico et al. 2005). The advent of Nile tilapia into novel river systems in southern Africa is a cause for concern for the conservation of indigenous congenerics that are at an extirpation risk through hybridization and competition arising from habitat and trophic overlaps (Chifamba 1998; Canonico et al. 2005; Cambray and Swartz 2007; Zengeya and Marshall 2007; Weyl 2008). In areas where Nile tilapia has established, it has rapidly replaced indigenous congenerics, to the extent that some populations have become extirpated (Goudswaard et al. 2002; Chifamba 1998; De Vos et al. 1990). For example, Nile tilapia appeared in the Kafue River in the mid-1990s after escaping from nearby fish farms and has subsequently supplanted the threespot tilapia *O. andersonii* from the Kafue River system (Schwank 1995; Chikopela et al. 2011). Subsequent introduction occurred in the adjacent Lake Kariba, where the Nile tilapia has become abundant at the expense of the native Kariba tilapia *O. mortimeri* which has significantly declined in abundance from some parts of the lake (Chifamba 1998; Marshall 2006). As a result, Kariba tilapia is now listed as Critically

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Endangered (CR) on the IUCN Red List of threatened species (Marshall and Tweddle 2007; IUCN 2011). Further introductions have occurred into most medium to small impoundments within the middle Zambezi catchment where Nile tilapia has supplanted most indigenous congenerics (Marshall 2000; Zengeya and Marshall 2007). It has also been implicated in hybridisation with indigenous congenerics (Moralee et al. 2000; van der Waal and Bills 2000; D' Amato et al. 2007). Another adverse impact of Nile tilapia is its potential to transmit diseases, habitat destruction and water quality changes into novel water systems as seen elsewhere (Figueredo and Giani 2005; McCrary et al. 2007).

In South Africa, Nile tilapia was initially introduced in the Cape Flats area near Cape Town in the Western Cape Province, and in the KwaZulu-Natal Province in the 1950s for aquaculture (van Schoor 1966). Its range has since expanded to include the Limpopo River and other eastern flowing rivers in South Africa and Mozambique where it is now established and spreading (van der Waal and Bills 1997, 2000; Weyl 2008). The advent of Nile tilapia in the Limpopo River and other eastern river systems poses a threat to the other native tilapia species such as the Mozambique tilapia, *O. mossambicus*, that is now listed as Near-Threatened (NT) on the IUCN Red List of threatened species as a result of reduced abundance and hybridisation with the Nile tilapia (Cambray and Swartz 2007; D' Amato et al. 2007; Tweddle and Wise 2007; Weyl 2008; IUCN 2011).

The ecology of seasonal rivers within the Limpopo River system is poorly understood and as a result of the lack of earlier information on the hydrology as well as biota, recent changes and environmental deterioration have not been recorded (van der Waal 1997; van der Mheen 1997). The effect that Nile tilapia may have on these indigenous congenerics is, therefore, largely unknown. It may be especially severe in rivers, which are already threatened by activities

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such as dam construction, water abstraction, pollution, siltation, invasive alien weeds and habitat destruction. It is, therefore, critical to identify areas within the Limpopo river basin where Nile tilapia has been introduced, predict which river system(s) are vulnerable and possibly at risk of further Nile tilapia invasions, and more importantly, what can be done to stop its spread and reduce its impact.

Study objectives

The objective of this thesis was to study the extent and impact of Nile tilapia on the indigenous fish fauna in the Limpopo River basin, South Africa. The thesis, therefore, aims to contribute towards understanding those factors that contribute towards Nile tilapia to be such a successful invasive species and give insights into the rate and implications of invasion by Nile tilapia in South Africa.

Description of study area

The study area was 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*. 22ºS - 24ºS; 26ºE –31º 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).

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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 that 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). As a result of the highly seasonal and semi-arid climate, the Limpopo river system supports a relatively depauperate fish faunal assemblage comprised of only 83 species, relative to other river systems within the Zambezian ichthyofaunal region such as the upper Zambezi that contains 134 species that equate to the highest lotic diversity in southern Africa (Skelton 1994; Bills and Marshall 2004). Indigenous tilapiines in the Limpopo river system include the banded tilapia *Tilapia sparrmanii*, black tilapia *O. placidus*, Mozambique tilapia *O. mossambicus*, and redbreast tilapia *T. rendalli*. The greenhead tilapia *O. macrochir*, is only known from one occurrence record (Kleynhans and Hoffman 1992) and might have failed to establish.

Key study questions

The key research questions in this study include:

1) Is it possible to distinguish hybrids of Mozambique and Nile tilapias from respective pure morpho-specimens by either morphometrics and/or genetic analysis?

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2) To what extent has the Nile tilapia established and spread within the Limpopo river basin, South Africa?

3) How do environmental factors (temperature, precipitation, seasonality of river flows) affect the realised and potential distributional range of Nile tilapia within the Limpopo River basin, South Africa?

4) What biological aspects of Nile tilapia allow it to be such a highly successful invasive species? For example, does Nile tilapia have a competitive advantage relative to indigenous congenerics over food and suitable habitats for spawning and brooding? and to,

5) What is the spatial extent of the hybridisation between indigenous Mozambique and invasive Nile tilapia, within the Limpopo River basin, South Africa?

Hypotheses and predictions

The above research questions were used to formulate five main research hypotheses/predictions that are addressed separately as stand-alone chapters:

Hypothesis 1

H_o: Null hypothesis

- There is no variation in meristic and morphometric characters between the hybrids of Nile tilapia, *Nile tilapia,* and Mozambique tilapia, *O. mossambicus*.

- There is no evidence of establishment and further spread of Nile tilapia within the Limpopo river basin.

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H_a: Alternative hypothesis,

- Meristic and morphometric variability obscures the identification between the hybrids of Nile, *O. niloticus*, and Mozambique tilapia, *O. mossambicus*, tilapia hybrids.

- The Nile tilapia is now established and spreading within the Limpopo river basin.

Hypothesis 2

H_o: Null hypothesis

- There are no patterns of food resource partitioning among tilapiine fishes in the Limpopo river system.

- Resource partitioning among tilapiines species is not influenced by ontogeny and seasonal changes in resource availability.

- The Nile tilapia does not adversely affect co-occurring indigenous congenerics through predation and/or competition.

H_1: Alternative hypothesis,

- Co-existence of sympatric species in most tropical ecosystems is often achieved through niche differentiation and it is therefore envisaged that tilapiine fishes in the Limpopo river system partition food by specializing on certain food items.

- Tropical river systems experience highly seasonal patterns of precipitation and hydrology that influence fish growth, availability of habitats and food resources,

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therefore, changes in habitat and food resources availability are likely to influence patterns of resource partitioning among tilapiines.

- Indigenous congenerics are likely to become extirpated from the river system through hybridization and competition arising from habitat and trophic overlaps with that of Nile tilapia.

Hypothesis 3

$H_{o:}$ Null hypothesis

- There are no differences in the predictive power of models trained with different environmental variables.
- Nile tilapia occupies the same environments (niche space) in its native and introduced ranges.
- The invasive potential of Nile tilapia within river systems in southern Africa is localised and restricted to river systems with known introductions from aquaculture and recreational fishing activities. Further range expansion into novel river systems is restricted by lack of suitable habitats.

H_a: Alternative hypothesis,

- Model performance and degree of niche conservatism varies according to variables selected to build the model and spatial distribution of suitable and unsuitable environmental variables between the native and introduced ranges.

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- Nile tilapia is considered to be an extremely hardy and eurytopic invader, it is expected to reveal a broad invasive potential that will completely overlap the natural range of most indigenous congenerics in river systems in southern Africa.

Hypothesis 4

H_o: Null hypothesis

- The use of habitat suitability measures such as physiological tolerance to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern is inadequate to identify river systems that would be suitable and vulnerable to Nile tilapia establishment.

H_1: Alternative hypothesis,

- The natural fitness of Nile tilapia in recipient river system is strongly influenced by favourable environmental conditions such as optimal water temperature, availability of aquatic habitats, and the presence or absence of competing species. It is expected that the measured habitat suitability variables would be adequate to identify river systems that would be suitable and vulnerable to Nile tilapia establishment.

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Thesis approach

The research approach adopted for this thesis was multidisciplinary and included molecular, morphometric, and ecological analyses. Firstly, the realised distribution of Nile tilapia was confirmed through a fish survey of the Limpopo River and its associated tributaries within the Limpopo Province of South Africa. One major constraint in the conservation of *Oreochromis* spp. in river systems in southern Africa is that their morphological identification is often difficult because of considerable variation and broad interspecific overlaps in meristic and morphometric characters that are used in species descriptions. Therefore, the presence or absence of Nile tilapia within a given river segment was verified through genetic and morphological identification of sampled populations. Morphometric and molecular techniques were used in conjunction to assess levels of gene transfer among conspecific *Oreochromis* species, species diversity, their distribution, translocation/introduction into novel areas and hence identification of areas and/or species at risk of hybridisation.

This was followed by a similar integrated analysis of the trophic ecology of Nile tilapia and co-occurring tilapiine species in the Limpopo river system. The dual use of stable isotopes and stomach content analysis allowed for a better understanding of trophic dynamics in the river system, as the two methods gave point-in-time and time-averaged changes occurring in food web structure and functioning.

Ecological niche models were developed to predict the potential of Nile tilapia to extend and establish populations in novel African river systems with a particular focus on river systems within sub-Saharan Africa. This was achieved by matching the environmental conditions associated with known occurrence records of Nile tilapia in its native and introduced ranges to predict potential suitable novel areas where it will likely to establish. Geo-referenced occurrence

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data for Nile tilapia in African river systems were obtained from various sources including, museum specimen records, biodiversity databases such as FishBase (http://www.fishbase.org), Global Biodiversity Information Facility (GBIF; http://www.gbif.org), published literature, and fish survey data from various fisheries departments in southern African countries. The ecological niche model was further extended towards a deductive qualitative risk assessment model approach that utilised the life history traits and environmental tolerances of Nile tilapia.

A qualitative ecological risk assessment was based on known physiological tolerance limits of Nile tilapia in relation to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern to identify river systems that would be suitable for the invasive species establishment. Ideally, ecological risk assessments should be quantitative but in cases such as the Limpopo River basin where there is insufficient data on fish community structure and functioning, qualitative approaches can be applied successfully as an alternative to identify river systems that are vulnerable and at risk to Nile tilapia invasion.

Thesis outline

This thesis consists of two sections. The first contains two chapters (Chapters 2 and 3) and explores those factors that make Nile tilapia such a highly successful invasive species by investigating some aspects such as hybridisation and trophic interrelationships with indigenous *Oreochromis* species in the Limpopo River Basin. The second (Chapters 4 and 5) develop predictive models to evaluate the invasive potential of Nile tilapia to establish into novel systems in southern Africa by identifying river system(s) that are vulnerable and at serious risk of Nile tilapia invasions.

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In Chapter 2, morphometric and genetic variation between Nile tilapia, indigenous congenerics and their associated hybrids are investigated. The spatial extent of Nile tilapia invasion into the Limpopo Province was also determined, 10 years after its introduction, by identifying populations of *O. mossambicus* that have not yet received introgression from Nile tilapia. This can help stake-holders and managers to focus future conservation and management efforts.

Chapter 3 investigates stable isotope ratios and seasonal changes in diet of tilapiine cichlids in the Limpopo River to determine patterns of resource partitioning. This chapter uses two complementary methods- stomach content and isotope analysis. The practical limitations of conventional stomach content analysis in previous dietary studies of tilapiines elsewhere have often led to equivocal conclusions. 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. 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 gave point-in-time and time-averaged estimates of a given species diet.

Chapter 4 develops a correlative model that compares the associations between environmental variables and known species' occurrence localities to predict potential areas where a given species is likely to establish. It evaluates the potential of Nile tilapia to extend and establish populations in novel African river systems with a particular focus on river systems within sub-Saharan Africa where it is now established and spreading. Computational tools such as niche models have been shown to be useful in predicting potential geographical areas at risk

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of fish invasions elsewhere, but have not been widely applied to African inland fisheries despite continued widespread fish introductions.

Chapter 5 extends the ecological niche model towards a deductive qualitative risk assessment model approach that utilises the life history traits and environmental tolerances of an organism. This study outlines a qualitative ecological risk assessment based on known physiological tolerance limits of Nile tilapia in relation to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern to identify river systems that would be suitable for the invasive species establishment. The model provides a useful preliminary logistic framework for the identification of river systems that are vulnerable to a Nile tilapia invasion where conservation measures should be directed and implemented to prevent its introduction and spread within the river systems.

Chapter 6 concludes the thesis with a general discussion on the findings of the research and relates our current understanding of the impact of invasive species in novel river systems on indigenous congenerics in African river systems and the factors that promote their introduction, establishment and spread. It also highlights how current management protocols may be developed and/or improved by highlighting areas of uncertainty in invasive species management where future research should directed and gives recommendations to the management of Nile tilapia invasions in the Limpopo river basin.

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Chapter 2

Morphometric variability and hybridization of invasive and endemic tilapia in a sub-tropical African river system (Limpopo River, South Africa) obscures the identification of the three *Oreochromis* species.[1]

Tsungai A. Zengeya • Armanda D. S. Bastos • Anthony J. Booth • Christian T. Chimimba

Abstract

1. The introduction of the invasive Nile tilapia (*Oreochromis niloticus*) into the Limpopo River basin in northern South Africa is a cause for concern for the conservation of indigenous congenerics as adverse ecological impacts such as decreased abundance and species loss through hybridisation have been observed in adjacent catchments within southern Africa. One major constraint in the conservation of *Oreochromis* spp. in river systems in southern Africa is that interspecific morphological identification is often difficult because of considerable variation and broad interspecific overlaps in meristic and morphometric characters.

2. In this study, we used two complementary methods mitochondrial DNA (mtDNA) analysis and morphometrics to assess molecular and morphometric variation between Nile tilapia and an indigenous congeneric Mozambique tilapia *O. mossambicus*. We also determined the spatial extent of Nile tilapia invasion within the Limpopo Province, 10

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[1] This chapter is presented as it was prepared for submission to *Aquatic Conservation: Marine and Freshwater Ecosystems*. Zengeya TA, Bastos ADS, Booth AJ, Chimimba CT. 2012. Morphometric variability and hybridization of invasive and endemic tilapia in a sub-tropical African river system (Limpopo River, South Africa) obscures the identification of the three *Oreochromis* species.

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years after its introduction, by identifying populations of Mozambique tilapia that have not yet hybridised with Nile tilapia.

3. Based on a conflict between mitochondrial lineages and morphological species assignment, we were able to identify areas in which hybridisation between Nile and Mozambique tilapias has occurred. Hybrids (*O. mossambicus* × *O. niloticus*) were found mainly from the main channel of the Limpopo River and the immediate reaches of associated tributaries. Morphometric analyses revealed no clear morphological shape distinction between specimens typed genetically as *O. niloticus* × *O. mossambicus* hybrids from hybridization zones from either Mozambique or Nile tilapia as the hybrids resembled both parental species.
4. Comparison of mtDNA Limpopo specimens sequences with reference sequences from Genbank revealed the presence *O. andersonii* mitochondrial haplotypes in the Limpopo river system in South Africa

5. Of concern are river systems in the upper bushveld sub-catchment (Mokolo, Lephalala and Mogalakwena Rivers) that are still free of Nile tilapia but remain vulnerable. Concerted conservation and management efforts should focus on these river systems as they are likely to offer “refugia” for native populations of indigenous Mozambique tilapia in the advent of Nile tilapia invasion.

Key words: Mitochondrial DNA, morphometrics, indigenous *Oreochromis*, invasive Nile tilapia, southern Africa

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Introduction

Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758), was introduced for aquaculture into several countries within southern Africa between the 1950s and 1980 (Skelton 1994). As a result of subsequent escapes from aquaculture facilities and its continued intentional propagation into novel river systems, Nile tilapia is now prevalent in most river systems in southern Africa and are now widely distributed and common in rivers such as the Kafue, Middle Zambezi, Nata (Makgadikgadi/Okavango), Runde-Save, Buzi and Limpopo river systems (Schwank 1995; van der Waal and Bills 1997, 2000; Marshall 2006; Tweddle and Wise 2007; Weyl 2008; Zengeya and Marshall 2008).

The advent of Nile tilapia into these novel river systems is a cause for concern for the conservation of indigenous congenerics as adverse ecological impacts such as decreased abundance and hybridisation have been observed (Chifamba 1998; Canonico et al. 2005; Cambray and Swartz 2007; Weyl 2008; Zengeya and Marshall 2007). For example, in Lake Kariba, Nile tilapia appeared in the mid-1990s after escaping from *in situ* cage-culture fish farms and has become abundant at the expense of the native Kariba tilapia, *Oreochromis mortimeri*, which has significantly declined in abundance from some parts of the lake (Chifamba 1998; Marshall 2006). As a result, Kariba tilapia is now listed as Critically Endangered (CR) on the 2011 IUCN Red List of threatened species (Marshall and Tweddle 2007; IUCN 2011). In South Africa, Nile tilapia 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 flowing rivers in South Africa and Mozambique where it is now established and spreading (van der Waal and Bills 1997, 2000; Weyl 2008). The advent of Nile tilapia in the Limpopo River and other eastern river

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systems poses a threat to other native tilapia species such as Mozambique tilapia, *O. mossambicus*, that was recently listed as Near-Threatened (NT) by the IUCN as a result of reduced abundance and hybridisation with Nile tilapia (Cambray and Swartz 2007; D' Amato et al. 2007; Tweddle and Wise 2007; Weyl 2008; IUCN 2011).

One major constraint in the conservation of *Oreochromis* spp. in river systems in southern Africa is that their morphological identification is often difficult because of considerable variation and broad interspecific overlaps in meristic and morphometric characters that are used in species descriptions (Trewavas 1983). As a result, species distribution has often been used as a decisive factor in the identification of different *Oreochromis* spp. (Jubb 1967; Trewavas 1983; Bell-Cross and Minshull 1998; Skelton 2001). However, the extensive introduction of formerly allopatric *Oreochromis* spp. into artificial sympatry within most river systems has resulted in hybridisation between congenerics (Feresu-Shonhiwa and Howard 1998; Gregg et al. 1998; Moralee et al. 2000; D' Amato et al. 2007). This has inherently made morphological distinction of species and/or their hybrids difficult. This is especially true for both Mozambique and Nile tilapias that are known to interbreed and produce hybrids that are difficult to identify morphologically because back-crosses resemble parental species (Trewavas 1983). This clearly poses a serious problem in the control and management of the invasive Nile tilapia as it is often difficult to identify aquatic systems where it has established, using standard morphological identification.

To circumvent these problems, several studies have used a combination of genetic and morphometric analyses to elucidate the genetic diversity of congeneric *Oreochromis* spp. within the Limpopo river system (Moralee et al. 2000; Van der Bank and Deacon 2010; D' Amato et al. 2007). Moralee et al. (2000) utilised allozyme analysis to verify the presence of the Nile tilapia

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within the Limpopo River and determined that hybridisation was evident. Recent analyses have indicated that within the Limpopo River system, allozyme data are no longer useful for differentiating between back-crossed Mozambique and Nile tilapia specimens (Van der Bank and Deacon 2010). In a comparable study, D' Amato et al. (2007) genotyped the control region of mitochondrial DNA (mtDNA) and used a panel of five microsatellites to assess the genetic diversity of Mozambique and Nile tilapia, confirming the value of these as more appropriate genetic markers for identifying hybrids.

These studies only assessed the genetic diversity in localities with known Nile tilapia introductions and have largely ignored those areas where it is yet to establish. Despite its widespread introduction into river systems within sub-Saharan Africa, several river systems within the Limpopo River are still free of Nile tilapia but remain vulnerable. While Nile tilapia has established feral populations along the main arm of the Limpopo River and the immediate reaches of its associated tributaries east of the Shashe/Limpopo rivers confluence it is yet to establish in most river systems in the upper bushveld sub-catchment (van der Waal and Bills 1997, 2000; Zengeya et al. 2011). These river systems are likely to offer “refugia” for native populations of indigenous congenerics and genetic knowledge of such populations is crucial to their future conservation and management.

The objectives of this study were therefore to expand on the previous work of Moralee et al. (2000), D' Amato et al. (2007), and Van der Bank and Deacon (2010) by using a mitochondrial marker in combination with classical morphology and morphometrics to determine invaded and non-invaded zones and to use these results as a basis for guiding interpretation of morphometric analyses, with the aim of developing a taxonomic protocol capable of distinguishing hybrids from respective pure strains, by genetic and/or morphometric

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analyses. We used and expanded on a comprehensive morphometric protocol developed by Barel et al. (1977) and Snoeks (2004) that has been previously successfully applied to elucidate the taxonomy and morphology of cichlids within the ancient lakes of East Africa. We also assessed the spatial extent of Nile tilapia invasion into the Limpopo Province in South Africa, 10 years after it was first reported in the system, by identifying those river systems that only contain Mozambique tilapia mitochondrial signatures, and that are yet to be introgressed with Nile tilapia.

Methods

Study area

Fish were sampled from 20 sites in the central sub-catchment of the Limpopo River basin in northern Limpopo Province, South Africa between September 2008 and December 2009 (Table 1). The study area comprised 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 together with their associated tributaries (*ca*. 22ºS - 24ºS; 26ºE –31º E; Fig. 1). A detailed description of the study area is provided by is Zengeya *et al*. (2011). 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 SAMUS 725MP electrofisher (Samus Special Electronics, Warsaw, Poland). Large and deep river channels, farm dams and weirs were sampled overnight using three fleets of gill nets 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. Specimens were sorted according to locality for genetic and morphological analysis and preserved in absolute ethanol.

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Table 1. The locality and specific geographic coordinates of 20 sampling locations of Nile tilapia *Oreochromis niloticus* along the Limpopo River, South Africa and its associated tributaries.

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, Luvhuvhu River	22°59'S-30°30'E
17	Xikundu Weir, Luvhuvhu 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

Genetic characterisation and analysis

Genomic mitochondrial DNA (mtDNA) was extracted from either kidney or caudal peduncle muscle of all *Oreochromis* specimens using the High Pure Roche PCR template preparation kit according to the manufacturer's prescribed protocol. A fragment of the displacement loop (D-loop) in the mtDNA control region was amplified using primers H16498 5' CCT GAA GTA GGA ACC AGA TG 3' (Meyer et al. 1990) and L19 5' CCA CTA GCT CCC AAA GCT A 3' (Bernatchez et al. 1992). The cycling conditions consisted of a denaturation cycle at 94° C for 3 min, followed by 35 cycles of denaturation for 94° C for 30 s, 40 s at 51° C and extension at 72° C for 5 min. An amplicon approximately 400 bp in length was obtained and purified using the High Pure Roche PCR product purification kit according to the manufacturer's prescribed protocol.

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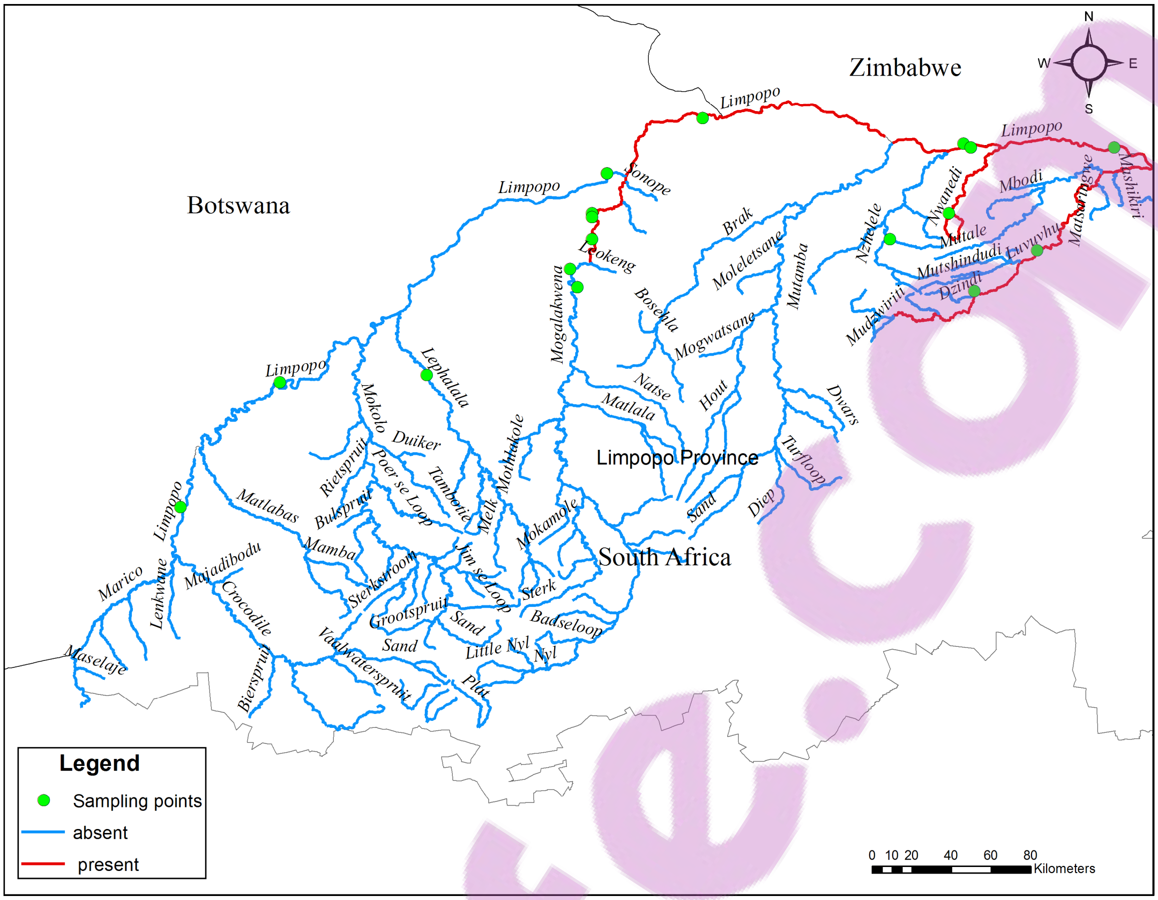

Figure 1. The Limpopo River and its associated tributaries in South Africa and the location of 20 sites (1-20; see Table 1.) sampled in the present study. River sections in red show the current range of Nile tilapia (*Oreochromis niloticus*), while rivers sections in blue show areas that are currently free from invasion.

Nucleotide sequences were determined by cycle sequencing with version 3.1 of the Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) at an annealing temperature of 51° C, with each of the PCR primers run in separate reactions. Sequences were precipitated and run on an ABI PRISM™ 3100 Analyser (Applied Biosystems).

A 119-taxon dataset was generated, consisting of *Oreochromis* specimens from several river systems in the Limpopo River basin, South Africa (Table 2). The dataset was subsequently reduced to 24 taxa representing the sequence diversity within the sampled river systems on a per-

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species, per-river and per-haplotype basis. This Limpopo River dataset was complemented with 30 reference sequences of congeneric *Oreochromis* species from several river systems in central and southern Africa that were taken primarily from Nagl et al. (2001) and D'Amato et al. (2007) (Table 2). *Tilapia rendalli* from the Mogalakwena River and a reference sequence *T. rendalli* AF328854 from the Zambezi River, Zimbabwe were used as an out-group. Phylogenies were inferred using Minimum Evolution (ME), Neighbour Joining (NJ) and Maximum Likelihood (ML) analyses in Mega 5 (Tamura et al. 2011). Nodal support was assessed by 10,000 bootstrap replications for ME, NJ and 1000 for ML analyses respectively.

Morphometrics

Standard measurements are based on Barel et al. (1977) and Snoeks (2004). A total of 23 measurements and 15 counts were recorded from each specimen examined. All measurements were made point- to-point unless otherwise stated. Head measurements were expressed as percentage of head length (HL), whereas body measurements were expressed as a percentage of standard length (SL).

Body measurements (Figure 2a)

The following body measurements were recorded:

1) Standard length (SL) - from rostral tip of upper jaw to mid-point of origin of caudal fin (Figure 2a. n°1). The origin of the caudal fin can be located by folding the caudal fin slightly at its presumed origin and the fold indicates the posterior border of the hypurals;

2) Body depth - maximum depth of the body in front of pelvic fin, starting from base of first dorsal-fin ray to a perpendicular point along belly (Figure 2a. n°2);

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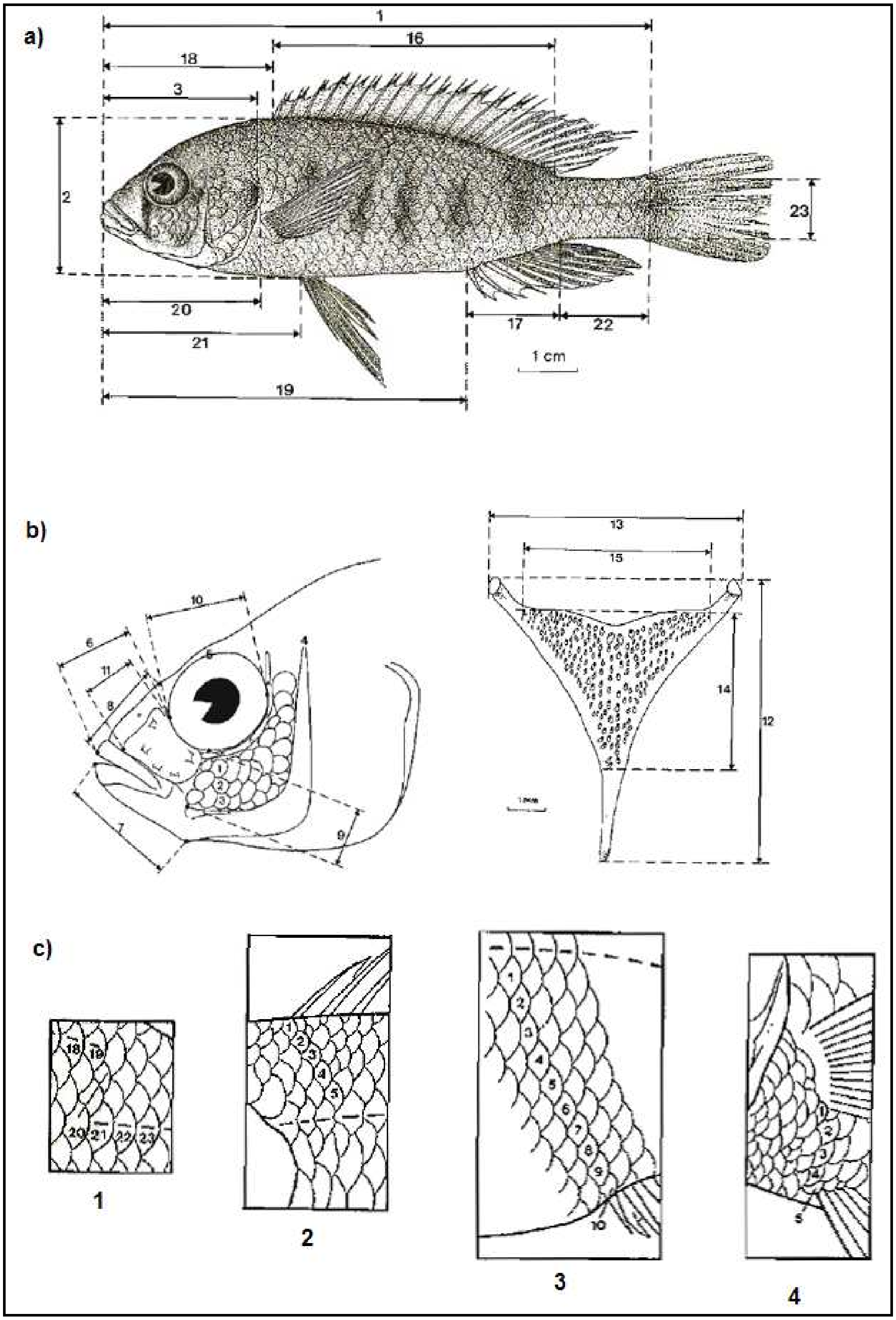

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Figure 2. a) Head and **b)** body measurements recorded from all *Oreochromis* specimens**.** (1) standard length, (2) body depth, 3) head length, (4) head width, (5) inter-orbital distance, (6) snout length, (7) lower-jaw length, (8) premaxillary pedicel length, (9); cheek depth and cheek scales, (10) eye diameter, (11) lachrymal depth, (12) lower pharyngeal length, (13) lower pharyngeal length, (14) dentigerous area length, (15) dentigerous area width, (16) dorsal fin-base length, (17) anal-fin base length, (18) pre-dorsal length, (19) pre-anal length, (20) pre-pectoral length, (21) pre-pelvic length, (22) caudal-peduncle length, and (23) caudal-peduncle depth. **c)** Meristic counts recorded from *Oreochromis* specimens. (1) total number of scales along lateral line, presented as ($x + y$): where x = scales on upper lateral line and y = scales on lower lateral line;(2) scales between dorsal fin and lateral line, (3) scales between anal fin and upper lateral line, and (4) scales between pectoral and pelvic fin lateral line (after Snoeks 2004).

3) Dorsal fin-base length - from anterior to posterior tip of dorsal fin base (Figure 2a. n°16);

4) Anal-fin base length - from anterior to posterior tip of the anal fin base (Figure 2a. n°17);

5) Pre-dorsal length - from anterior tip of the snout to anterior base of the first dorsal fin ray (Figure 2a. n°18);

6) Pre-anal length - from anterior tip of snout to anterior border of anus (Figure 2a. n°19);

7) Pre-pectoral length - from anterior tip of snout to anterior tip of pectoral fin base (Figure 2a. n°20);

8) Pre-pelvic length - from anterior tip of snout to anterior tip of pelvic fin base (Figure 2a. n°21);

9) Caudal-peduncle length - from posterior base of last anal fin ray to caudal border of the hypurals (Figure 2a. n°22);

10) Caudal-peduncle depth - minimum vertical distance across caudal peduncle (Figure 2a. n°23).

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Head measurements (Figure 2b)

11) Head length (HL) - from anterior tip of snout (premaxillae) to tip of posterior border of operculum (Figure 2a. n°3);

12) Head width - top-view distance measure between the bony elements of the pterotics (not shown);

13) Inter-orbital distance - minimum distance between bony orbits of both eyes (Figure 2b. n°5);

14) Snout length - from anterior tip of snout (premaxillae) to rostral point of bony orbit of the eye (Figure 2b. n°6);

15) Lower-jaw length - from anterior tip of lower jaw to ventro-caudal tip of lower jaw (Figure 2b. n°7);

16) Premaxillary pedicel length - from anterior tip of snout (pre-maxillae) to the ascending process of the premaxilla (Figure 2b. n°8);

17) Cheek depth - from ventral point of bony margin of orbit to dorsal corner of the lower jaw/ suspensorium articulation (Figure 2b. n°9);

18) Eye diameter - greatest horizontal distance across the bony orbit of eye (Figure 2b. n°10);

19) Lachrymal depth - from the rostral corner of bony orbit to rostral corner of lachrymal (Figure 2b. n°11);

20) Lower pharyngeal length - from anterior caudal tips of horns to the rostral tips of anterior lamella, including the cartilage (Figure 2b. n°12);

21) Lower pharyngeal length - distance between the lateral lips of the horns (Figure 2b. n°13);

22) Dentigerous area length - from rostral margin of the most rostral tooth or tooth socket to caudal margin of the most caudal tooth or socket (Figure 2b. n°14); and

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23) Dentigerous area width - distance between lateral margins of left and right teeth or sockets (Figure 2b. n°15).

Meristics (Figure 2c)

The following meristic data were recorded:

1) Total number of scales along lateral line, presented as ($x + y$) - where x = scales on upper lateral line and y = scales on lower lateral line (Figure 3. n°1);
2) Scales on upper lateral line (Figure 3. n°1);
3) Scales on lower lateral line (Figure 3. n°1);
4) Scales between dorsal fin and lateral line - number of scales from the anterior base of the dorsal fin base to the point at which the posteriorly directed scale row meets the lateral line (Figure 3. n°2). The lateral line scale is not included;
5) Scales between anal fin and upper lateral line - number of scales from anterior base of anal fin base to the point at which the anterior directed scale row meets the upper lateral line (Figure 3. n°3). The lateral line scale is not included;
6) Scales between pectoral and pelvic fin lateral line - number of scales from ventral base of pectoral fin to anterior base of pelvic fin (Figure 3. n°4);
7) Cheek scales - series of scales on cheek, ventral to eye (see Figure 2b).

The dorsal fin, pectoral fin and anal fin ray counts (8-12) were recorded. Fins were stretched and held against a light source to facilitate correct counting. Fin-ray counts were expressed in Roman numerals for spines and unbranched rays, and Arabic numbers for branched rays.

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13) First upper-arch gill rakers (UR) - number of gill rakers on the shorter, upper arm of anterior side of first gill arch;

14) First lower-arch gill rakers (LR) - number of gill rakers on the longer, lower arm of anterior side of first gill arch; and

15) Total number of gill rakers on first gill arch expressed as total = UR/1/LR. A dissection of the first branchial arch was necessary to obtain an accurate binocular count of the gill rakers. Additional observations were made on the following: colour and shape of dorsal fin; presence of stripes on dorsal, caudal and anal fins and the colour patterns on the body profile.

All generated morphometric data were subjected to the *a priori* principal components analysis (PCA) and Unweighted-pair group arithmetic average (UPGMA) cluster analysis of standardized data (Sneath and Sokal 1973; Humphries et al. 1981, Bookstein et al. 1985). The PCA was based on a product-moment correlation matrix among variables (Sneath and Sokal 1973). Size-free comparison of log-transformed data for all the specimens was achieved by discarding the first principal component (PC1) as mainly a size component (Humphries et al. 1981, Bookstein et al. 1985). The UPGMA cluster analysis was based on Euclidean distances among groups (Sneath and Sokal 1973). The genetically-identified phenotypic assemblages obtained *a priori* were further subjected to *a posteriori* analyses in order to assess their statistical integrity (Sneath and Sokal 1973). This included canonical variates (discriminant) analysis (CVA; Sneath and Sokal 1973) of the morphometric data based on a variance-covariance matrix. This procedure which maximizes the variation between groups and minimizes variation within them is useful for classifying unknown specimens (Sneath and Sokal 1973). All CVAs were always followed by a multivariate analysis of variance (MANOVA) in order to test for statistically significant differences between the designated group centroids. Other analyses in the

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study included the generation of standard descriptive univariate statistics for each delineated phenetic group.

All the above morphometric analyses were based on the meristics (15 meristics), the log-transformed measurements (23 measurements) and percentages (23 measurements) [Measurements on the head are expressed as percentage of head length (HL) whereas measurements on the body were expressed as a percentage of standard length (SL)] for important characteristics which can be used to separate and distinguish the respective species. All meristic, log-transformed, and percentages data were analysed separately in all multivariate analyses. All morphometric analyses were undertaken using algorithms in STATISTICA version 7 (StatSoft, 2000). Hybrid specimens (*O. mossambicus* × *O. niloticus*) were identified based on conflict between mitochondrial and morphological species assignments (Table 2).

Results

Genetic analysis

The 54-taxon in-group dataset contained 252 variable and 129 parsimony informative sites across the 394 nucleotide (nt) fragment of the displacement loop (D-loop) region that was characterised in this study. The model of sequence evolution selected under the AIC_C was the Tamura-Nei (1993) model with a gamma shape parameter (G) of 0.235, and nucleotide base frequencies of A = 0.314, C = 0.116, G = 0.207, T = 0.362, corresponding to an A+T bias of (68 %). The model adjusted transition: transversion ratio (R) was 2.06. The D-loop gene tree (Figure 3) recovered four monophyletic tilapiine lineages from the Limpopo River basin. Nodes that had ≥ 50% bootstrap support in phenetic analyses (Neighbour Joining) generally had high levels of support in the Maximum Likelihood analyses (Figure 3).

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Table 2. List of mtDNA Dloop sequences generated from this study and sequences accessed from GenBank for congeneric *Oreochromis* species occurring in the Limpopo River basin and the reference sequences used for genetic analysis. *Denotes samples that were identified on the basis of mtDNA sequences alone.

Species		Genbank accession number	N	Sampling locality	references
Molecular identification	Morphological				
*O. andersonii**	*O. andersonii*	AF296487	1	Okavango Delta, Botswana	Nagl et al. 2001
O. andersonii	*O. andersonii*	AF296488	1	Okavango Delta, Botswana	D'Amato et al. 2007
O. andersonii x Oreochromis	*O. andersonii*	AY833492-93	2	Olifants and Limpopo Rivers	D'Amato et al. 2007
O. andersonii x Oreochromis	*O. andersonii*	AY833498	1	Limpopo River , South Africa	D'Amato et al. 2007
O. andersonii x Oreochromis	*O. andersonii*	AY833500	1	Limpopo River , South Africa	D'Amato et al. 2007
O. andersonii x Oreochromis	*O. andersonii*	AY833502	1	Limpopo River , South Africa	D'Amato et al. 2007
*O. aureus**	*O. aureus*	AF328851	1	Lake Manzala, Egypt	Nagl et al. 2001
*O. esculentus**	*O. esculentus*	AF296485	1	Ladies Lake, Lake Victoria system	Nagl et al. 2001
*O. karongae**	*O. karongae*	AF328844	1	Lake Malawi	Nagl et al. 2001
*O. mortimeri**	*O. mortimeri*	AF328845	1	Zimbabwe	Nagl et al. 2001
*O. mossambicus x O. niloticus**	*O. mossambicus*		1	Namwedi Dam, South Africa	This study
*O. andersonii**	*O. mossambicus*		11	Mogalakwena River, South Africa	This study
*O. andersonii**	*O. mossambicus*		13	Limpopo River, South Africa	This study
*O. andersonii**	*O. mossambicus*		10	Nandoni Dam, South Africa	This study
*O. andersonii**	*O. mossambicus*		11	Namwedi Dam, South Africa	This study
*O. andersonii**	*O. mossambicus*		3	Lephalala River ,South Africa	This study
*O. andersonii**	*O. mossambicus*		4	Shingwedzi River, South Africa	This study
*O. andersonii**	*O. mossambicus*		10	Luvhuvhu River , South Africa	This study
*O. mossambicus**	*O. mossambicus*	AY597335	1	China	Chen at al. (unpublished)
O. mossambicus	*O. mossambicus*	AY833441	1	Olifants River, South Africa	D'Amato et al. 2007
O. mossambicus	*O. mossambicus*	AY833443-45	3	Boesmans River, South Africa	D'Amato et al. 2007
O. mossambicus	*O. mossambicus*	AY833447-48	2	Makathini and Sucoma Rivers, Malawi	D'Amato et al. 2007
O. mossambicus	*O. mossambicus*	AY833450-52	3	Kasinthula River, Malawi	D'Amato et al. 2007
O. mossambicus	*O. mossambicus*	AY833454	1	Olifants River, South Africa	D'Amato et al. 2007
*O. mossambicus x O. niloticus**	*O. niloticus*		13	Limpopo River, South Africa	This study
*O. niloticus**	*O. niloticus*		14	Limpopo River, South Africa	This study
*O. niloticus**	*O. mossambicus*		3	Limpopo River, South Africa	This study
O. niloticus	*O. niloticus*	AY833472	1	Limpopo River, SA and Nile River, Egypt	D'Amato et al. 2007
O. niloticus	*O. niloticus*	AY833473	1	Nile River, Egypt	D'Amato et al. 2007
O. niloticus	*O. niloticus*	AY833466	1	Olifants River, SA and Nile River, Egypt	D'Amato et al. 2007
O. niloticus	*O. niloticus*	AY833482-83	2	Olifants River, SA and Nile River, Egypt	D'Amato et al. 2007
O. niloticus	*O. niloticus*	AY833486	1	Nile River, Egypt	D'Amato et al. 2007
*O. niloticus**	*O. niloticus*	AF328849	1	Lake Manzala, Egypt	Nagl et al. 2001
*O .mossambicus**	*O. mossambicus*	AF328843	1	Zambezi River, Zimbabwe	Nagl et al. 2001
*T. rendalli**	*T. rendalli*		2	Mogalakwena River	This study
*T. rendalli**	*T. rendalli*	AF328854	1	Zambezi River, Zimbabwe	Nagl et al. 2001

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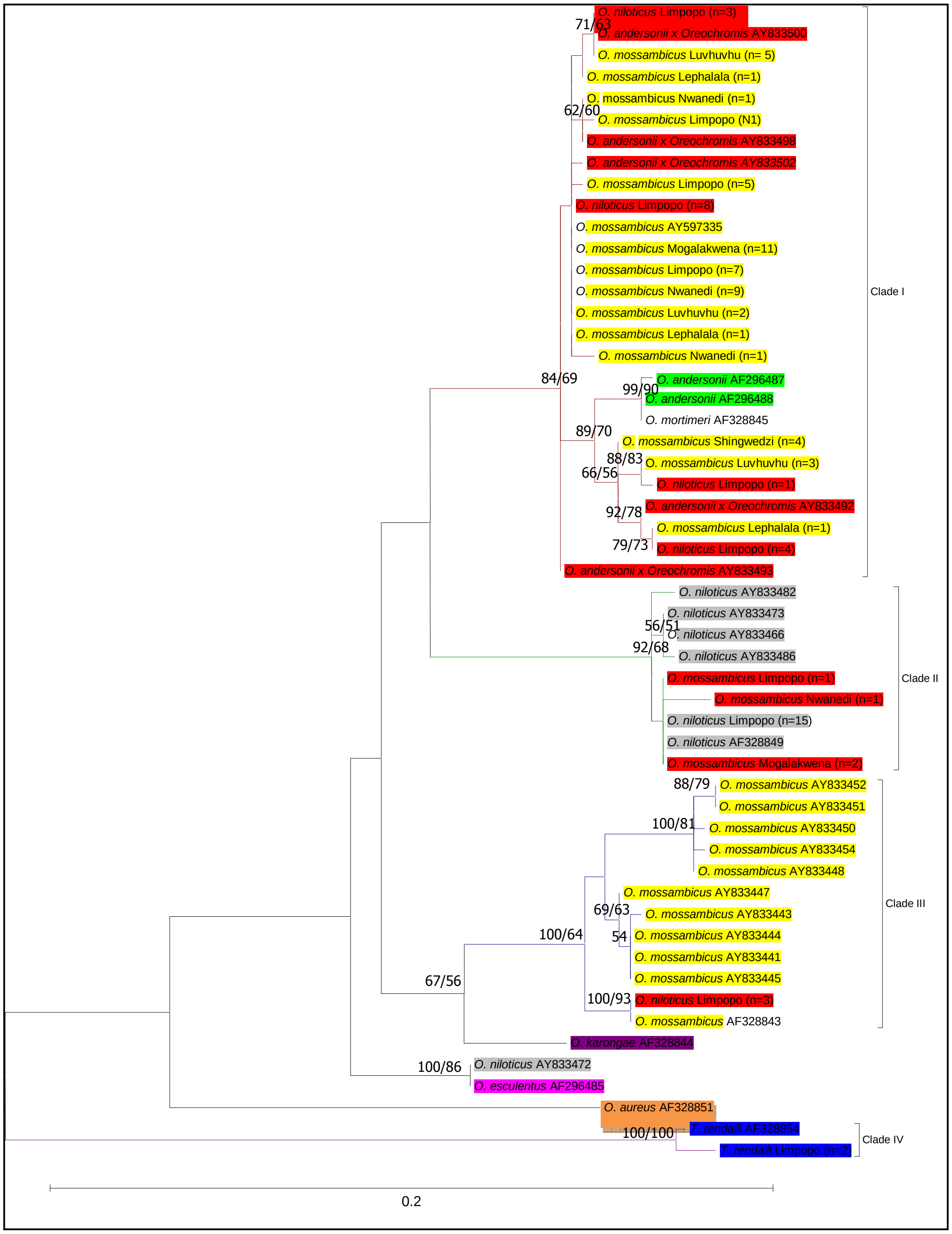

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Figure 3. A D-loop gene tree inferred using a partial fragment sequence data (394 bp) and depicting the genetic relatedness of invasive Nile tilapia, *Oreochromis niloticus* and indigenous tilapiine species from river systems in southern Africa. *Tilapia rendalli* from the Mogalakwena River and a reference sequence *T. rendalli* AF328854 from the Zambezi River, Zimbabwe was used as an out-group. Taxon names for sequences generated from this study comprise species name (based on morphology), followed by river of origin and number of individuals characterised. Taxonomic names for sequences obtained from Genbank comprise of species name and sequences accession number. Nodal support are given in percentages are indicated as NJ/ML next to relevant nodes. Taxa are colour coded were green = *O. andersonii*, yellow = Mozambique tilapia, grey = Nile tilapia, red = *O. mossambicus* × *Oreochromis* hybrids, pink = *O. esculentus*, white = *O. mortimeri*, orange = *O. aureus*, purple = *O. karongae* and blue = *Tilapia rendalli* is the out-group.

Clade I comprised of 68 Limpopo river system specimens characterised in this study of which 52 were morphologically identified as Mozambique tilapia and were sampled from the Limpopo, Lephalala, Mogalakwena, Luvhuvhu and, Shingwedzi rivers and from the Namwedi and Nandoni dams. The remaining 16 individuals were morphologically identified as Nile tilapia from the Limpopo River. The reference sequences that fall within clade I correspond to a congeneric Zambezi species: Kariba tilapia *O. mortimeri* (AF328845) from the middle Zambezi River, Zimbabwe, threespot tilapia *O. andersonii* (AF296487, AF296488) from the Okavango Delta in Botswana; and five *O. andersonii* × *Oreochromis* spp. hybrids from the Olifants and Limpopo rivers in South Africa (Nagl et al. 2001; D’Amato et al. 2007).

Clade II comprised of 15 individuals morphologically identified as Nile tilapia from the Limpopo River. The group also included reference sequences of Nile tilapia from Lake Manzala, Egypt (AF328849), Olifants River, South Africa and Nile River, Egypt (AY8333466, AY833482-3, AY8333486 and AY833473), as well as four specimens identified morphologically as Mozambique tilapia specimens, which were sampled from the Namwedi dam and Mogalakwena and Limpopo rivers (this study).

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Clade III comprised of reference sequences of Mozambique tilapia from various river systems from southern Africa that include: the Makathini, Kasinthula and Sucoma Rivers in Malawi and the Boesmans and Olifants Rivers in South Africa. This group also included three specimens morphologically identified as Nile tilapia from the Limpopo River (this study) and a reference sequence for *O. karongae* from the Lake Malawi (Nagl et al. 2001).

Clade IV comprised of two specimens morphological identified as *T. rendalli* from the Mogalakwena River (this study) and a reference sequence *T. rendalli* AF328854 from the Zambezi River, Zimbabwe. Three reference sequence for Nile tilapia AY833472 from the Limpopo River, South Africa (D'Amato et al. 2007) and Nile River, Egypt; *O. esculentus* AF296485 from Ladies lake (a satellite of Lake Victoria, Tanzania); and *O. aureus* AF328851 from Lake Manzala, Egypt each represent discrete lineages.

Morphometrics

The ordinated results of the log-transformed measurements (Fig. 4a) and a UPGMA cluster analysis (Fig. 5a) based on the 23 of all *Oreochromis* specimens examined (*n* = 71) were similar and showed complete overlaps among the monophyletic lineages identified by mtDNA data analysis as Mozambique and Nile tilapias, and specimens of *O. mossambicus* × *O. niloticus* hybrids, respectively. The first Principal Component (91 % of the total variance), second Principal Component (2 % of the total variance) and subsequent generated PCA axes all showed complete overlaps between the specimens of the two congenerics and hybrids. Similarly, the PCA and UPGMA cluster analysis based on 23 measurements expressed as percentages of SL and HL of all *Oreochromis* specimens examined (*n* = 71) also failed to classify the respective specimens of the two congenerics and hybrids (not shown).

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In contrast to the PCA and UPGMA cluster analysis of log-transformed measurements, the CVA of log transformed measurements produced a 95 % *a posteriori* classification and differentiated specimens of the two congenerics and their associated hybrids (Fig 6a). Mozambique tilapia was separated from Nile tilapia and specimens of hybrids on the first canonical variate axis (86 % of explained variance) and the most important measurements were snout length, head width, head length, body depth, dorsal fin base length, anal fin base length, caudal peduncle length, caudal peduncle depth and lower pharyngeal jaw width (Table 3). The second canonical variate explained the remaining 14 % of the total variance and further differentiated between Nile tilapia and specimens of hybrids. The most important loading on the second CVA axis were lachrymal depth, snout length, inter-orbital distance, head length, caudal peduncle length and lower pharyngeal jaw width (Table 3). A MANOVA indicated a statistically significant morphological difference between the centroids of the three delineated phenetic groups ($F_{(28,118)} = 4.63$; $P < 0.01$). The results of the CVA are also reflected in the standard descriptive univariate statistics for each delineated phenetic group as summarized in Table 5.

Similarly, the CVA based on measurements expressed as percentages of SL and HL of all *Oreochromis* specimens examined ($n = 71$) also showed complete separation between specimens of the two congenerics and specimens of the hybrids on the first and second CVA axis (not shown). The first CVA axis explained 79 % of the total variance and the most important variables with relatively high loading were eye diameter, cheek depth, dorsal fin base length, inter-orbital width, head width, anal fin base length and pre-pectoral length. The second canonical variate explained 21 % of the total variance and the most important loading variables were lower jaw length, eye depth, cheek depth and pre-pelvic length. The CVA produced a 99 %

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a posteriori classification and a MANOVA indicated a statistically significant morphological difference between the centroids of the three delineated phenetic groups ($F_{30,144} = 6.10$; $P < 0.01$).

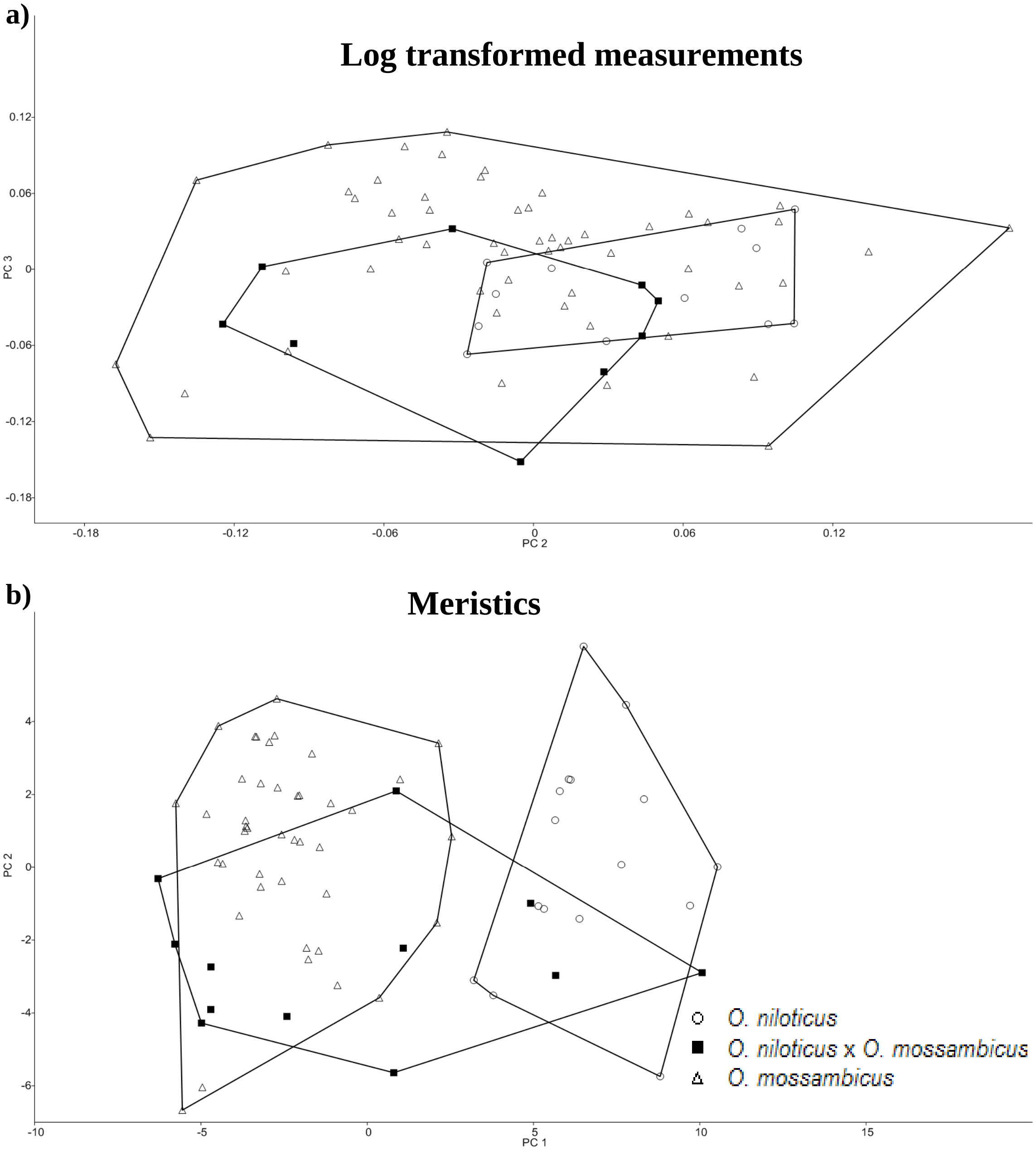

Figure 4. A principal component analysis of: a) 23 log-transformed measurements; b) 15 meristics counts for all genotyped specimens of *Oreochromis mossambicus* (Δ), *O. niloticus* (○), *O. mossambicus* × *O. niloticus* hybrids (■) from the Limpopo River basin, South Africa.

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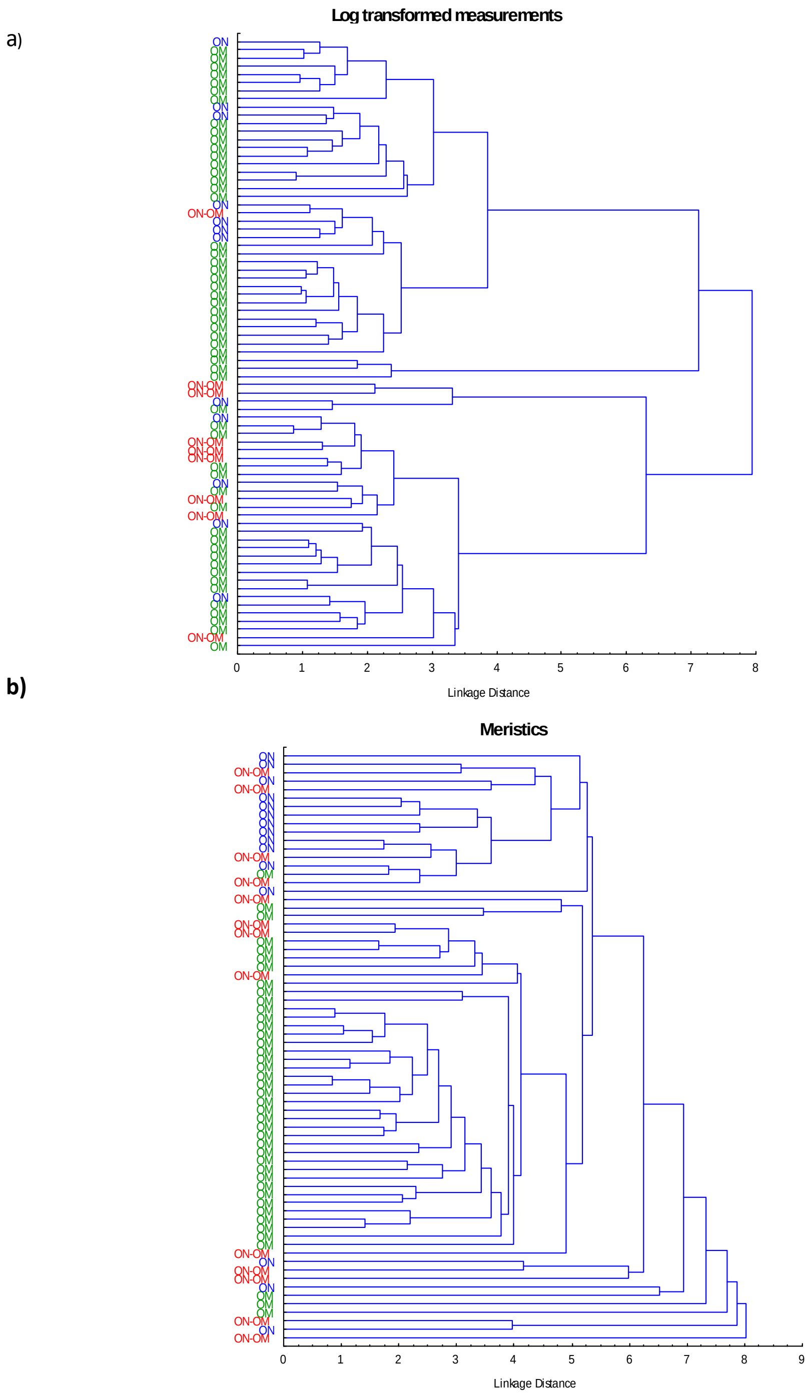

Figure 5. An Unweighted-pair group arithmetic average (UPGMA) cluster analysis of standardized data for: a) 23 log transformed data; and b) 15 meristics counts for all genotyped *Oreochromis* specimens from Limpopo River basin, South Africa. Specimens indicated in green = *O. mossambicus* (OM), blue = *O. niloticus* (ON), and red = *O. mossambicus* × *O. niloticus* hybrids (ON-OM).

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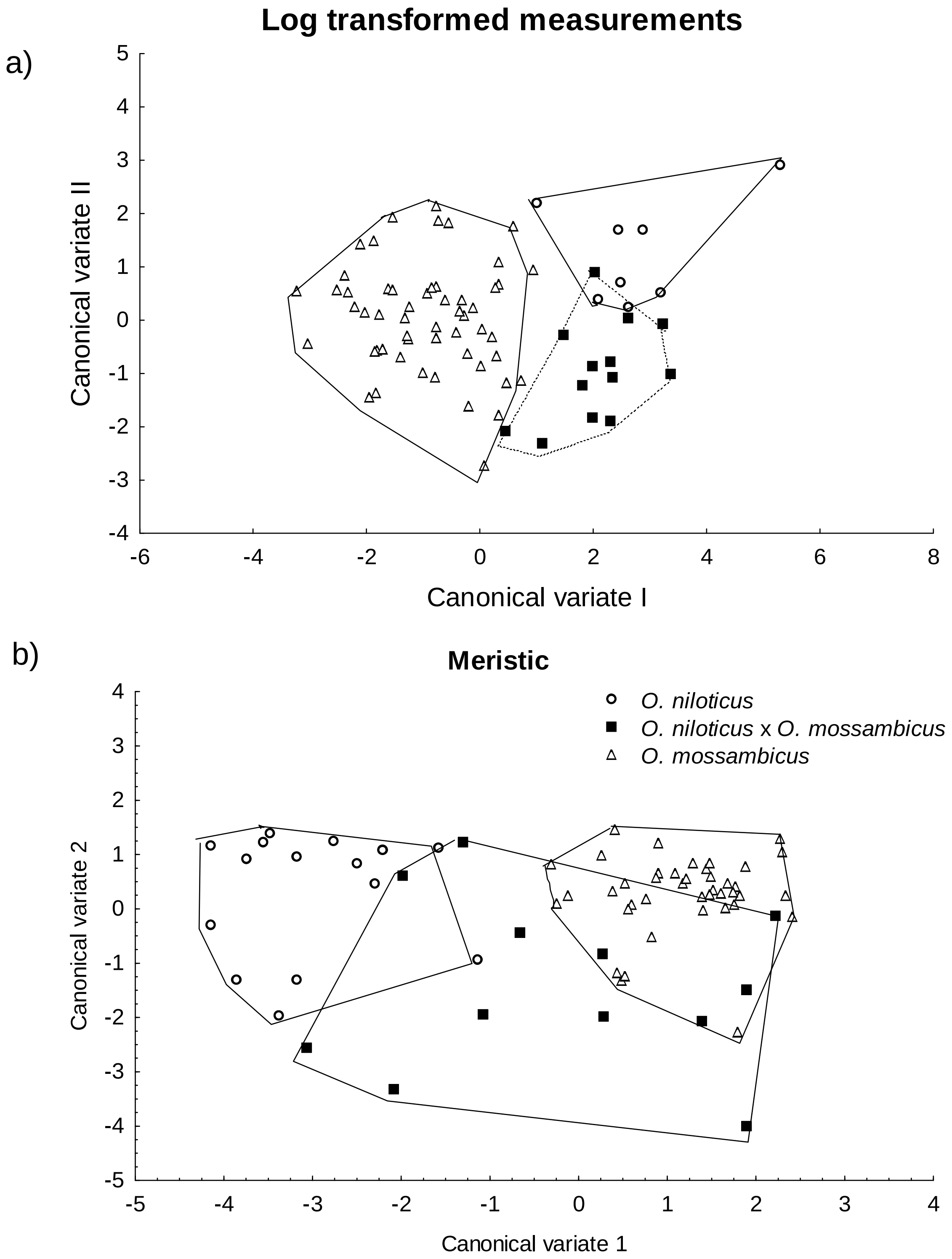

Figure 6. A canonical variates (discriminant) analysis (CVA) of: a) 23 log transformed data; and b) 15 meristics counts for all genotyped specimens of *Oreochromis mossambicus* (Δ), *O. niloticus* (○), *O. mossambicus* × *O. niloticus* hybrids (■) from the Limpopo River basin, South Africa.

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Table 3. Loadings for the first two canonical variates (CV), their eigenvalues and explained variance on 23 log transformed measurements examined of all *Oreochromis* specimens (*n* = 71). The discriminate analysis method was forward stepwise and 10 measurements which contributed the least to the discrimination of the three phenetic groups (*O. niloticus*, *O. mossambicus* and *O. mossambicus* × *O. niloticus* hybrids) were excluded from the final analysis. The most important variables are shown in bold.

Variable	CV1	CV2
Lachrymal depth	-0.55	**1.00**
Snout length	**-1.21**	**-1.03**
Eye diameter	0.90	-0.67
Inter-orbital distance	-0.84	**1.31**
Head width	**4.22**	-0.53
Head length	**2.42**	**4.48**
Body depth	**-1.68**	-0.64
Dorsal fin base length	**2.03**	0.63
Anal fin base length:	**-1.81**	0.89
Prepectoral length:	-0.82	-1.01
Caudal peduncle length	-0.11	**-3.39**
Caudal peduncle depth	**1.66**	-0.78
Lower pharyngeal jaw width	**-1.65**	**-1.75**
Eigenvalue	2.25	0.36
Cumulative variance explained	0.86	1.00

Meristics

The ordinated results of the meristic PCA (Fig. 4b) and a UPGMA cluster analysis (Fig. 5b) were similar and revealed clear separation between specimens of Mozambique and Nile tilapias. However, both analyses showed overlaps between specimens of hybrids and the two congeners. The first principal component explained 59 % of the total variance and the most important meristic characteristics on this axis with relatively high loadings were the hypobranchial arch gill rakers and total number of gill rakers (UR/1/LR) (Table 4). The second principal component explained 21 % of the total variance, and did not differentiate the two morpho-species and their

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hybrids. The standard descriptive univariate statistics of the meristics data for each delineated phenetic group are summarized in Table 6.

Table 4. Loadings for the first two principal components (PC), their eigenvalues and explained variance on 15 meristic variables examined of all *Oreochromis* specimens (*n* = 71). The most important meristics are shown in bold.

Variable	PC1	PC2
Total number of scales along the lateral line	0.21	**0.79**
Scales on upper lateral line	0.14	0.36
Scales on lower lateral line	0.06	0.39
Scales between dorsal fin and lateral line	0.01	-0.01
Scales between anal fin and upper lateral line	-0.01	0.19
Scales between pectoral and pelvic fin lateral line	0.01	0.00
Cheek scales	0.00	0.03
Spines on the dorsal fin	0.03	-0.01
Branched soft rays on the dorsal fin	0.10	-0.01
Branched soft rays on the pectoral fin	0.02	-0.02
Spines on the anal fin	0.00	0.00
Branched soft rays on the anal fin	-0.06	0.01
Epibranchial arch gill rakers	0.19	-0.03
Hypobranchial arch gill raker	**0.56**	-0.15
Total number of gill rakers (UR/1/LR)	**0.76**	-0.19
Eigenvalue	21.49	7.78
Cummulative variance explained	0.59	0.81

Similarly, the CVA based on meristic counts of all *Oreochromis* specimens examined (*n* = 71) showed complete separation between specimens of the two congenerics on the first (87 % of the total variance) and second canonical variate (23 % of the total variance) axes and the hybrids also plotted intermediate between the two congenerics (Fig. 6b). The most important loading variables with relatively high loading were total gill rakers on the first CVA axis and scales between anal fin and upper lateral line on the second CVA axis. CVA produced an 89 % *a*

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posteriori classification and a MANOVA indicated a statistically significant morphological difference between the centroids of the delineated phenetic groups ($F_{16, 160} = 10.39$; $P < 0.01$).

In summary, all the *a priori* analyses (PCA and UPGMA cluster analysis) did not distinguish the three genetically-identified specimens of Mozambique and Nile tilapias and specimens of *O. mossambicus* × *O. niloticus* hybrids based on morphometric measurements. However a *posteriori* canonical variates (discriminant) analysis discriminated all genetically-identified specimens. This implies that without prior knowledge of a specimen identity (as confirmed by DNA analysis), body morphological can lead to equivocal specimen identification. In contrast, specimens of Mozambique tilapia can be easily distinguished from Nile tilapia based on meristic characters (total number of gill rakers on the first gill arch) irrespective of which statistical analysis is used. However, specimens of *O. niloticus* × *O. mossambicus* hybrids had intermediate meristic characters making their morphological differentiation from either Mozambique or Nile tilapia difficult as they resembled both parental species.

Realised and potential distributional range

Given the genetic and morphometric data presented above the current distribution of Nile tilapia within Limpopo Province in South Africa seem to be mainly centered in the main river channel and the immediate reaches of associated tributaries. Its distributional range appears to have expanded to include several river systems in the upper Bushveld catchment. These include the following rivers: Mogalakwena, Namwedi, and Luvhuvhu (Figure 1).

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Table 5. Standard descriptive statistics for specimens of *O. niloticus* (min = 89.4mm, max = 194.6mm, mean = 144.6mm, n = 8, SD = 36.8), *O. mossambicus* (min = 90.0 mm, max = 181.0mm, mean = 134.3mm, n = 55, SD = 41.9) and *O. mossambicus* × *O. niloticus* hybrids (min = 104.0 mm, max = 286.4mm, mean = 173.4mm, n = 12, SD = 26.0) from the Limpopo River Basin, South Africa.

Morphometrics	*O. niloticus*					*O. mossambicus*					*O. mossambicus* × *O. niloticus*				
	min	max	mean	n	SD	min	max	mean	n	SD	min	max	mean	n	SD
Standard length (mm)	89.4	194.6	144.6	8	36.8	90.0	181.0	134.3	55	41.9	104.0	286.4	173.4	12	26.0
As percentages of SL															
Body depth	35.7	43.2	39.2	8	2.7	33.8	43.8	39.1	55	2.2	35.5	46.7	39.1	12	2.9
Dorsal fin-base length	57.4	63.0	60.6	8	1.8	51.9	62.6	57.7	55	2.2	57.6	63.2	59.9	12	1.7
Anal-fin base length:	18.6	20.4	19.4	8	0.7	16.6	22.3	19.5	55	1.3	17.6	21.2	19.4	12	1.0
Predorsal length	33.4	38.6	36.3	8	1.8	32.9	59.0	37.3	55	3.4	35.1	42.7	37.2	12	2.2
Prepectoral length:	32.7	38.3	34.9	8	1.7	30.0	36.9	33.0	55	1.7	30.3	41.5	34.9	12	3.0
Prepelvic length:	39.7	44.2	41.9	8	1.4	35.3	46.1	40.5	55	2.1	38.7	46.8	41.6	12	2.4
Preanal length	70.0	75.0	72.4	8	1.7	62.9	73.4	69.1	55	2.6	66.2	77.4	70.8	12	3.1
Caudal peduncle length	15.1	17.2	16.3	8	0.8	14.7	19.5	17.2	55	1.1	16.1	20.1	17.8	12	1.2
Caudal peduncle depth	14.3	16.0	15.1	8	0.5	12.9	16.7	14.5	55	0.9	14.2	18.2	15.5	12	1.1
As percentage of CPL															
Caudal peduncle depth	85.9	98.9	92.7	8	4.2	73.5	97.2	84.5	55	5.8	75.4	99.9	87.2	12	7.7
As percentages of HL															
Lachrymal depth:	16.2	22.8	18.3	8	2.4	12.9	24.2	20.7	55	2.6	16.4	21.8	18.8	12	1.8
Snout length:	19.7	35.7	29.0	8	4.9	24.0	43.1	33.9	55	3.6	24.4	34.7	30.7	12	3.1
Lower jaw length	28.1	33.7	31.2	8	1.9	26.7	44.2	36.3	55	3.2	30.8	38.7	33.7	12	2.6
Premaxillary pedicel length:	16.1	22.4	20.1	8	2.3	17.6	37.5	23.2	55	2.9	18.0	22.7	21.0	12	1.3
Cheek depth	18.2	20.5	19.1	8	0.8	17.2	26.3	20.9	55	1.9	16.2	24.7	21.5	12	2.6
Eye diameter:	22.9	32.7	28.5	8	3.6	21.5	33.7	26.1	55	3.0	23.4	44.2	30.5	12	5.1
Inter-orbital distance	34.6	40.6	37.8	8	2.4	35.2	47.1	40.8	55	2.4	32.4	44.7	39.0	12	3.7
Head width	34.0	38.5	35.7	8	1.5	31.1	37.0	33.5	55	2.5	31.6	38.1	34.2	12	3.1
Head length	50.8	54.8	52.6	8	1.5	43.8	57.2	51.3	55	1.6	48.8	58.3	53.8	12	2.0
Lower pharyngeal jaw length	31.3	38.1	34.8	8	2.3	27.4	39.9	35.2	55	2.5	31.2	38.0	35.1	12	1.8
As percentages of PHJL															
Lower pharyngeal jaw width	82.8	99.8	91.5	8	5.3	85.0	120.7	93.1	55	6.3	82.7	107.0	95.6	12	6.7

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Table 6. Standard descriptive meristics statistics of specimens of *O. niloticus* , *O. mossambicus* and *O. mossambicus* × *O. niloticus* hybrids from the Limpopo River Basin, South Africa.

Meristics	*O. niloticus*				*O. mossambicus*				*O. mossambicus* × *O.*			
	min	max	mean	n	min	max	mean	n	min	max	mean	n
Total number of scales along the lateral line	31	41	36	20	28	38	35	42	30	37	33	8
Scales on upper lateral line	17	24	21	20	17	22	20	42	15	22	19	8
Scales on lower lateral line	11	18	15	20	11	17	15	42	12	16	14	8
Scales between dorsal fin and lateral line	5	6	5	20	5	5	5	42	5	5	5	8
Scales between anal fin and upper lateral line	11	15	14	20	11	16	15	42	10	15	13	8
Scales between pectoral and pelvic fin lateral line	8	9	8	20	8	9	8	42	7	8	8	8
Cheek scales	2	3	3	20	2	4	3	42	2	3	3	8
Spines on the dorsal fin	15	17	16	20	15	17	16	42	15	17	16	8
Branched soft rays on the dorsal fin	11	14	13	20	10	12	11	42	11	13	12	8
Branched soft rays on the pectoral fin	12	15	14	20	12	14	13	42	13	15	14	8
Spines on the anal fin	3	3	3	20	2	4	3	42	3	3	3	8
Branched soft rays on the anal fin	8	10	10	20	8	12	10	42	9	11	10	8
Epibranchial arch gill rakers	3	6	5	20	1	4	3	42	0	4	3	8
Hypobranchial arch gill raker	19	26	22	20	14	21	18	42	15	19	17	8
Total number of gill rakers (UR/1/LR)	25	31	28	20	19	25	21	42	18	24	21	8

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Discussion

Genetic variation

Mitochondrial DNA genotyping represents a valuable tool to detect hybrids especially in areas where hybridisation has occurred as a result of secondary contact (D'Amato *et al*., 2007). In this study mitochondrial DNA was able to confirm the introduction of Nile tilapia and another un-documented introduction of threespot tilapia into the Limpopo River system in agreement with previous studies of (D'Amato *et al*., 2007). A large proportion (93 %) of morphologically identified specimens of Mozambique tilapia obtained from this study and covering a large spatial extent of river systems within the Limpopo River system had maternal mtDNA that is linked to a reference sequence for threespot tilapia, a species whose establishment and spread in the system is largely un-documented. These confounding results may either reflect the complex hydro-geographical history of Limpopo River Basin or indicate that possible mis-identifications based on morphology may have occurred. Fish communities in the Zambezi ichthyofaunal region reflect a history of complex geo-morphological changes to major river courses and river captures with neighbouring systems (Skelton, 1994). Threespot tilapia is part of the tropical 'Zambezian' ichthyofauna and its natural range is restricted to the Upper Zambezi region, i.e., upstream of Victoria Falls and including the Cunene, Okavango and Kafue rivers (Trewavas, 1983; Skelton, 2001). The upper Zambezi, Okavango and Limpopo rivers were once connected to form the headwaters of a large drainage system (Moon and Dardis, 1988; Moore and Larkin, 2001). This resulted in a considerable mixing of fish communities between the systems and could account for the clustering of threespot tilapia with specimens identified morphologically as Mozambique tilapia from the Limpopo river system, as the two species might share a common ancestor.

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Alternatively the presence of threespot tilapia in the Limpopo river system could be explained by its introduction into the Shashe dam in Botswana for aquaculture (De Moor and Bruton 1988). It however appears to have either have failed to establish itself or is extremely rare because there are no known/documented records (based on morphology) of the species within the Limpopo River in South Africa. A possible explanation is that these species may have become established but this has gone documented, due to the similar and overlapping morphometric characteristics between Mozambique tilapia and threespot, which are not easily distinguishable in the field. There is therefore a critical need for further surveys combining molecular and morphometric analyses of *Oreochromis* congenerics within the Limpopo River system to ascertain the potential presence of threespot tilapia and the extent of the Nile tilapia invasion.

Nile tilapia was first recorded in the Limpopo River inside the Kruger National Park in 1996 and then later in 1998 at the confluence of the Limpopo and Shashe Rivers (Van Der Waal and Bills, 1997, 2000). It is interesting to note that 10 years later, several *Oreochromis* samples obtained from upper catchment river tributaries had mtDNA of Nile tilapia. This strongly suggests that the distributional ranges of Nile tilapia and/or hybrids have expanded from the main channel of the Limpopo River and the immediate reaches of associated tributaries to include middle reaches of several river systems in the upper Bushveld catchment such as the Mogalakwena, Nwanedi and Luvhuvhu rivers. Several reasons could account for such a slow dispersal into the upper catchment. These may include limited natural dispersal pathways and inadequate sampling. As a result of the semi-arid climate and the unpredictable rainfall, rivers and streams within the Limpopo River basin have seasonal and episodic/ephemeral surface water flows (FAO, 2004). The Limpopo River and associated tributaries are therefore characterized as ‘seasonal sand bed

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rivers' that recede into long stretches of dry sand, interspaced by a staggered series of residual pools, river weirs and farm dams during the dry season (van der Waal, 1997; Minshull, 2008). This often leads to a high degree of river fragmentation and in the case of Nile tilapia, may fortuitously retard its movement into novel river systems further up-stream.

In agreement with

Preliminary evidence points to directional hybridisation among *Oreochromis* congeners within the Limpopo River system. This hypothesis is supported by the fact that a large proportion (83 %, *n* = 23) of specimens of *O. niloticus* × *Oreochromis* hybrids identified in this study were morphological identified as Nile tilapia but had a conflicting mtDNA lineage. Since the mitochondrial DNA is inherited in a strictly matrilineal way (with a few exceptions), an Mozambique tilapia with an threespot tilapia lineage was likely to have been the maternal parent for all the specimens of all the Nile tilapia hybrids. Male reproductive competition for fertilisation is common among many lek-spawning fish species (DeWoody and Avise, 2001). Fish species within the genus *Oreochromis* are lek-spawning where an individual male builds a 'nest' and defends a defined territory around the 'nest' to which females are attracted to spawn (Trewavas, 1983). After successful spawning, females mouth-brood the eggs and provide parental care to subsequent juveniles. The reproductive success of these two species is therefore likely to be dependent on male-male competitive interactions for spawning grounds and/or female mate choice (Johnstone and Earn, 1999; Fessehaye *et al*., 2006). Nile tilapia is an aggressive competitor that is known to attain large body sizes (±6 kg) and large males often out-compete other congeneric species for limited spawning and nursing grounds (Lowe-McConnell, 2000).

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The sexual selection hypothesis for unidirectional hybridization in hybrid zones (Wirtz, 1999) may explain the proximate basis of heterospecific matings between *Oreochromis* spp. and Nile tilapia in the Limpopo River. It is hypothesised that bigger males of Nile tilapia may have a competitive advantage over spawning grounds and in female mate choice. Mate choice by *Oreochromis* spp. females is based on spawning site characteristics (size and shape) and body size of males but they may occasionally mismate with Nile tilapia when their options are limited (e.g., limited access to conspecific males in spawning grounds due to competitive exclusion of "*Oreochromis* spp." males by Nile tilapia males. Conversely, females of Nile tilapia will likely reject males of the "*Oreochromis* spp." such that these reciprocal crosses rarely transpire. Unidirectional hybridisation of fish is common in hybrid zones (see Writz, 1999) and within the Limpopo river system further studies on the fish reproductive biology (behavioural and genetic paternity studies) are needed in order to confirm this hypothesis.

Morphometric variation

Our study found no clear morphological distinction of hybrid specimens of Nile tilapia and *Oreochromis* specimens morphological identified as Mozambique tilapia. All the *a priori* analyses (i.e., without knowledge of a specimen's genetic identity) showed no separation between the genetically-identified specimens of the two congeners and associated hybrids. Equivocal results were further observed in an attempt to distinguish specimens of hybrids from the respective morpho-specimens of the two congeners based on meristic characters. Specimens of Mozambique tilapia can be easily distinguished from Nile tilapia based on the total number of gill rakers on the first gill arch. Specimens of hybrids, however, revealed intermediate meristic characters making their morphological differentiation from either Mozambique or Nile tilapia difficult because they resembled both parental species. Similarly, previous studies have also

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failed to identify with certainty specimens of Mozambique and Nile tilapia hybrids from morpho-specimens of the two congeners based on either morphometrics and/or meristics (Trewavas, 1983; Moralee *et al*., 2000; Van der Bank and Deacon, 2010).

In southern Africa, biogeography is often used as a deciding factor in species identification because of the localized distribution of indigenous *Oreochromis* species (Jubb, 1967; Trewavas, 1983; Bell-Cross and Minshull, 1998; Skelton, 2001). The extensive movement of formerly allopatric *Oreochromis* spp. into artificial sympatry within most river systems has made the morphological distinction of the different species and/or their hybrids difficult. For example, Kariba tilapia *O. mortimeri* is endemic to the middle Zambezi and Luangwa River systems but the identity of *Oreochromis* spp. in this catchment is now uncertain owing to the widespread introduction of Mozambique and Nile tilapia (Zengeya and Marshall, 2007). Body morphology of Mozambique and Kariba tilapia are similar and the only difference is in male breeding dress-Mozambique tilapia turns into deep greyish black with a white lower head and throat, while Kariba tilapia the body colour profile is either iridescent olive blue or bronze with the dusky green or black throat (Skelton, 2001).

Caveats/ limitations

One major limitation of mtDNA analysis is that it can only demonstrate that genetic material of a species is present but cannot provide definite evidence that it is absent because the paternal component is not considered. As a result of this limitation, recent studies on hybridisation in cichlids have used mtDNA in combination with either microsatellite markers (e.g. D’ Amato et al. 2007) or with nuclear DNA (Angienda et al. 2011). This was not done in this study, but morphometric analysis was used instead to try to morphological identify morpho-specimens of Nile and Mozambique tilapia to determine if morphological species assignment was congruent

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with mitochondrial lineages. The hypothesis that an *Oreochromis* specimen was not a hybrid could only be proved if they was no conflict between mitochondrial lineages and morphological species assignment. However, morphology may not have been the ideal deterrent either, because there was strong evidence of hybridisation among the three congenerics- Mozambique, Nile and threespot tilapia. This therefore places into doubt the genetic integrity of the comparative material and the Limpopo *Oreochromis* cichlid population may already comprise a hybrid swarm. Furthermore, the *Oreochromis* specimens for morphological analysis were limited to the Limpopo river system. Ideal the *Oreochromis* specimens from the Limpopo system should be compared with specimens from regions where there is clear evidence of pure genetic material such as the Eastern Cape for Mozambique tilapia and Nile region for Nile tilapia. Additional reproductive biology studies including behavioural and genetic paternity studies are required to evaluate the postulated sexual selection hypothesis. Given the highlighted limitations of mtDNA analysis future genetic paternity studies should use a combination on mtDNA, nuclear DNA, microsatellites and morphometric analysis. Unidirectional hybridisation will likely have a major impact on the conservation of indigenous congenerics as they are likely to be extirpated from the river system and replaced by an admixture of introgressed hybrids.

There is therefore a need to characterize the genetic diversity of indigenous *Oreochromis* spp. within the Limpopo river system. These data can assist stake-holders and conservation managers to focus future conservation and management efforts on river systems where indigenous *Oreochromis* species are most vulnerable to invasion by congeneric Oreochromis species. In the Limpopo river basin, of concern are river systems in the upper bushveld sub-catchment (Mokolo, Lephalala and Mogalakwena rivers) and east flowing coastal rivers which have been designated as 'reservoir' areas for the conservation of Mozambique tilapia (Nel *et al.*,

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2011). If maintained ecologically intact and free of invasive fishes, the reservoir areas are likely to offer ‘genetic refugia’ for native populations. Furthermore, better knowledge of extant variability among indigenous congenerics may help shed light on their adaptive life history traits.

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Chapter 3

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

Tsungai A. Zengeya • Anthony J. Booth • Armanda D. S. Bastos • Christian T. Chimimba

Abstract

The stable isotope ratio and seasonal changes in the diet of two indigenous (Mozambique tilapia Oreochromis *mossambicus*, redbreast tilapia *Tilapia rendalli*) and one exotic (Nile tilapia *Oreochromis niloticus*) tilapiine cichlids in the subtropical Limpopo River, South Africa were investigated to determine patterns of resource partitioning. Stomach contents of Nile and Mozambique tilapia 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 δ^{13}C-depleted Nile tilapia indicates a phytoplankton-based diet, while the δ^{13}C-rich Mozambique tilapia 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. Redbreast tilapia was largely macrophagous and fed mainly on aquatic macrophytes and had a low dietary overlap with both Nile and Mozambique tilapia. In

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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.

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), Nile tilapia 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 Nile tilapia to be a highly successful invader. Feral populations are now established in most river catchments in the southern African

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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 2008; 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 congenerics 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, Nile tilapia was initially introduced in the Cape Flats area (Cape Town, Western Cape Province) and in the 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 2008). The advent of Nile tilapia 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 Nile tilapia (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 Nile tilapia 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 Nile tilapia 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

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between the exotic Nile tilapia 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 Nile tilapia 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*. 22ºS - 24ºS; 26ºE –31º 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

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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).

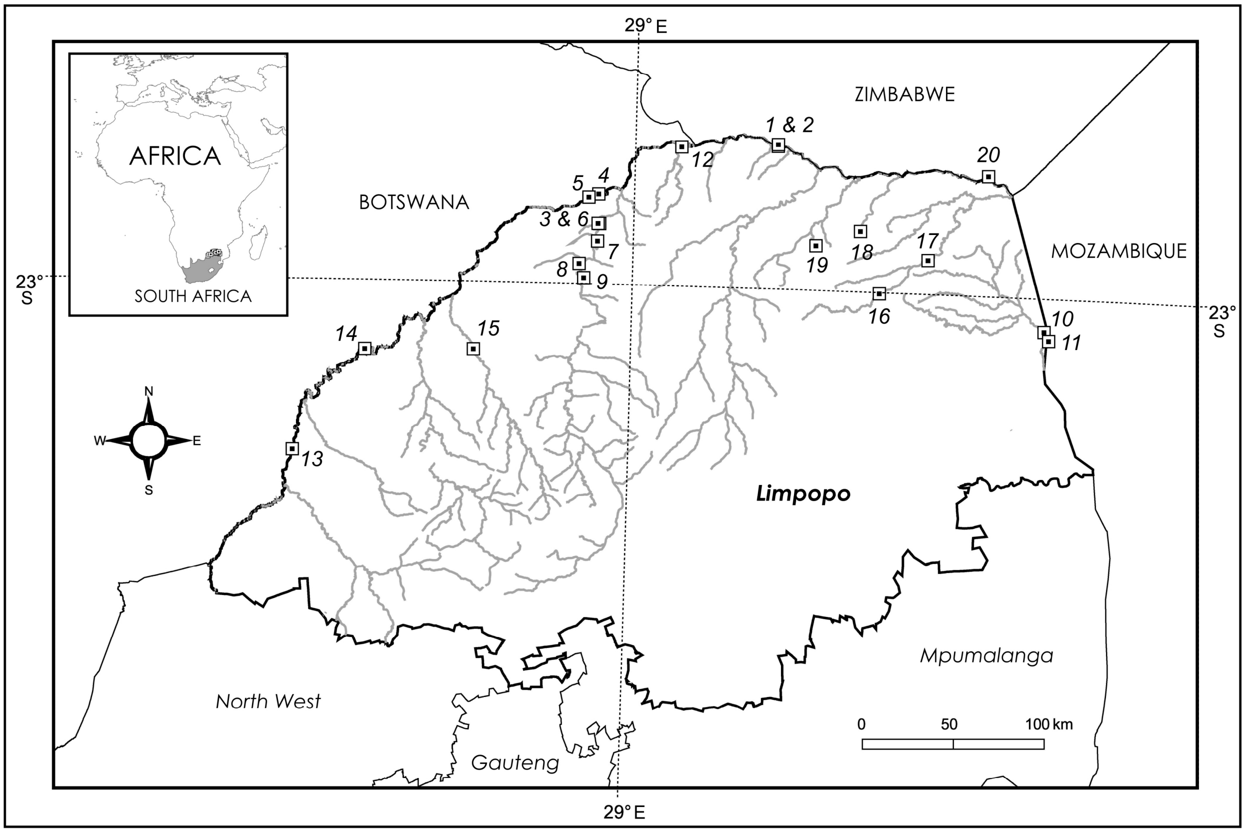

Figure 1. The Limpopo River in South Africa with its major tributaries and the location of sampling sites (1-20; see Table 1 for specific sites) in the present study.

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 SAMUS 725MP electrofisher (Samus Special Electronics, Warsaw, Poland). Large and deep river

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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 approximately 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.

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, Luvhuvhu River	22°59’S-30°30’E
17	Xikundu Weir, Luvhuvhu 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

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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 (such as 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 were the three species coexist. Fish muscle samples were stored frozen prior to been oven-dried at 60º C for 48 hrs, ground into a powder and analysed for δ^{13}C and δ^{15}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:

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

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

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overlap coefficient (Pianka 1974). Indices 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 (five food categories) was analysed by a fixed factor 3-way MANOVA (season, habitat and size class), and Scheffe's 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 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. Mozambique tilapia was the most abundant and widespread (80% of the total catch and > 70% of all sampling stations) while redbreast was widespread but not abundant (15% of the total catch and > 70% of all sampling stations). Nile tilapia 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. Banded tilapia was rare (< 1% of the total catch and >10% of all sampling stations). Greenhead tilapia, which first appeared in the river system in 1992 (Kleynhans and Hoffman 1992), was not recorded.

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Muscle tissue from all tilapiine species was δ^{15}N enriched (range = 9.3–16.5 ‰) and δ^{13}C-depleted (range = –23.2 – –30.5 ‰). Nile tilapia exhibited the most δ^{13}C-depleted and was significantly different from both Mozambique and redbreast tilapia (Figure 2) (ANOVA: $F_{2,\,74}$ = 22.36; n = 77; P < 0.001). Stomach content analysis (five food categories, three species) revealed significant differences in diet among the three tilapiine species [repeated MANOVA: $F_{8,.868}$= 24.24; n = 230; P < 0.001). Redbreast tilapia was a macrophage which fed primarily on living aquatic macrophytes while Mozambique and Nile tilapia 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 δ^{15}N (Table 3.). Redbreast tilapia was macrophagous and fed mainly on macrophytes across all size classes and seasons.

The diet of Nile tilapia 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 detrivores (Table 2). In contrast, Nile tilapia exhibited significant relationship for δ^{15}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 redbreast tilapia 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 δ^{15}N values for Mozambique tilapia showed no ontogenic changes in diet for δ^{15}N (r^2 = 0.20; P > 0.20) (Fig. 2). The diet of Mozambique tilapia was opportunistic and consisted of a wide range of prey items across all size classes (Table 2). Based on δ^{13}C all the 3 species showed no ontogenetic dietary shifts (Fig. 2). Seasonal shifts in diet (5 food categories and 2 seasons) were observed for Mozambique (repeated MANOVA: $F_{4,\,420}$ = 5.25; n = 88; P <

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0.001) and Nile tilapia (repeated MANOVA: $F_{4,\ 385} = 7.60$; $n = 79$ $P < 0.001$), but not for redbreast tilapia (repeated MANOVA: $F_{4,\ 255} = 2.03$; $n = 53$; $P > 0.09$) (Fig. 3). Significant interactions between diet and habitat were observed for Mozambique [5 food categories and 3 habitats (repeated MANOVA: $F_{8,\ 425} = 3.82$; $n = 88$; $P < 0.001$)] and Nile tilapia [5 food categories and 2 habitats (repeated MANOVA: $F_{4,\ 385} = 7.60$; $n = 79$ $P < 0.001$)] but not for redbreast tilapia [5 food categories and 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 (Nile and Mozambique tilapia) fed primarily on vegetative detritus and had high niche overlap across all size classes and seasons (Table 3.). Redbreast tilapia was macrophagous and fed mainly on macrophytes across all size classes and seasons.

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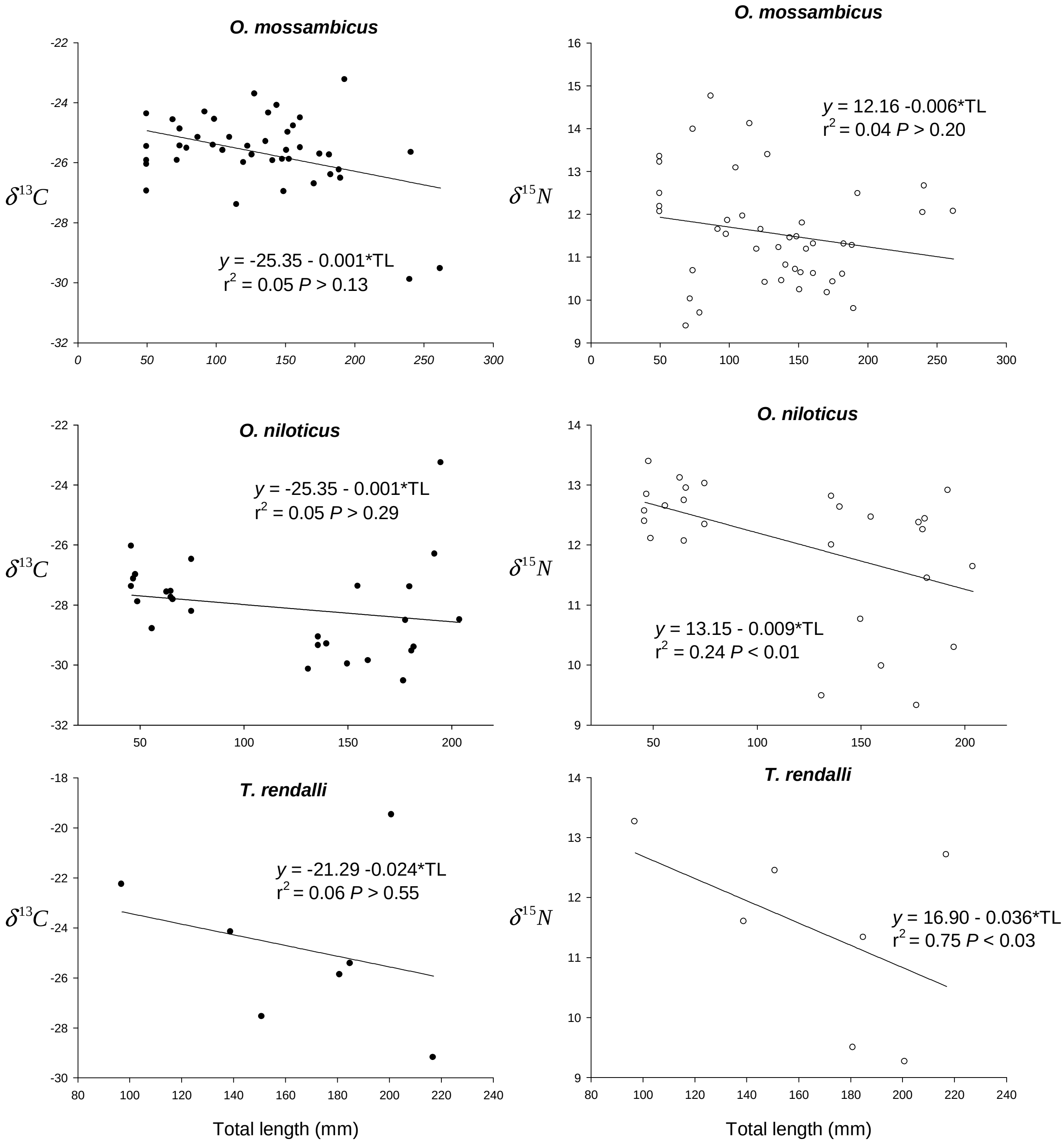

Figure 2. The relationship between isotopic carbon 13 ($\delta^{13}C$) (●) and nitrogen 15 ($\delta^{15}N$) (○) against total length (TL; mm) for tilapiine fish species (*O. mossambicus*, *O. niloticus* and *T. rendalli*) from the Limpopo River and associated tributaries, Limpopo Province, South Africa.

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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)	*N*	Cyanophyta	Green algae	Diatoms	Periphyton	Fine detritus	Plant detritus	Macrophytes	Zooplankton	Macrofauna	INB
O. mossambicus	< 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
Nile tilapia	< 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
T. rendalli	< 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	*T. rendalli*		*Nile tilapia*	
		Wet	Dry	Wet	Dry
<10mm TL	*O. mossambicus*	0.52	0.17	**0.91**	**0.95**
	T. rendalli			0.46	0.15
>10 mm TL	*O. mossambicus*		0.04	**0.88**	**0.94**
	T. rendalli			0.29	0.06

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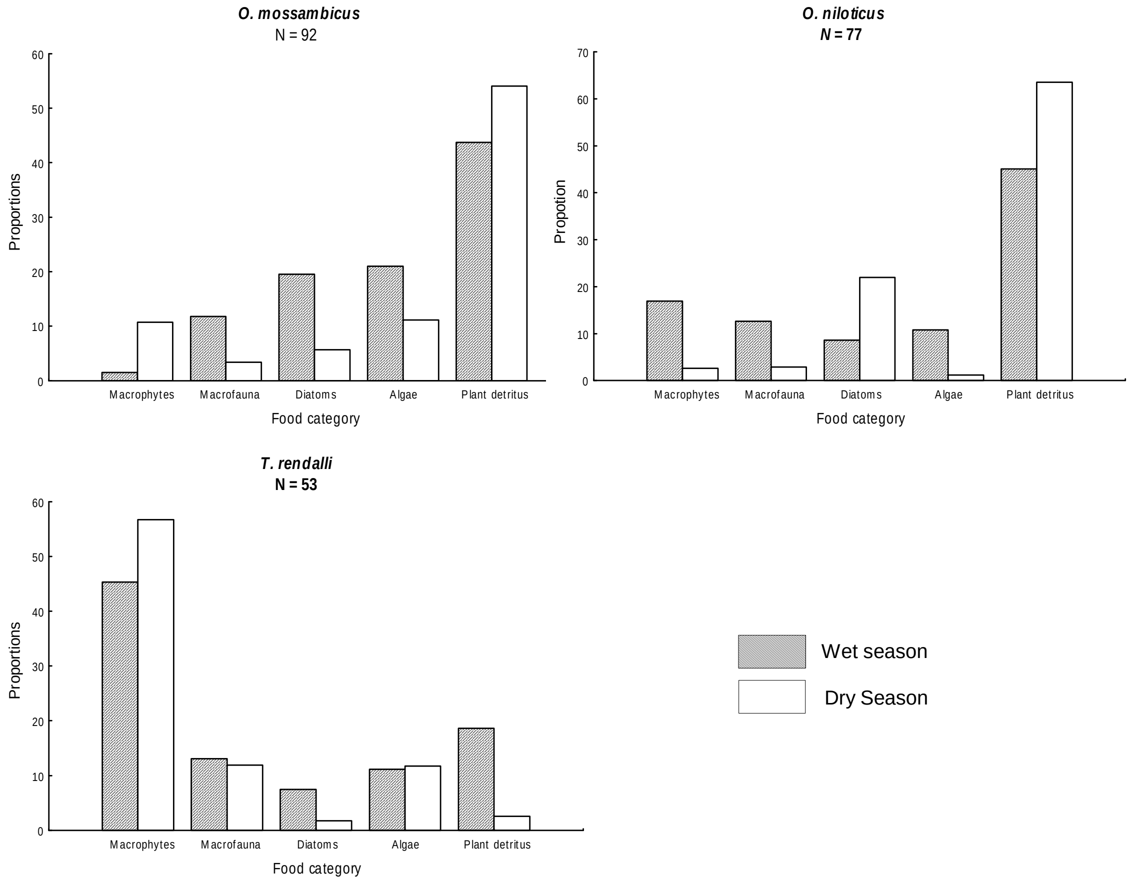

Figure 3. The proportion (by volume) of food items in the diet of three cichlid species (*Oreochromis mossambicus*, *O. niloticus*, and *T. rendalli*) in relation to season in the Limpopo River Basin, South Africa.

Discussion

The similarity in stomach contents combined with the difference in isotopic composition between Nile and Mozambique tilapia reveal fine-scale patterns of food resource partitioning that could be achieved through selective feeding. The depleted carbon isotopic composition of Nile tilapia indicates a phytoplankton-based diet while the enriched composition in Mozambique tilapia suggests a diet dominated by vegetative detritus and periphyton. Phytoplankton communities in tropical rivers typically have δ^{13}C values of between -40 and -25‰ while detrital aggregate of macrophytes, benthic algae and soil organic matter usually range between -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.

Nile tilapia is known to selectively feed on phytoplankton (Moriarty and Moriarty 1973; Getabu 1994; Bwanika et al. 2004; Zengeya and Marshall 2007) whereas Mozambique tilapia 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 this study, Mozambique and Nile tilapia 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 Nile and Mozambique tilapia may reflect the importance of vegetative detritus in the species diet during

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this time; vegetative detritus is often the most abundant food resource in most tropical rivers and it is usually available throughout the year (Winemiller and Winemiller 2003). Redbreast tilapia 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 Nile and redbreast tilapia, 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, Mozambique tilapia 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 Mozambique tilapia, likely use omnivory, among other adaptive life history traits, to survive these seasonal perbutations 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

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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, and 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

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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 Nile tilapia and indigenous *Oreochromis* species have subsequently implied that inter-specific competition could partly explain, among other factors, the successful establishment and spread of Nile tilapia 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 Nile tilapia in invading novel tropical hydrological systems worldwide does, however, provide strong circumstantial evidence to support the hypothesis of inter-specific competition. Owing to its hardy nature and wide range of trophic and ecological adaptations, Nile tilapia 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). Nile tilapia 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

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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). Nile tilapia 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 2008). However, whether Nile tilapia 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 (approximately >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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Chapter 4

Ecological niche modeling of the invasive potential of Nile tilapia, *Oreochromis niloticus* in African river systems: concerns and implications for the conservation of indigenous congenerics?[3]

Tsungai A. Zengeya • Mark P. Robertson • Anthony J. Booth • Christian T. Chimimba

Abstract

This study applied ecological niche models to determine the potential invasive range of Nile tilapia, *Oreochromis niloticus*, with a particular focus on river systems within southern Africa where it is now both established and spreading. Computational tools such as niche models are useful in predicting potential range of invasive species, but there are limitations to their application. In particular, models trained on native records may fail to predict the full extent of an invasion. This failure is often attributed to changes in either the niche of the invading species or variables used to develop the models. In this study, we therefore, assessed for differences in the predictive power of models trained with different environmental datasets and the ability of Nile tilapia to survive in conditions not represented by its native range. Niche models were constructed using Maxent and the degree of niche similarity and conservatism was assessed using the Schoener index and Hellinger distance. Null models were used to test for significance. Model performance and the degree of niche conservatism between the native and introduced

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ranges of Nile tilapia varied significantly with variable selection. This indicates that the spatial distribution of suitable and unsuitable environmental variables varies between the two regions. Nile tilapia exhibited a broad invasive potential over most of southern Africa that overlaps the natural range of other endemic congenerics. Of particular concern are areas that are free of exotic species but are now vulnerable due to the promotion of fish introductions mainly for aquaculture and sport fishing.

Key words: Ecological niche modeling, Invasion, Indigenous congenerics, Maximum entropy models (Maxent), Nile tilapia, Southern Africa

Introduction

Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758), is an endemic African freshwater cichlid that is native to the Nile River basin, south-western Middle East and the Niger, Benue, Volta and Senegal Rivers, lakes Chad, Tanganyika, Albert, Edward, and Kivu (Trewavas 1983; Daget et al. 1991). Owing to its hardy nature, and its wide range of trophic and ecological adaptations, it has been world widely introduced for aquaculture, augmentation of capture fisheries, and sport fishing (Trewavas 1983; Welcomme 1988). It is currently among the most world widely distributed invasive fish and has established feral populations in most tropical and sub-tropical environments to which it has gained access (Welcomme 1988; Costa-Pierce 2003; Canonico et al. 2005).

Within Africa, Nile tilapia was initially introduced into Lake Victoria in the 1950s and its distribution has since expanded to include most river systems in eastern and southern Africa (Skelton 1994). It was imported into Zambia in 1982 for aquaculture purposes and appeared in

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the Kafue River in the mid-1990s after escaping from nearby fish farms (Schwank 1995). Subsequent aquaculture introductions occurred in Lake Kariba. It is now widely distributed and is common in most sub-catchments of the Middle Zambezi, Nata (Makgadikgadi/Okavango), Runde-Save, Buzi and Limpopo River systems (van der Waal and Bills 1997, 2000; Tweddle and Wise 2007; Weyl 2008; Zengeya and Marshall 2008). The advent of Nile tilapia into novel river systems is a cause for concern for the conservation of indigenous congenerics that are at an extirpation risk through hybridization and competition arising from habitat and trophic overlaps (Chifamba 1998; Canonico et al. 2005; Cambray and Swartz 2007; Weyl 2008; Zengeya and Marshall 2007).

Invasive aquatic species, once established, are practically impossible to eradicate (Lockwood et al. 2007). This is of particular concern with a highly invasive and wide ranging species such as Nile tilapia where prevention should be the preferred remediation method (Wise et al. 2007). As is often the case with highly invasive fish species, after their establishment, the only practical management option is often only to predict the species eventual distributional range and adopt measures to either stop or slows down its dispersal across river systems (Chen et al. 2007).

Despite its widespread distribution within sub-Saharan Africa, several river systems are still free of Nile tilapia but remain vulnerable. These areas currently act as reserves for the conservation of indigenous congenerics and it is important to identify such areas to prevent further Nile tilapia introductions. These areas include the Cunene, Okavango and the Upper Zambezi Rivers where the endemic threespot (*O. andersonii*) and greenhead tilapia (*O. macrochir*) are naturally restricted (Trewavas 1983; Skelton 2001). The localised distribution of endemic tilapiines within southern Africa predisposes them to high invasion risk as the

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introduction of Nile tilapia into a given catchment is likely to have a significant impact on species with limited natural ranges. Examples include: 1) Kariba tilapia (*O. mortimeri*) that is naturally confined to the middle Zambezi and Luangwa River systems; 2) black tilapia (*O. placidus*) that is naturally confined to the east coastal plains from the Lower Zambezi to the Mkuze Rivers in KwaZulu-Natal Province, South Africa; and 3) Mozambique tilapia (*O. mossambicus*) that occurs naturally from the lower Zambezi River system to the Bushmans River system of Eastern Cape Province, South Africa, spreading far inland within the Limpopo River Basin, but south of the Phongolo River system, and is naturally confined to the closed estuaries and coastal reaches of rivers (Skelton 2001).

This study applied ecological niche models to predict the potential geographic distribution of Nile tilapia outside its native range, with a particular focus on river systems within southern Africa where it is now both established and spreading its range. Computational tools such as niche models have been shown to be useful in predicting potential geographic areas at risk of fish invasions elsewhere (Igushi et al. 2004, McNyset 2005; Zambrano et al. 2006; Chen et al. 2007; DeVaney 2009). Ecological niche models utilize associations between environmental variables and known species' occurrence localities to predict potential areas where a given species is likely to establish (Guisan and Thuiller 2005). Several approaches have been used to predict potential distributions (Guisan and Zimmermann 2000; Guisan and Thuiller 2005; Elith et al. 2006; Elith and Leathwick 2009). The varied array of available modelling techniques, however, makes it inherently difficult to choose an "ideal" modeling approach especially when comparisons among the different approaches have revealed that different techniques may perform better under certain situations (Elith et al. 2006; Guisan et al. 2007). Despite these inconsistent differences in model performances, novel techniques, such as maximum entropy

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models (Maxent), have been shown to consistently out-perform more established correlative approaches (Elith et al. 2006).

Recently, some authors have highlighted the limitations of niche models for forecasting the potential range of invasive species (Elith et al. 2006; Fitzpatrick et al. 2007). In particular, several studies have shown that niche models developed using native range occurrences may fail to predict the full extent of an invasion. This failure has often been attributed to changes in the niche of the invading species (Fitzpatrick and Hargrove 2008) and/or the choice of environmental variables used to train the models (Peterson and Nakazawa 2008; Rödder et al. 2009; Rödder and Lötters 2009, 2010).

Two critical assumptions in ecological niche modelling are that the range of a study species is in equilibrium within its environment (Araujo and Pearson 2005) and that its niche is conserved across space and time (Pearman et al. 2008). Recent studies have found mismatches between species' native and invasive range in terms of climatic niches (Broennimann et al. 2007; Fitzpatrick et al. 2007; Broennimann and Guisan 2008). Such apparent niche shifts during biological invasions violate the basic assumption behind the application of ecological niche models and weaken their reliability (Rödder and Lötters 2010). Evidence for niche shifts is, however, still equivocal, and the entire issue of niche conservatism is still under debate (Rödder and Lötters 2009; Peterson 2011).

The predictive ability of ecological niche models is correlated with the selection of variables used to build them (Peterson and Nakazawa 2008). Climatic conditions in different geographic regions may show variation in environmental parameters that can affect the natural fitness of the study species, i.e., range-restricting variables and also other variables that may have little or no influence (Rödder and Lötters 2009). It is, therefore, likely that different variable sets

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will incorporate different ecological niche space suitable for a species. It is considered prudent to assume that differences in the predictive abilities of models will occur if a species occupies a different niche space in its native and introduced range (Fitzpatrick et al. 2008).

In this paper, we evaluated the potential of Nile tilapia to extend and establish feral population in novel areas outside it natural distributional range. We specifically asked three research questions: 1) are there differences in the predictive power of models trained with different environmental datasets? 2) does Nile tilapia occupy the same environments in its native and introduced ranges? and 3) what are the major concerns and implications for the conservation of indigenous congenerics as a result of Nile tilapia invasions?

Methods

Environmental data sources

The selection of predictor variables influences the performance of ecological niche models (Peterson and Nakazawa 2008; Warren et al. 2010). To test if there are differences in the predictive power of models trained with different environmental datasets, we used two main types of predictor variables (Table 1). The first dataset comprised of proxy bioclimatic variables that have been widely used in ecological niche modeling of freshwater systems (Igushi et al. 2004; McNyset 2005; Zambrano et al. 2006; Chen et al. 2007). These variables represent annual trends (mean annual temperature and annual precipitation), seasonality (annual range in temperature and precipitation) and either extreme or limiting environmental factors (temperature of the coldest and warmest months and precipitation of the wet and dry quarters) and were obtained from the world climate database (WorldClim; Hijmans et al. 2005; http://www.worldclim.org). The second dataset comprised of annual predictions of mean

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monthly water temperature (maximum, median, minimum and range) that are based on air temperature estimates by the Centre for Resource and Environmental Studies (CRES), Australian National University, Australia and the Food and Agriculture Organization (FAO) of the United Nations (UN; Jenness et al. 2007). Additional data summarising aspects of topography (elevation, slope, aspect, flow accumulation, flow direction and topographic index) and main river systems in Africa were obtained from the United States Geological Survey's Hydro-1k dataset (http://edcdaac.usgs.gov/gtop30/hydro). Estimates of water temperature variables have previously been successfully applied (Kapetsky 1994) to identify areas where temperature might be a limiting factor to the aquaculture production of Nile tilapia and sharptooth catfish (*Clarias gariepinus*) in Africa. In addition, within each variable set, predictor variables were divided into four groups: 1) a comprehensive group comprising of the most predictor variables; 2) an extreme minimalistic set describing the availability of water and energy and lastly, groups (3-4) comprised of temperature and precipitation-related parameters, respectively (Table 1). Finally, in each group, variables were evaluated using correlation analysis to exclude variables that were highly correlated ($r > 0.8$).

Specimen data sources

Georeferenced occurrence data for Nile tilapia were obtained from various sources including, museum specimen records, biodiversity databases such as FishBase (http://www.fishbase.org), Global Biodiversity Information Facility (GBIF; http://www.gbif.org), the published literature, and fish survey data from various fisheries departments in southern African countries that included Botswana, Mozambique, Zambia and Zimbabwe. A combined total of 92 occurrence points were obtained for Nile tilapia in its native range and 81 localities in its introduced range

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(Fig. 1). We defined its native range as the area where it is known to occur naturally as defined by both Trewavas (1983) and Daget et al. (1991) (Fig 1.) All occurrence records outside the species natural distributional range were considered to be introductions and as such indicated the potential introduced range of the species. We assumed that the occurrence records were accurate and reliable because they are available in the public domain and as such are open to peer review and evaluation, and occurrence records from museum collections are typically recorded within a logical framework (i.e., with a reference voucher number with locality, collector, date and identifier).

Table 1. List of environmental variables used in the ecological niche modelling of Nile tilapia (*Oreochromis niloticus*) in Africa.

Group	Variable set	
	Bioclimatic variables	Water estimates
Comprehensive	BIO1 = Annual mean temperature	BIO12 = Annual Precipitation
	BIO2 = Mean monthly temperature range	BIO13 = Precipitation of Wettest Month
	BIO3 = Isothermality	BIO14 = Precipitation of Driest Month
	BIO4 = Temperature seasonality	BIO15 = Precipitation Seasonality
	BIO5 = Maximum temperature warmest month	BIO16 = Precipitation of Wettest Quarter
	BIO6 = Minimum temperature coldest month	BIO17 = Precipitation of Driest Quarter
	BIO7 = Temperature annual range	BIO18 = Precipitation of Warmest Quarter
	BIO8 = Mean temperature wettest month	BIO19 = Precipitation of Coldest Quarter
	BIO9 = Mean temperature driest month	Maximum Monthly Water Temperature (Annual)
	BIO12 = Annual Precipitation	Median Monthly Water Temperature (Annual)
	BIO13 = Precipitation of Wettest Month	Minimum Monthly Water Temperature (Annual)
	BIO14 = Precipitation of Driest Month	Range of Monthly Water Temperature (Annual)
	BIO15 = Precipitation Seasonality	Flow accumulation
	BIO18 = Precipitation of Warmest Quarter	Flow direction
	BIO19 = Precipitation of Coldest Quarter	Compound Topographic Index
Minimalistic	BIO1 = Annual mean temperature	BIO12 = Annual Precipitation
	BIO5 = Maximum temperature warmest month	BIO13 = Precipitation of Wettest Month
	BIO6 = Minimum temperature coldest month	BIO14 = Precipitation of Driest Month
	BIO12 = Annual Precipitation	Maximum Monthly Water Temperature (Annual)
	BIO13 = Precipitation of Wettest Month	Minimum Monthly Water Temperature (Annual)
	BIO14 = Precipitation of Driest Month	
Temperature	BIO1 = Annual mean temperature	Maximum Monthly Water Temperature (Annual)
	BIO2 = Mean monthly temperature range	Median Monthly Water Temperature (Annual)
	BIO3 = Isothermality	Minimum Monthly Water Temperature (Annual)
	BIO4 = Temperature seasonality	Range of Monthly Water Temperature (Annual)
	BIO5 = Maximum temperature warmest month	
	BIO6 = Minimum temperature coldest month	
	BIO7 = Temperature annual range	
Precipitation	BIO12 = Annual Precipitation	
	BIO13 = Precipitation of Wettest Month	
	BIO14 = Precipitation of Driest Month	
	BIO15 = Precipitation Seasonality	

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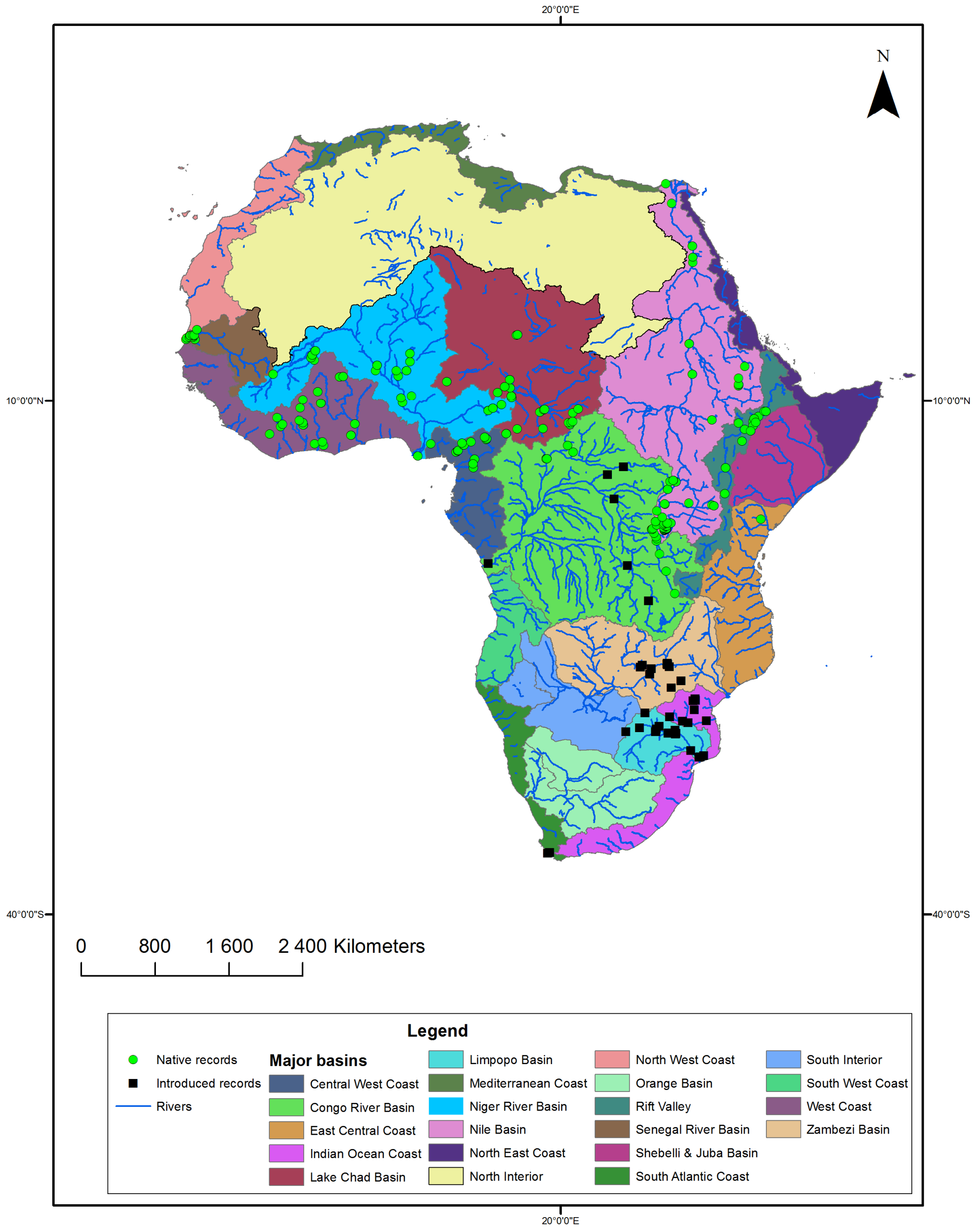

Figure 1. The major hydrological basins in Africa together with main rivers, georeferenced native occurrence records (●) and known introduction (■) records of Nile tilapia (*Oreochromis Niloticus*) outside its native range. (Source: African Water Resources Database (AWRD; Jenness et al. 2007; http://www.fao.org/geonetwork).

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Model building

Maxent uses occurrence records (representing species presence) and a set of background records in order to predict the potential distribution of a species. This requires the user to define the region from which the background records are drawn that can influence model performance (van der Wal et al 2009; Anderson and Raza 2010). We therefore performed an exploratory analysis to evaluate the relationship between the geographical extent from which the background records are drawn and how the model performed. In each case, models were trained with 10000 pseudo-absence points drawn from a background whose area was defined incrementally as distances from the occurrence points (25, 50, 75, 100, 150, 200 km). Model performance, as reflected by the Area Under the Curve (AUC) statistic, increased as background size expanded from 10 to 75 km but subsequent expansions resulted in only minor increases in AUC. Therefore, the background was drawn from a radius of 75 km from each known occurrence point. For all models, the algorithm's parameters were set to a maximum number of 500 iterations, a regularization multiplier of 1, autofeatures and a convergence threshold of 0.00001. The logistic output format was used for the suitability maps. Values range from 0 (indicating complete unsuitability) to 1 that indicates perfect suitability for a species within a particular grid cell.

We considered two approaches to develop ecological niche models to test if Nile tilapia occupied the same and/or different environments in its native and introduced ranges within African river systems. First, we constructed niche models for Nile tilapia within its native range that included the Nile River basin, Lake Chad, south-western Middle East and the Niger, Benue, Volta and Senegal Rivers, and Lake Tanganyika (Fig. 1). Ten native range predictions were made for each group of variables (see Table 1 for variable classification) and, in each model, all

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occurrence records were partitioned into a calibration set (training set) and a testing set (validation set) using *k*-fold partitioning (Phillips et al. 2006). The average model performance was obtained by repeating the process for 10 iterations such that each of the groups could be used for testing. A consensus map was then created as an average of the 10 native range projection maps for each group of environmental variables (Figs. 3-4). Second, we then projected the potential range of Nile tilapia onto novel river systems in Africa such as the Congo, Cunene, Zambezi, Okavango and Limpopo Rivers, and Lake Malawi. Ten predictions were made for each group of variables (see Table 1 for variable classification) and in each model, all occurrence records of known introductions were partitioned into a calibration set (training set) and a testing set (validation set) using the same *k*-fold method as done for the native range. A consensus map was also created as an average of the 10 introduced range projection maps for each group of environmental variables (Figs. 3-4).

Niche overlap, equivalence and similarity

We then analysed niche similarity between ecological niche models of Nile tilapia from its native and invaded ranges using measures of niche similarity using ENMTools (Warren et al. 2010). ENMTools quantifies niche similarity using two measures introduced by Warren et al. (2008): Schoener's index of niche breadth (*D*) and Hellinger's distance (*I*). Both *D* and *I* are quantitative measures of differences in habitat suitability between two potential distribution models and values range from 0 (indicating that niche models are completely different) to 1 (indicating that niche models are identical). The significance of the *D* and *I* values was then evaluated using null models of niche similarity and equivalence.

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For niche equivalence, we tested the hypothesis that ecological niche models produced for Nile tilapia populations within its native and introduced ranges were identical. Pseudo-replicate datasets were constructed by combining the georeferenced occurrence data points from both populations and randomising their identities. This process was repeated to generate 100 pseudo-replicate datasets. The number of replicates determines the statistical resolution of the test, with 100 replicates being adequate to generate a statistical resolution of 0.01 (Warren et al. 2008). An ecological niche model was then created for each pseudo-replicate to produce a null distribution of overlap scores between populations from the shared distribution. The actual observed measures of niche similarity were then compared with percentiles of these null distributions to test the hypothesis that ecological niche models from native and invasive records were not significantly different. The hypothesis was rejected when the empirically observed values of *D* and/or *I* were either lower or higher than values obtained from pseudo-replicate datasets, resulting in a Type 1 error (α) of 0.01.

For niche similarity, we tested the hypothesis that ecological niche models drawn from entirely non-overlapping Nile tilapia populations (native and introduced ranges) are any different from one another than expected by random chance given the underlying environmental difference between the two regions. This was conducted by comparing ecological niche models based on native records of Nile tilapia but trained on a background randomly drawn around known occurrence points in its introduced range (i.e., as opposed to using actual occurrence points). The random samples from the introduced range were drawn from a background whose area was defined as a radius of 75 km from each known occurrence point (see model building section for justification of background selection). The same process was repeated in the other direction by building ecological niche models based on known introduced records and trained on a randomly

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drawn background from its native range. This process was repeated in either direction (native ↔ introduced) to generate 100 pseudo-replicate datasets. The observed measures of niche similarity (D and I values) from the two original populations (native and introduced ranges) were then compared with percentiles of these null distributions. The hypothesis that niche similarity (or divergence) is different from that expected by chance between Nile tilapia populations in its native and introduced ranges based on the availability of habitats was rejected using the same randomisation test procedure as outlined for niche similarity.

Model evaluation

We evaluated the performance niche models of Nile tilapia from its native and invaded ranges trained with different environmental predictors using three different methods of model evaluation. These are:

1) **Maximum test AUC:** AUC defines the discrimination ability (between presence and background) of the models where values range from 0 (indicating random distribution) to 1.0 (indicating perfect prediction), with values > 0.5 being considered to indicate that the model discriminates better than random (Mantel et al. 2001). All AUC model performance measures were calculated in Maxent and predictions with an AUC value greater than 0.9 were considered to be acceptable (Swets 1988; Fielding and Bell 1997).
2) **Information criteria**: We also used the Bayesian Information Criteria (BIC) and small sample corrected Akaike Information Criteria (AIC_c; Burnham and Anderson 2002) model performance measures in ENMTools (Warren et al. 2010) for model selection. Ecological niche models were ranged according to their normalised relative likelihood values ($w_{i=}$ *Akaike weights*). The w_i was then interpreted as the probability that i was the

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best model given the data and set of candidate models. The confidence set of candidate models was taken as Akaike weights that are within 10% of the highest, which is the minimum cut-off point for evaluating strength evidence (Royall, 1997). Hence, as a result of the large difference in w_i between models in the native and introduced ranges, the comparisons among models was limited to each respective region and not across the two regions.

3) **Minimum difference between training and test data (AUC$_{\text{diff}}$):** Over-fit models generally perform well on training data than on test data and by minimising the difference between training and test data, we minimize the risk that our models are over-parameterised in such a way as to be overly specific to the training data (Warren and Seifert 2011).

Ecological risk

We defined the invasion risk of Nile tilapia on other indigenous congenerics as the product of the likelihood of Nile tilapia becoming successfully established in a given novel river system and the associated adverse ecological consequences (National Research Council 2002). The highest risk scenarios are likely to unfold when there is a high probability of Nile tilapia establishment in recipient river systems and the associated adverse ecological impacts are significant.

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Results

Comparison of bioclimatic variables

The variation in climatic variables between the native and introduced ranges of Nile tilapia are summarised in Fig. 2. The range of variation for climatic variables in the native range is relatively larger than observed values in the introduced range, and as a result most of the observed climatic conditions in the introduced range, with the exception of “range of monthly water temperature”, fall within the variation gradient of climatic variables in the native range. Model performance differed significantly with variable selection (seven variable sets and two predictor categories) used for model training (Repeated-measures multivariate analysis of variance (MANOVA): $F_{6,\ 108} = 4053.60$; $n = 140$; $P < 0.001$) (Figs. 3-4). The relative contribution of each variable in each set of environmental predictors is summarised in Table 2. In general irrespective of sampling region (native or introduced), the ‘comprehensive set’ recorded the best model performance for models trained with either bioclimatic variables (AUC = 0.958) and/or water estimates (AUC = 0.950). Models developed from the precipitation subset recorded the lowest model performance (AUC = 0.865). There were, however, no significant differences (repeated-measures MANOVA: $F_{1,\ 18}= 0.675$; $n = 120$; $P > 0.416$) in model performance between models trained with bioclimatic and water temperature estimates. Overall model performance for all variables set was significantly higher (repeated measures MANOVA: $F_{1,\ 18}= 1833.1$; $n = 140$; $P < 0.001$) in the introduced range compared to the native range for all variable sets (Table 3).

A similar pattern in model performance was evident using BIC and AICc where models trained with ‘comprehensive sets’ recorded the best model performance for both the native and introduced ranges (Table 4). Models trained on the remaining variable sets (minimalistic,

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temperature and precipitation) recorded low probabilities ($P < 0.03$) and were considered as poor predictions of Nile tilapia in its native and introduced ranges.

Niche overlap, equivalence and similarity

Realised niche overlap, equivalence and similarity tests between Nile tilapia's native and introduced ranges are summarised in Table 3. I ranged between 0.46 and 0.85, and D between 0.24 and 0.61. The variable set with the highest niche overlap was 'precipitation' ($I = 0.85$, $D = 0.61$) followed by 'minimalistic - bioclimatic' set ($I = 0.853$, $D = 0.606$). The lowest overlap was recorded for comprehensive datasets from bioclimatic ($I = 0.502$, $D = 0.242$) and water estimate variables ($I = 0.46$, $D = 0.22$). The null hypothesis of niche equivalence was rejected ($P < 0.05$) for all environmental data sets.

The niche similarity between invasive records compared with the native background revealed that environmental conditions described by 'precipitation', 'minimalistic - water estimates' and 'temperature-water estimates' were similar for both the D and I indices ($P < 0.01$) (Table 2). The 'temperature - bioclimatic' and 'comprehensive-water estimates' were not statistically significant ($P > 0.01$) for either D or I, while the variable 'minimalistic-bioclimatic' revealed no significant differences ($P > 0.01$) for D but was significantly different for I ($P < 0.01$) . Results based on the native records compared to the introduced background revealed that the variable sets 'comprehensive-bioclimatic', 'minimalistic-bioclimatic' and 'temperature-water estimates' were more different than expected by chance for both D and I measures ($P < 0.01$). Three variable sets - 'minimalistic-water estimates', 'comprehensive-water estimates' and 'temperature-bioclimatic' - were not significantly different ($P > 0.01$). The variable set

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'precipitation-bioclimatic' revealed no significant difference for *I* ($P > 0.01$) but was significantly different for *D* ($P < 0.01$).

A significant relationship was observed between niche overlap and model performance, with AUC values decreasing significantly with increasing *D* and *I* (Fig 5). In contrast, omission error decreased significantly ($P < 0.01$) with increasing *D* and *I* (Fig 6). For all variable sets, the training AUC was significantly higher than the test AUC in the introduced range while in the native range, the inverse was true (Fig 7).

Realised and potential distributional range

The realised distribution of the Nile tilapia within its native range covers most of north Africa (Nile River basin, Niger River basin, Lake Chad basin, Central West Coast basin, West Coast, and Senegal River basin) but is limited to the north by the Sahara desert, and extends south-wards to include most of the great lakes (Tanganyika, Albert, Edward and Kivu) (Figs. 3-4). The predicted potential geographical range of the Nile tilapia reveals a broad invasive potential over most of central and southern Africa. Its potential distributional range covers the entire basins of Zambezi and Limpopo Rivers and the continent's coastal rivers along the Indian Ocean. Nile tilapia is also predicted to potentially occur in the East Central Coast of Africa and the Shebelli and Juba Basins (Figs.3-4). The models, however, predicted low suitability for most of the Congo and Orange River basins and west-flowing rivers in the south Atlantic Coast of Africa.

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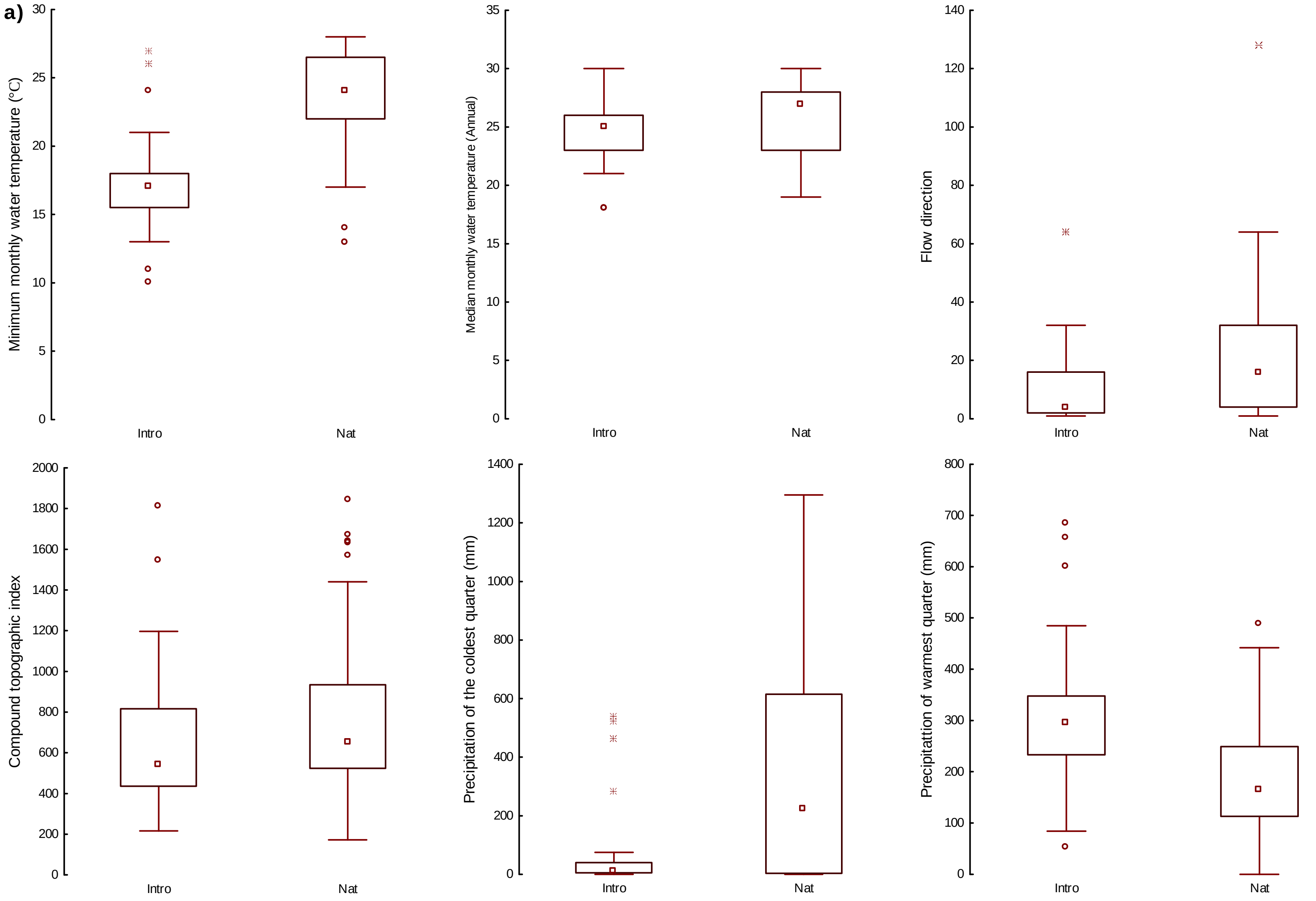

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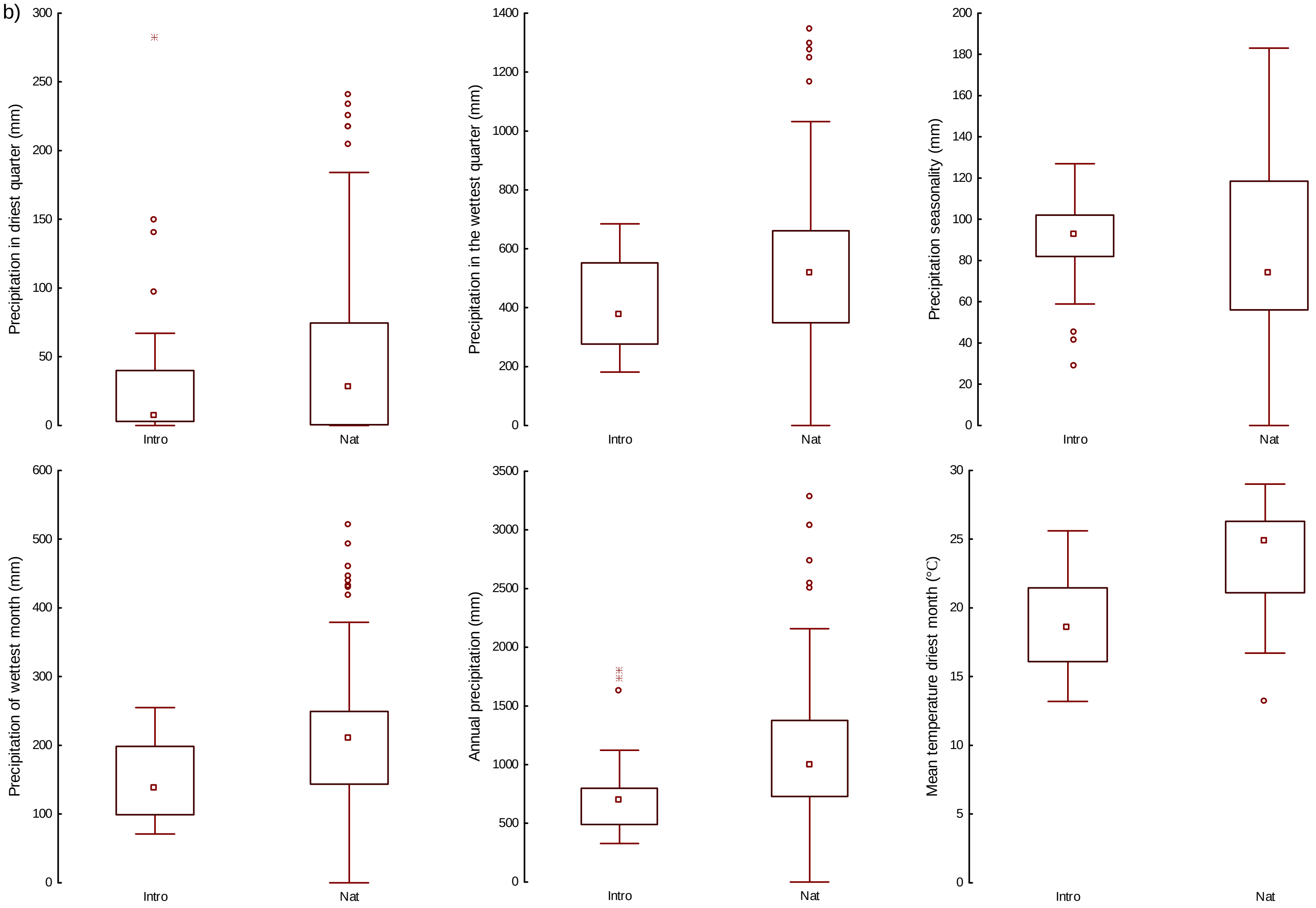

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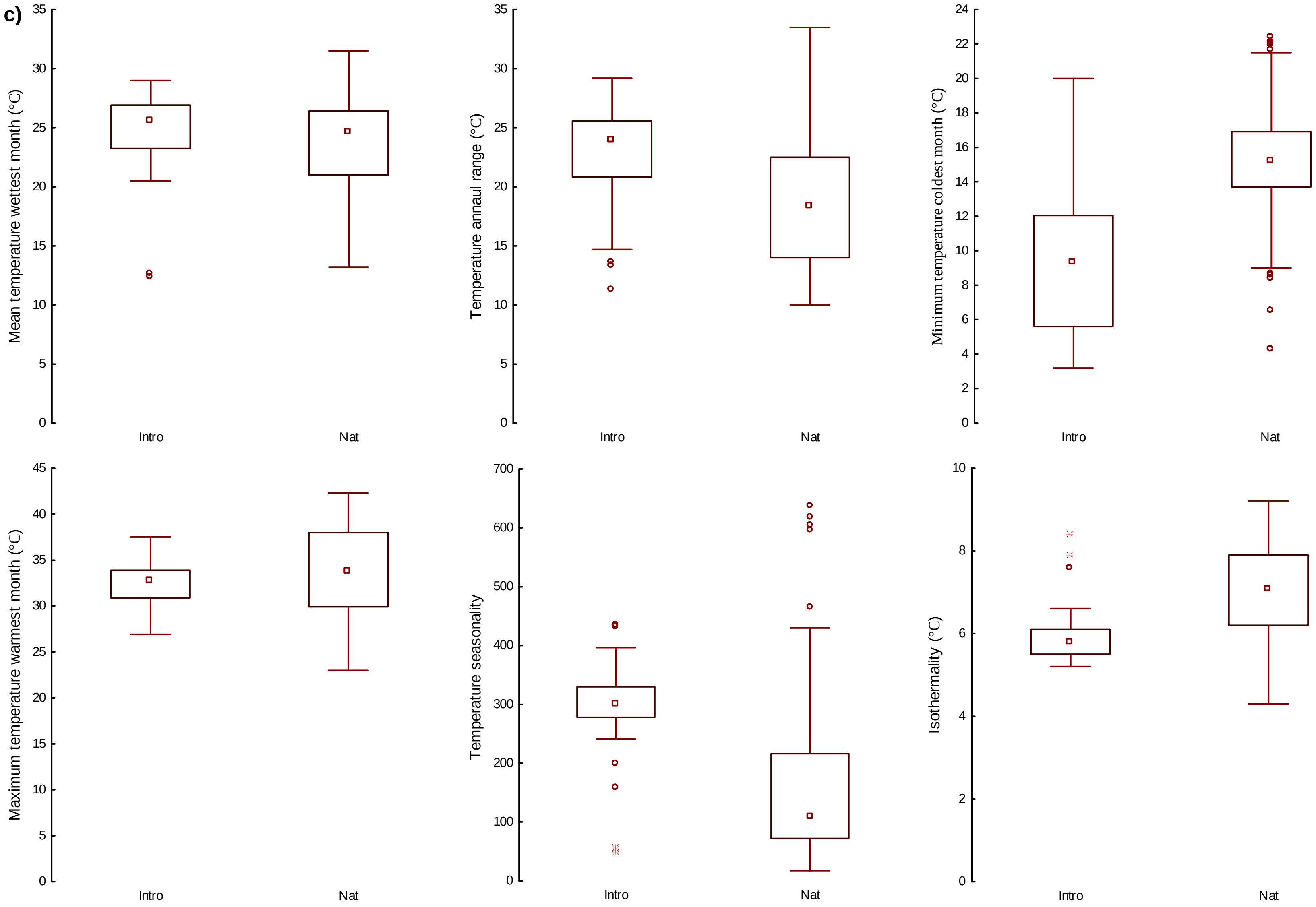

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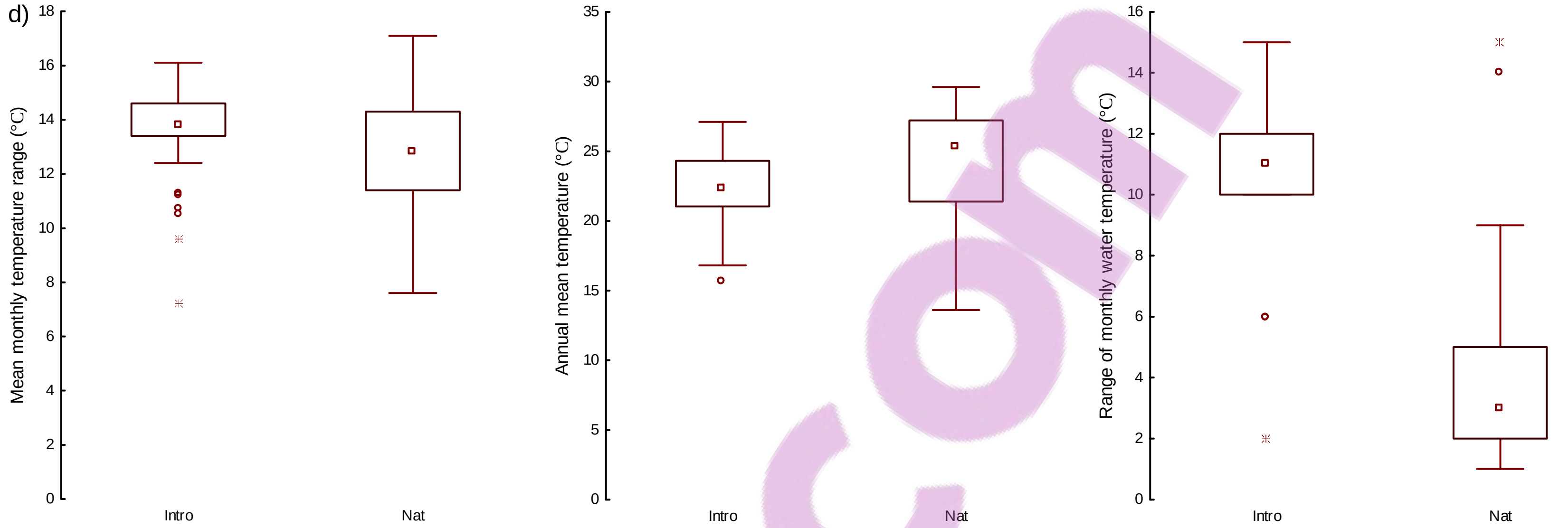

Figure 2. Variation in climatic variables extracted from known native and introduced occurrence records of Nile tilapia (*Oreochromis niloticus*) in African river systems. The box values represent the 1st and 3rd quartiles, the median is indicated by the central small box, whiskers indicate non-outlier range and the circles and crosses indicate the outlier range.

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Table 2. Environmental variable sets and the relative contribution (%) of each variable used to build ecological niche models of Nile tilapia (*Oreochromis niloticus*) in river systems within its native and introduced ranges in Africa. The variable which explained the most variation in model performance are shown in bold.

Environmental variables	**Native**						
	bioclimatic variables				water temperature estimates		
	comprehensive	minimalistic	temperature	precipitation	comprehensive	minimalistic	temperature
BIO1 = Annual Mean Temperature	0.3	5.7	4.0				
BIO2 = Mean Diurnal Range	1.5		5.7				
BIO3 = Isothermality	0		1.9				
BIO4 = Temperature seasonality	**22.2**		**39.0**				
BIO5 = Maximum temperature of warmest month	0.7	13.1	8.5				
BIO6 = Minimum temperature of coldest month	18.4	**51.3**	**33.6**				
BIO7 = Temperature annual range	2.4		7.0				
BIO8 = Mean temperature of wettest month	5						
BIO9 = Mean temperature of driest quarter	3.6						
BIO10 = Mean temperature of warmest quarter							
BIO11 = Mean temperature of coldest quarter							
BIO12 = Annual Precipitation	0.8	4.6		**18.1**	0.5	**34.1**	
BIO13 = Precipitation of Wettest Month	0.7	**20.5**		**55.0**	0.4	5.3	
BIO14 = Precipitation of Driest Month	0.7	4.9		7.8	0.6	4.6	
BIO15 = Precipitation Seasonality	4.5			**19.1**	2.7		
BIO16 = Precipitation of Wettest Quarter					1.8		
BIO17 = Precipitation of Driest Quarter					0.7		
BIO18 = Precipitation of Warmest Quarter	**23.1**				18.7		
BIO19 = Precipitation of Coldest Quarter	16.1				12.1		
Maximum Monthly Water Temperature					11.5	17.5	**24.4**
Median Monthly Water Temperature					2.4		5.0
Minimum Monthly Water Temperature					13.5	**38.5**	**22.6**
Range of Monthly Water Temperature					**30.1**		**47.9**
Flow accumulation					2.3		
Flow direction					1.0		
Compound Topographic Index					1.9		

Environmental variables	**Introduced**						
	bioclimatic variables				water temperature estimates		
	comprehensive	minimalistic	temperature	precipitation	comprehensive	minimalistic	temperature
BIO1 = Annual Mean Temperature	1.1	23	24.4				
BIO2 = Mean Diurnal Range	4.2		8.9				
BIO3 = Isothermality	0.1		0.2				
BIO4 = Temperature seasonality	**29.5**		**54.2**				
BIO5 = Maximum temperature of warmest month	0	0.9	11.6				
BIO6 = Minimum temperature of coldest month	0	**21.8**	0.3				
BIO7 = Temperature annual range	0.2		0.4				
BIO8 = Mean temperature of wettest month	6.7						
BIO9 = Mean temperature of driest quarter	7.5						
BIO10 = Mean temperature of warmest quarter							
BIO11 = Mean temperature of coldest quarter							
BIO12 = Annual Precipitation	2.7	**43.7**		**71.6**	5.9	3.5	
BIO13 = Precipitation of Wettest Month	3.2	6.7		14.4	1.6	10.7	
BIO14 = Precipitation of Driest Month	0.4	4.0		6.1	0.3	3.1	
BIO15 = Precipitation Seasonality	0.0			7.9	0.0		
BIO16 = Precipitation of Wettest Quarter					0.2		
BIO17 = Precipitation of Driest Quarter					2.3		
BIO18 = Precipitation of Warmest Quarter	**40.4**				**34.9**		
BIO19 = Precipitation of Coldest Quarter	3.9				3.8		
Maximum Monthly Water Temperature					3.5	29.1	**26.4**
Median Monthly Water Temperature					0.0		0.2
Minimum Monthly Water Temperature					4.7	**53.6**	4.5
Range of Monthly Water Temperature					**37.8**		**68.9**
Flow accumulation					0.3		
Flow direction					3.2		
Compound Topographic Index					1.4		

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Table 3. Model performances (AUC), niche overlap in terms of Schoener's index of niche breadth (*D*) and (b) Hellinger' distance (*I*) values and assessment of niche identity and equivalency tests for Nile tilapia (*Oreochromis niloticus*) in it native and introduced ranges in Africa in relation to different environmental datasets.

Variable set	Group	Model fit (AUC)		I				D			
		Native	Introduced	Overlap	Identity	Background		Overlap	Identity	Background	
						inv→nat	nat→inv			inv→nat	nat→inv
Bioclimatic variables	Comprehensive	0.948	0.959	0.502	0.00	0.00	0.00	0.242	0.00	0.00	0.00
	Minimalistic	0.877	0.924	0.708	0.00	0.92	0.00	0.438	0.00	0.00	0.00
	Temperature	0.876	0.926	0.683	0.00	0.02	0.06	0.377	0.00	0.26	0.84
	Precipitation	0.869	0.893	0.853	0.00	0.00	0.02	0.606	0.00	0.00	0.00
Water estimates	Comprehensive	0.942	0.953	0.459	0.00	0.52	0.52	0.224	0.00	0.06	0.25
	Minimalistic	0.886	0.929	0.590	0.00	0.00	0.03	0.338	0.00	0.00	0.04
	Temperature	0.894	0.897	0.621	0.00	0.00	0.00	0.316	0.00	0.00	0.00

Table 4. Model performances in terms of small-sample size corrected Akaike Information Criteria (AIC_c) and Bayesian Information Criteria (BIC) for Nile tilapia (*Oreochromis niloticus*) in its native and introduced ranges in relation to different environmental datasets.

Region	Variable set	Group	AICc	Δ_i AICc	W_i	BIC	Δ_i BIC	W_i
Introduced	Water estimates	Comprehensive	2667.25	0.00	1.00	2697.49	0.00	0.97
		Minimalistic	2678.68	11.42	0.00	2715.35	17.87	0.00
		Temperature	2767.37	100.12	0.00	2790.03	92.54	0.00
	Bioclimatic	Comprehensive	2688.78	21.52	0.00	2704.40	6.91	0.03
		Minimalistic	2720.89	53.64	0.00	2758.74	61.26	0.00
		Temperature	2752.97	85.71	0.00	2787.99	90.50	0.00
		Precipitation	2774.93	107.68	0.00	2801.67	104.18	0.00
Native	Water estimates	Comprehensive	15463.93	55.94	0.00	15851.73	66.55	0.00
		Minimalistic	16090.65	682.66	0.00	16302.10	516.92	0.00
		Temperature	15994.82	586.83	0.00	16184.68	399.50	0.00
	Bioclimatic	Comprehensive	15407.99	0.00	1.00	15785.18	0.00	1.00
		Minimalistic	16003.08	595.09	0.00	16249.37	464.19	0.00
		Temperature	15941.69	533.70	0.00	16198.66	413.48	0.00
		Precipitation	16242.60	834.61	0.00	16436.97	651.79	0.00

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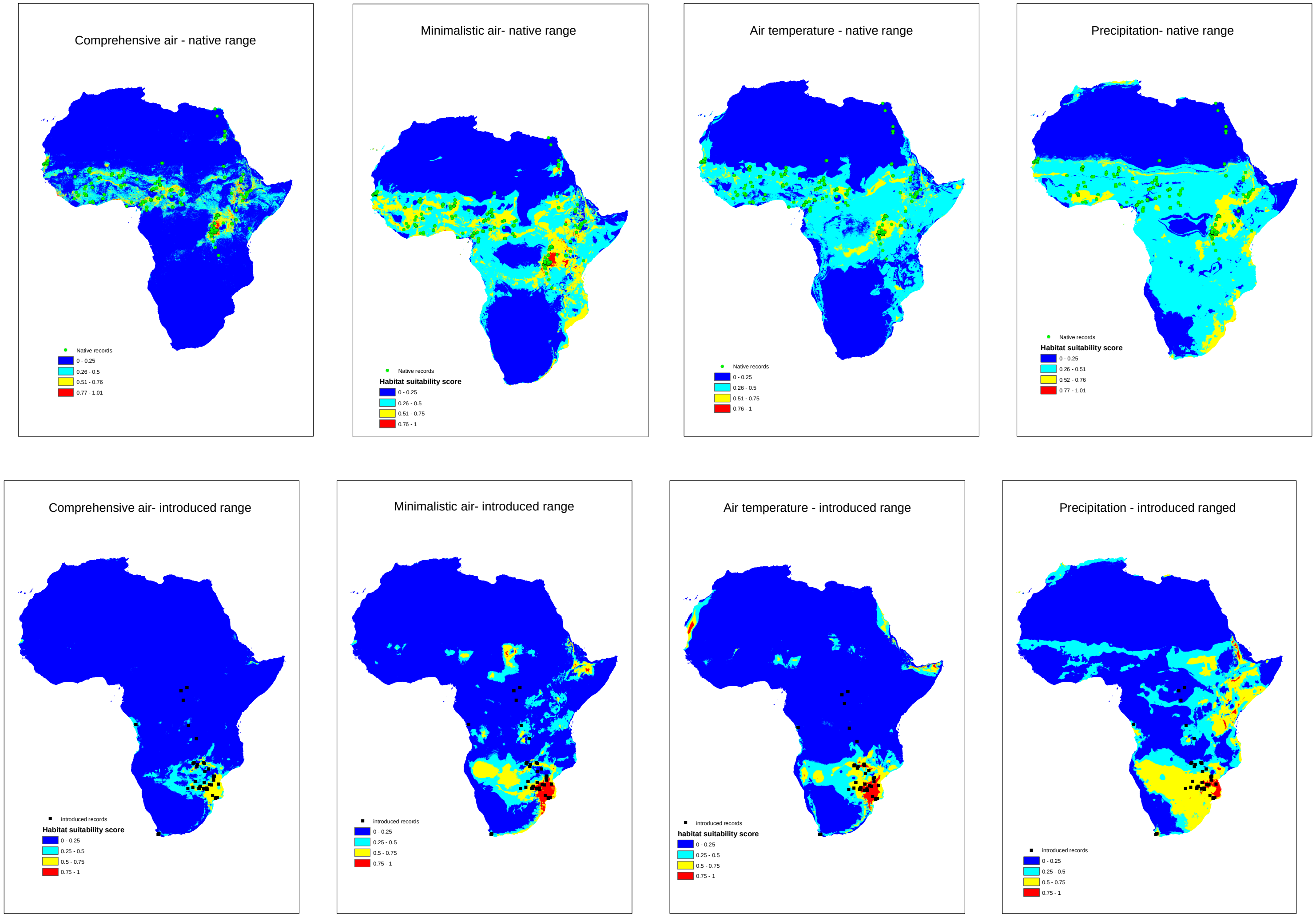

Figure 3. The projected distributional range of Nile tilapia (*Oreochromis niloticus*) in Africa based on ecological niche models trained with bioclimatic variables. Each map represents an average of 10 replicates for each group of variables and region (native and introduced) created using the *k*-fold partition method. Potential distribution is indicated by shaded areas, with red and blue indicating high and low probabilities of suitable conditions, respectively. Circles (●) indicate georeferenced native occurrence records and squares (■) indicate known introduction records.

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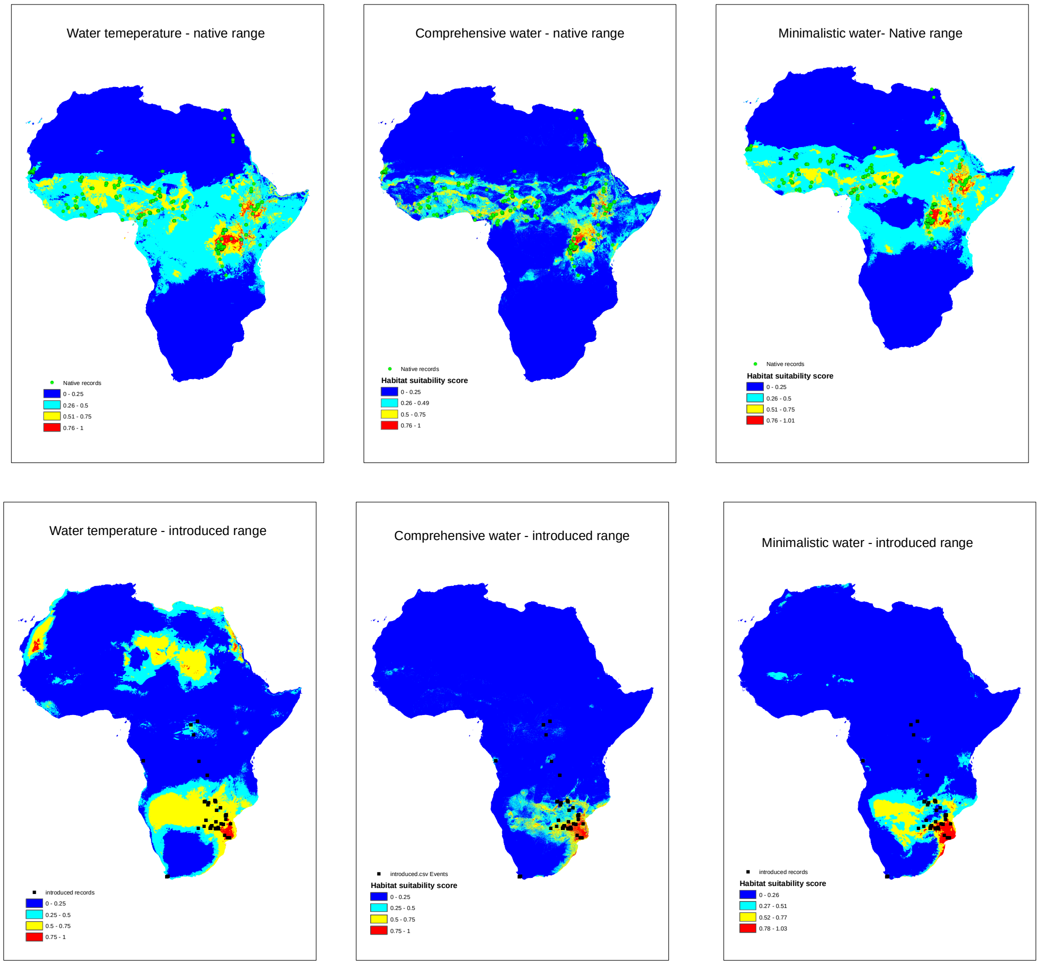

Figure 4. The projected distributional range of Nile tilapia (*Oreochromis niloticus*) in Africa based on ecological niche models trained with water temperature estimates. Each map represents an average of 10 replicates for each group of variables and region (native and introduced) created using the *k*-fold partition method. Potential distribution is indicated by shaded areas, with red and blue indicating high and low probabilities of suitable conditions, respectively. Circles (●) indicate georeferenced native occurrence records and squares (■) indicate known introduction records.

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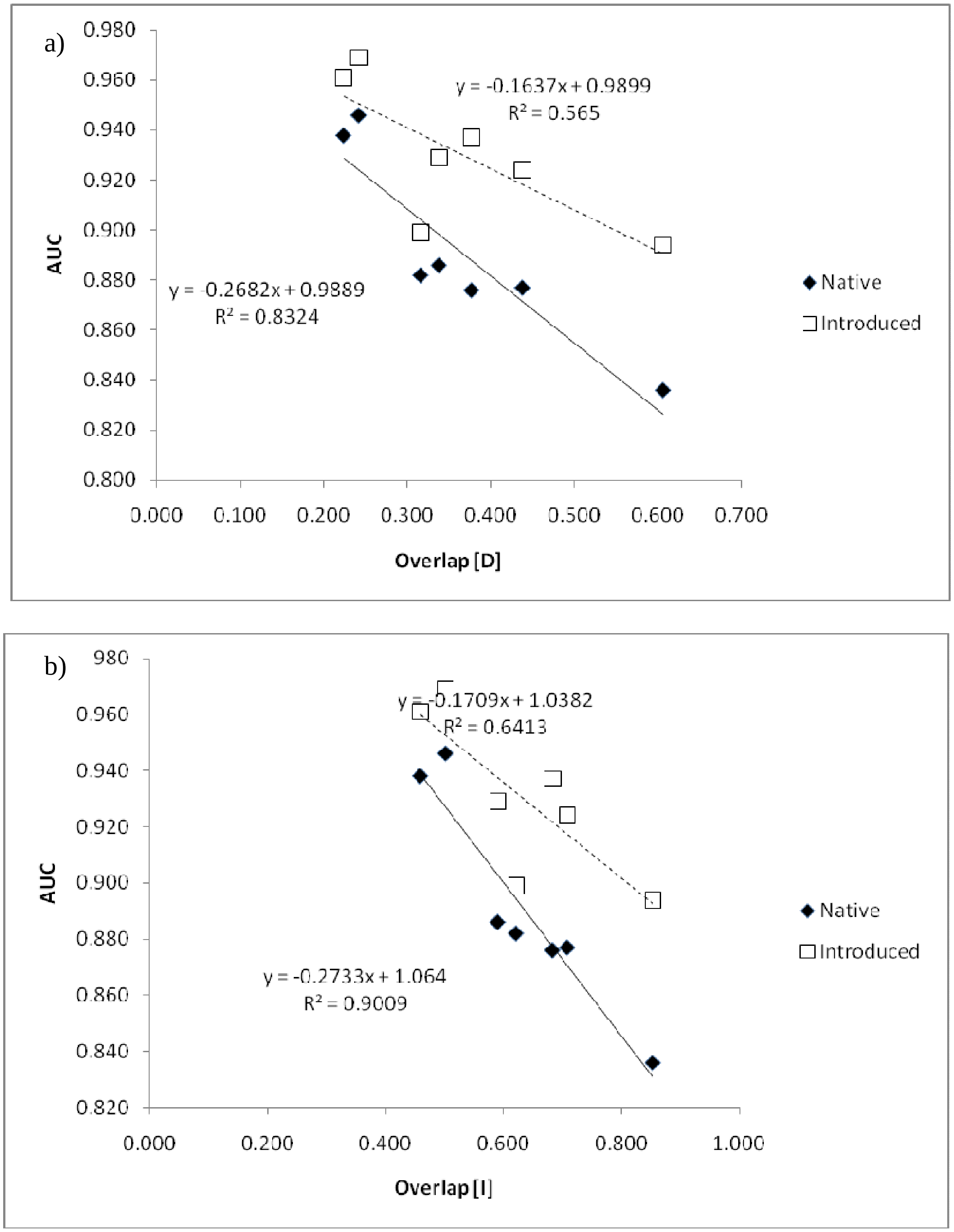

Figure 5. The relationship between niche overlap of native and introduced ranges of Nile tilapia (*Oreochromis niloticus*) in Africa in terms of (a) Schoener's index of niche breadth (*D*) and (b) Hellinger' distance (*I*) values with model performance (AUC). Diamonds (♦) indicate models trained with different variable sets and native occurrence records and squares (□) indicate models trained with different variable sets and known introduction records

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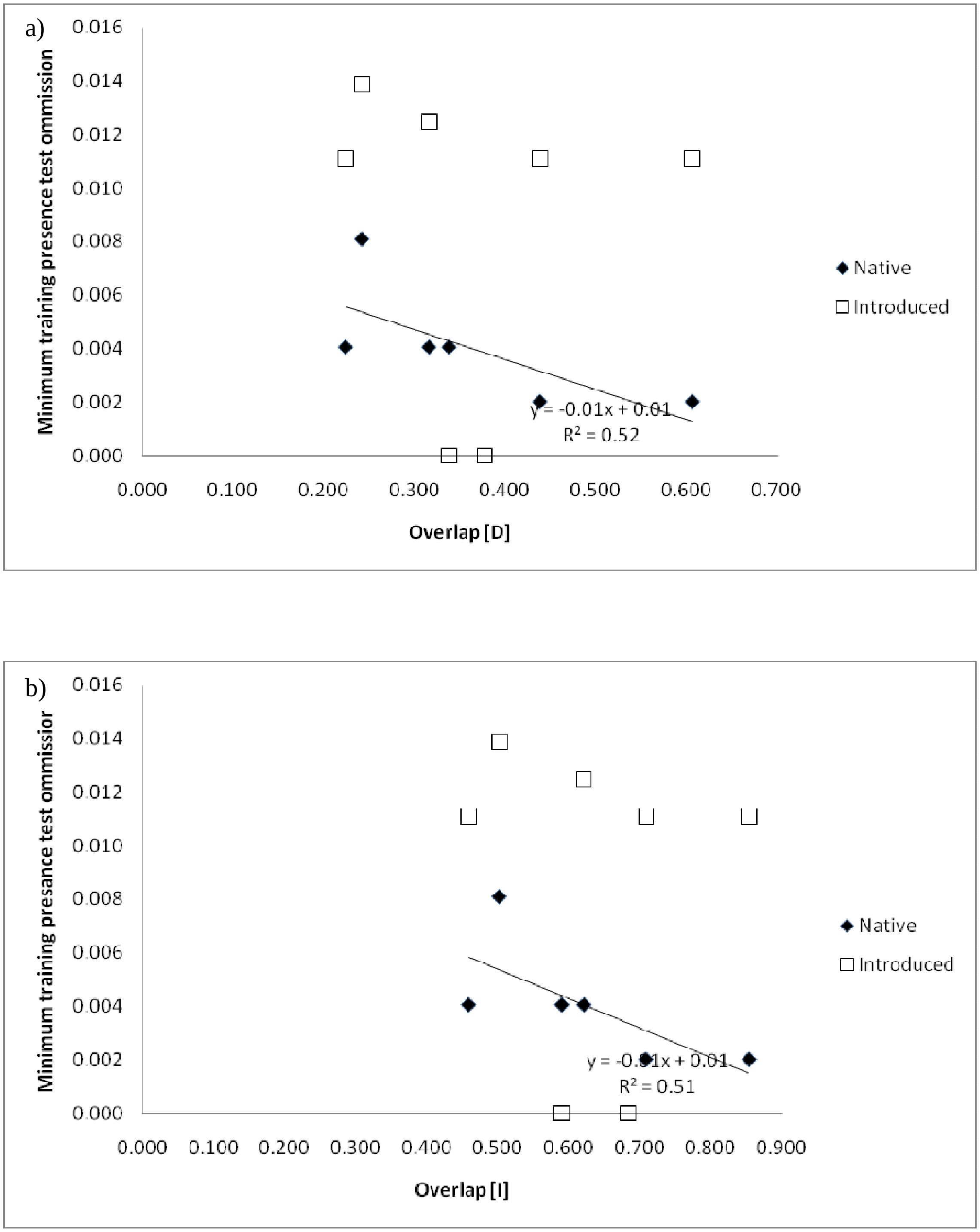

Figure 6. The relationship between niche overlap of native and introduced ranges of Nile tilapia (*Oreochromis niloticus*) in Africa in terms of (a) Schoener's index of niche breadth (*D*) and (b) Hellinger' distance (*I*) values with minimum training presence omission errors. Diamonds (♦) indicate models trained with different variable sets and native occurrence records and squares (□) indicate models trained with different variable sets and known introduction records.

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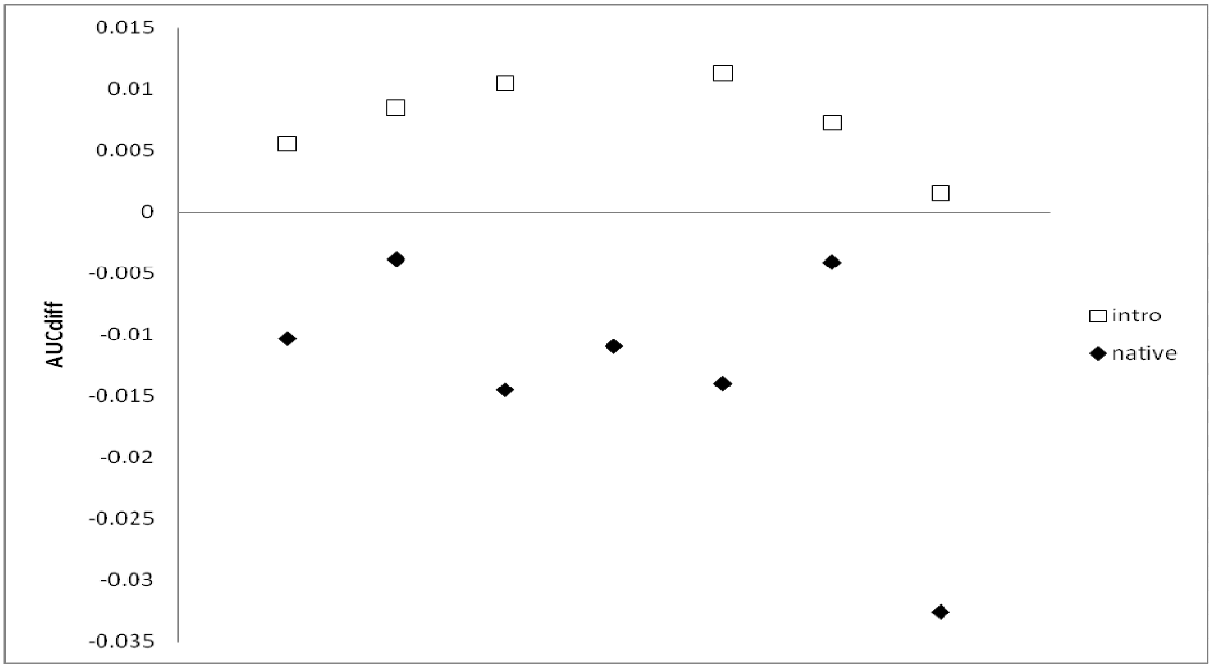

Figure 7. The minimum difference between training and test (AUC$_{diff}$) for ecological niche models of Nile Tilapia (*Oreochromis niloticus*) in its native and introduced ranges in relation to different environmental datasets. Diamonds (♦) indicate models trained with different variable sets and native occurrence records and squares (□) indicate models trained with different variable sets and known introduction records.

Discussion

Comparison of bioclimatic variables

The two major limitations for the application of ecological niche models in African freshwater systems is the lack of aquatic environmental data such as water quality variables, habitat availability and quality, and the lack of an up-to-date, accurate and easily accessible species occurrences records (McNyset 2005; Zambrano et al. 2006). To circumvent the lack of aquatic environmental data, we used proxy bioclimatic variables that have been successfully applied in previous studies in freshwater systems elsewhere (e.g., Igushi et al. 2004; McNyset 2005; Zambrano et al. 2006; Chen et al. 2007). We found no significant difference in the performance of models trained with proxy atmospheric variables relative to water temperature estimates as water temperature estimates are derived from air temperature bioclimatic variables (Jenness et al. 2007).

Recent studies have criticised the use of the AUC statistic to evaluate potential distribution of species despite its wide acceptance as a model evaluation tool (Lobo et al. 2007, Peterson et al. 2008; Jiménez-Valverde et al. 2011). In brief, the major limitations of AUC are that: 1) it ignores the goodness of fit of the models by assuming equal costs for commission and omission errors; 2) it is spatially independent, i.e., there is no information on the spatially distribution of errors; and 3) it is sensitive and positively related to the spatial extent of the study area.

These caveats are especially true when dealing with invasive species, particularly when evaluating models performance that are trained with different variable sets and in different geographic regions as this often leads to equivocal conclusions (Peterson 2011). For example, contrary to our findings, other comparable studies evaluating the potential distribution of invasive species, have recorded best model performance with minimalistic datasets relative more comprehensive variable sets (Rödder and Lötters 2009, 2010). This disparity in model performance may reflect the practical limitations of AUC as a measure of model performance for invasive species. In this study, this can be illustrated by examining the significant relationship between model performance and niche overlap. First, models trained with large numbers of variables recorded the highest AUC (indicating better model performance) but low niche overlap (indicating large differences in habitat suitability between the native and introduced ranges). This is likely to be a result of the inclusion of relaxed variables (i.e., variables that show large differences between the native and introduced ranges) leading to highly conserved models that predicted large areas of the background without known occurrence points as absences/unsuitable. The AUC statistic is known to be sensitive to the relative ratio of suitable to unsuitable habitat, and tends to increase dramatically when models are projected into an area that contains large areas of unsuitable habitat (Lobo et al. 2007). In contrast, minimalistic variable sets consisted of

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conserved variables (i.e., variables with the least difference between the native and introduced ranges) which produced models that predicted larger areas of the background as suitable relative to the comprehensive set. The AUC statistic, therefore, penalised these models trained with minimalistic variables for predicting areas as being suitable where there are no occurrences records, hence the low AUC values.

Second, we found significantly higher model performance in the introduced range than the native range for all variable sets. The low AUC values in the native range do not necessarily indicate that the models are poor but rather due to a methodological artefact. The training AUC was always higher than the test AUC in the introduced range while in the native range the inverse was true. Such a difference indicates that models in the introduced range are over-fitting in such a way as to be overly specific to the training data (Warren and Seifert 2011). In this study, the amount of habitat that is predicted to be suitable from introduced occurrence points is, therefore, less than that the predicted suitable habitats using native range points. It is possible that the observed differences in the distribution of suitable habitats and the occurrence of Nile tilapia populations both in its native and introduced ranges may be a result of differences in sampling effort between the two respective populations.

In southern Africa, Nile tilapia is still spreading and as a result the known occurrence records are unlikely to represent the whole range of environmental conditions that the species is capable of establishing itself (a major assumption of ecological niche models). In southern Africa, most occurrence data records are limited to monitoring surveys conducted by various national fisheries departments. These are generally limited in scope and only include major rivers and reservoirs with viable artisanal and commercial fisheries such as the Kafue River and lakes Kariba and Chicamba. Nile tilapia has been extensively propagated by farmers and anglers for recreational and sport fishing into small and medium reservoirs around the sub-

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region, often circumventing permitting processes. As a consequence, these introductions are not usually documented and monitored. This paucity of information inherently makes it difficult to ascertain exactly those areas where Nile tilapia has been introduced and to predict those areas where it is likely to spread to. While we consulted widely and managed to source georeferenced occurrence data for Nile tilapia from museum specimen records, biodiversity databases, the published literature, and fish survey data, only 173 occurrence points were obtained for the species (92 in its native range and 81 points in its introduced range) and we assume that these data represent the most up-to-date compilation of known occurrences of Nile tilapia within the African continent.

Given the limitations of the AUC, which models should be considered to be the most informative models? Although the comprehensive datasets have the highest AUC, they appear to be overly conservative. Final model-selection should be based on biologically meaningful and more conserved variables to avoid over-restriction (Rödder and Lötters 2009). In our view, we therefore, consider the models trained with the minimalistic dataset to be the most informative as it describes biologically relevant parameters such as the availability of water and thermal energy. In the minimalistic dataset, the variable which explained the most variation in model performance was minimum water temperature where the probability of Nile tilapia establishment was very low at temperature below < 20°C but increased exponentially with temperature up to maximum of ≥ 30°C. The areas that are highly suitable for the establishment of Nile tilapia were, therefore, mainly associated with a minimum monthly temperature of ≥ 20 °C. Nile tilapia is a eurythermal species which can tolerate a wide range of temperatures (8–42 °C) with a preferred optimal temperature range between 31 °C and 36 °C (Philippart and Ruwet 1982). However, the natural fitness of *Nile tilapia* in terms of respiration, feeding, growth and reproduction is reduced at sub-optimal

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temperatures below 20 °C (Amoudi et al. 1996; Ross 2000; Atwood et al. 2003; Charo-Karisa et al. 2005).

Annual precipitation influences the availability of surface water, habitats and food resources for aquatic fauna (Low-McConnell 2000) and, as such, indirectly influences the successful establishment of aquatic invasive species. It is therefore, not surprising that the geographic extent (realised distribution) of Nile tilapia within African river systems is limited by regions of moderate to low rainfall and extreme temperature ranges such as the Sahara desert in the north (native range) and the Kalahari and Namib deserts in the south (introduced range). Several approaches have been used to assess the accuracy of ecological niche models (see Liu et al. 2011 for a recent review). The varied array of available modelling techniques, however, makes it inherently difficult to choose an "ideal" model evaluation approach to use especially when comparisons among the different approaches have revealed that different techniques may perform better under certain situations (Elith and Leathwick 2009). The information criterion was included in this study for comparison purposes. Warren and Seifert (2011) found that AICc and BIC perform better at identifying models that are appropriately complex than AUC, as AUC is prone to over-parameterisation. In this study however, the information criterion appears to be subject to the same problems with the spatial extent of the study area as AUC, as both parameters produced identical model performance assessments. To reduce such discrepancy in the use of AUC when comparing models with different background sizes, Hijmans (2011) recently suggested a novel method for the removal of spatial sorting bias. Its application in ecological niche modelling still needs to be assessed further and its successful application will be of major interest as it is likely to change conclusions of previous studies. Furthermore, in order to improve the commonly used model evaluation metrics, Liu et al. (2011) recently proposed that for each model evaluation test

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statistic, a measure of its precision should be provided in the form of standard error and confidence intervals in order to statistically compare its accuracy between alternate models.

Niche conservation versus niche shift

Consistent with other studies comparing the distribution of invasive species in their native and introduced ranges (see Broennimann et al. 2007, Fitzpratick et al. 2007, Rödder and Lötters 2009; Medley 2010), we found that the degree of niche conservatism of Nile tilapia between the native and introduced ranges varies with the variable datasets used. Peterson (2011) recently argued that until empirical evidence of niche shifts is robust to alternative means of analysis, biological inferences about species niche conservatism should be made with caution. Nevertheless, the identity and niche similarity tests reveal that the two populations of Nile tilapia from its native and introduced ranges are not drawn from identical distributions of environmental variables. This indicates that the spatial distribution of suitable and unsuitable environmental variables vary among the native and invasive ranges. This is expected as the two populations are allopatric and there are a very few disjunct areas in the world that have sufficiently similar climates to produce a signal of niche equivalency (Warren et al. 2008). Nile Tilapia is extremely hardy, with a wide range of trophic and ecological adaptations, and adaptive life history characteristics (Welcomme 1988; Getabu 1994; Balirwa 1998; Njiru et al. 2004). These adaptive life history characteristics predisposes it to be a highly successful invader, and may partly explain among other factors its successful establishment in novel river systems in southern African as demonstrated in this study.

Conservation implications

The predicted potential geographical range of Nile tilapia within southern Africa overlaps with the complete native ranges of all six endemic congenerics. From previous evidence,

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these native congenerics are likely to become extirpated from the river systems through either competitive exclusion and/or hybridisation (de Vos 1990; Twongo 1995; Canonico et al. 2005; D'Amato et al. 2007; Zengeya and Marshall 2007). Of additional concern are those areas that have been free of exotic species but are now vulnerable due to the introduction of fish mainly for aquaculture and sport fishing. The first area of concern is the upper Zambezi and Okavango River systems. The upper Zambezi River is an ecologically diverse "reservoir river" with varied and extensive habitats (Jackson 1986). As a result, the area has the highest fish diversity within the Zambezian River system (Skelton 1994). The Upper Zambezi and the Okavango River systems are regarded as pristine areas where minimal fish introductions have occurred (Bills and Marshall 2004) but the ardent promotion of small-scale aquaculture as a means of poverty alleviation and livelihoods has put such river systems at serious invasion risk (Tweddle 2010). In Zambia, aquaculture projects rearing Nile tilapia have been keenly promoted within the Zambezi River system, and the inevitable fish escaping from such facilities, has led to the establishment of feral populations in river systems such as the Kafue River (Schwank 1995). The distributional range of Nile tilapia has also spread to include tributaries of the Upper Kapombo River and it is highly likely that it will reach and further spread within the upper Zambezi River, where three-spot and greenhead tilapia are at risk of being extirpated (Tweddle 2010).

Further downstream, within the middle Zambezi River, Nile tilapia is already established and appears to be displacing the indigenous Kariba tilapia, *O. mortimeri*. In Lake Kariba, Nile tilapia appeared in the mid-1990s after escaping from *in situ* cage-culture fish farms and has become abundant at the expense of Kariba tilapia that has declined significantly in abundance (Chifamba 1998; Marshall 2006). As a result, Kariba tilapia is now listed as Critically Endangered (CR) on the IUCN Red List of threatened species (Marshall and Tweddle 2007). In Zimbabwe, Nile tilapia has been extensively propagated by

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farmers and anglers for recreational and sport fishing. It is now prevalent in most catchments where it has supplanted indigenous tilapiines in most medium- to small-sized dams (Marshall 2000). In Lake Chivero, a medium-sized reservoir on the Upper Manyame River, a sub-catchment of the middle Zambezi, Nile tilapia has displaced greenhead tilapia and is now the dominant commercial species harvested (Zengeya and Marshall 2007). Further south in the Zimbabwean Lowveld, Nile tilapia is known to attain *ca.* 6 kg in Inyankuni, Mayfair and other smaller dams (J.L. Minshull, former Curator of Ichthyology, Natural History Museum of Zimbabwe, Bulawayo, personal communication). The ability of Nile tilapia to attain such a large body size could partially explain its success in displacing indigenous congenerics, such as Kariba tilapia. Nile tilapia is an aggressive competitor and large males often out-compete other congenerics for limited spawning and nursing grounds (Lowe-McConnell 2000).

The second area of concern comprises the Lower Zambezi River and other eastern river systems such as the Buzi, Save-Runde and the Limpopo River systems where it is now established and spreading. It poses a threat to the other native tilapia species. For example, Mozambique tilapia is listed as Near-Threatened (NT) on the IUCN Red List of threatened species (Cambray and Swartz 2007). Feral populations of Nile tilapia are already established in the Runde-Save, Buzi and Limpopo River systems where adverse ecological impacts such as reduced abundance of indigenous species and hybridisation have already been documented (D' Amato et al. 2007; Tweddle and Wise 2007; Weyl 2008).

The third area of particular concern is the Lake Malawi system which comprises of Lake Malawi, its affluents and the Shire River down to Kapachira Falls. Both the endemic chambo (*O. shiranus*, *O. squamipinnis*, *O. lidole*, and *O. karongae*) and the haplochromine complexes are therefore, at risk (Tweddle and Wise 2007). This has been noted in another Rift Valley lake, Lake Victoria. It is a comparatively similar system to the Lake Malawi system and the introduced Nile tilapia displaced the native *O. variabilis* and *O. esculentus*

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(Witte and Van Densen 1995; Cowx et al. 2003). Other areas of concern include pristine regions that have experienced little or no fish introductions due to civil strife. These include Angola, Mozambique and the Democratic Republic of the Congo (DRC). With the end of these conflicts, it is anticipated that increased development in the form of aquaculture projects may expose novel river systems to serious risk of invasion (Tweddle and Wise 2007). In Angola, potential river catchments for aquaculture development such as the Cuito, Cubango and Cunene Rivers were also predicted as potentially suitable systems for the establishment of Nile tilapia.

Despite the well-documented adverse ecological effects of Nile tilapia on recipient river systems (see Canonico 2005 and references therein), it is among one of the most widely cultured species in aquaculture and stock enhancements (Suresh 2003). Aquaculture is perceived as a means of protein security, poverty alleviation and economic development in many developing countries (NEPAD 2005). As such, decisions on exotic fish introductions are usually based on the trade-off between socio-economic benefits and potential adverse ecological effects (Cowx 1999). In most invaded systems, Nile tilapia has had a pronounced impact on fisheries in terms of increased food production and poverty alleviation by creating alternative aquaculture and fisheries livelihoods (Wise et al. 2007). Interestingly, the establishment of Nile tilapia in novel systems has not led to a decrease in overall yields, but rather a replacement of indigenous species (Ogutu–Ohwayo 1991; Twongo 1995; Balirwa et al. 2003; Shipton et al. 2008; Weyl 2008). In some cases, Nile tilapia has supplanted desirable species from the fishery setups such as in Lake Victoria where Nile tilapia is often regarded as being of inferior quality in comparison to the various haplochromines that it supplanted and therefore, commands lower market prices (Wise et al. 2007).

The impact of Nile tilapia on recipient novel river systems highlights problems associated with fish introductions for aquaculture. As such, the precautionary approach to

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capture fisheries and species introductions should be applied when deciding where and what species should be used for aquaculture. In principle, the use of exotic species for aquaculture often leads to inevitable escapes into the wild, and as such, introductions into aquaculture facilities (e.g., ponds and cages) should be considered as direct introductions into adjacent river systems. Therefore, in southern Africa, Nile tilapia should be restricted to catchments where it has already established and prohibited in pristine areas that are still free of invasion (Bills and Marshall 2004; Weyl 2008). In addition, and if possible, potential point sources of Nile tilapia should be eradicated in non-invaded river systems (Weyl 2008). Alternatively, the use of indigenous species should be promoted and enhanced production can be achieved through stock enhancement (e.g., selective breeding) and better farming methods (Bills and Marshall 2004). Experimental breeding trials using greenhead tilapia in Zambia (Gopalakrishnan 1998) and Mozambique tilapia in South Africa (Brink et al. 2002) have identified respective strains with superior growth characteristics with the potential to become superior stocks for aquaculture. It should, however, be noted that the alternative species should also not be introduced to novel river systems outside their native range as they would possibly pose the same invasion problems as Nile tilapia.

River systems traverse political boundaries and are often shared among riparian countries. As a result, the activities within one section of a catchment may have direct effects in other countries (Thys van den Audenaerde 1994). It is, therefore, crucial to formulate and implement a common regional (e.g., Southern African Development Community (SADC)) policy on the use and movement of exotic species among riparian states within the region). There is also a need to implement regular monitoring programmes in most river catchments and also to educate farmers and anglers about the ecological impacts that invasive species such as Nile tilapia have on indigenous congenerics. The highly significant prediction of the occurrence of Nile tilapia in novel fresh water systems in southern Africa suggests that niche

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models can be used to assess potential threats before an introduced species becomes invasive and as such preventive measures can be implemented. We recognize that in most tropical rivers the actual impact of introduced species is generally difficult to ascertain because data on the community structure and functioning prior to the introductions are often lacking. Despite this, the well-documented success of Nile tilapia in invading novel tropical river systems worldwide and associated adverse effects (see Canonico 2005 and references therein) does, however, provide strong circumstantial evidence to support the hypothesis of increased extirpation rates and hybridisation risk to indigenous congenerics in recipient river systems as a result of Nile tilapia invasions. We therefore, advocate that in areas that are predicted to be highly suitable for the establishment of Nile tilapia, the precautionary approach to capture fisheries and species introductions should be applied when deciding where and what species should be used for aquaculture.

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Chapter 5

A qualitative ecological risk assessment of the invasive Nile tilapia, *Oreochromis niloticus* in a sub-tropical African river system (Limpopo River, South Africa)[4]

Tsungai A. Zengeya • Mark P. Robertson • Anthony J. Booth • Christian T. Chimimba

Abstract

1. This study outlines the development of a qualitative risk assessment method and its application as a screening tool for determining the risk of establishment and spread of the invasive Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), within the central sub-catchment of the Limpopo River basin in northern South Africa.
2. The assessment utilised known physiological tolerance limits of *O. niloticus* in relation to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern to identify river systems that would be suitable for *O. niloticus* establishment.
3. River sections along the Limpopo main river channel and the immediate reaches of its associated tributaries east of the Limpopo/Lephalala river confluence along the Botswana-South Africa-Zimbabwe border were identified as being highly vulnerable to *O. niloticus* invasion. Rivers in the upper Bushveld catchment (Upper Limpopo, Mogalakwena, Lephalala, Mokolo, Matlabas and Crocodile rivers) were categorised as of medium ecological risk, while headwater streams were considered to be of low ecological risk. The decrease in vulnerability between lowveld and highveld river sections was mainly a function of low water temperatures (8-12° C) associated with increasing altitude.
4. *Oreochromis niloticus* is already established in the lower catchment of the Limpopo River basin where indigenous congenerics are at an extinction risk through hybridization and competition exclusion. *Oreochromis niloticus*, therefore, poses an ecologically unacceptable risk to novel river systems in the upper catchment where it is yet to establish. The current risk assessment model provides a useful preliminary logistic framework for the identification of river systems that are vulnerable to an *O. niloticus* invasion where conservation measures

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should be directed and implemented to prevent its introduction and spread within the Limpopo river system.

Key words: Risk assessment, Invasion, Indigenous *Oreochromis*, Nile tilapia, Southern Africa

Introduction

The adverse ecological impacts associated with fish introductions on recipient freshwater ecosystems worldwide have drawn attention to the need to control and manage the movement of invasive species (Sala et al. 2000; Cambray 2003; Njiru et al. 2005; Pimentel et al. 2005). This has become especially important with the advent of increased global trade, transport and tourism that have afforded an opportunity for organisms to spread beyond their natural ranges (Copp et al. 2005; Gozlan et al. 2010). In response to this threat, most countries have implemented legislation prohibiting new introductions and some have developed adaptive management strategies to identify and minimise the impact of invasive species (Kolar 2004; Vander Zanden and Olden 2008). Prevention is the major tenet behind most invasive species management protocols as it is often much easier and significantly less costly especially for invasive aquatic species that are practically impossible to eradicate once established (Simberloff 2003; Lockwood et al. 2007).

Ecological risk assessments have been widely used as a screening tool to identify potential invasive species and to assess the risk of adverse ecological impacts associated with a given species establishment and spread to ecosystem structure and functioning (National Research Council 2002). An ecological risk assessment for invasive species consists of two main components: risk identification and risk management (Anderson et al. 2004; Webb 2006). Risk identification is a process that evaluates the likelihood that adverse ecological effects may either occur or are occurring to indigenous congenerics as a result of exposure to

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introduced species. Risk of invasion is identified by either deductive and/or correlative methods. Deductive approaches utilise life history traits and environmental tolerances of an organism to evaluate the likelihood that a species will transit all the invasion stages (initial dispersal, establishment, spread and impact) (Lockwood et al. 2007). For example, Schleier et al. (2008) developed a risk assessment based on habitat suitability (minimum water temperature, indigenous fish species of concern and the presence or absence of dams) to identify river systems in Montana (USA) watersheds that would be suitable for the establishment of the introduced mosquito fish *Gambusia affinis*. The major advantages of using such an approach to screen invasive species is that it is applicable to a variety of ecosystems and is easy to implement, modify and improve on as new data become available. It also highlights areas for future research by identifying areas of uncertainty within the model. The disadvantages associated with these deductive methods are that model development is data-intensive, there is limited transferability of model predictions (i.e., predictions limited to study area), and there are limited data available on failed introductions (Kolar and Lodge 2002; Kolar 2004).

Ecological niche modeling is a correlative method that utilizes associations between environmental variables and known species' occurrence localities to predict potential areas where a given species is likely to establish (e.g., Guisan and Thuiller 2005; Elith et al. 2006; Elith and Leathwick 2009). It has been successfully applied to a varied array of ecological disciplines that include ecology and evolutionary biology, impacts of climatic change, invasion biology and conservation biology (see Guisan and Thuiller 2005 for a review on the development and applications of ecological niche models). Ecological niche models have been successfully applied to predict the potential distribution of invasive fish species in novel systems (e.g., Igushi et al. 2004; McNyset 2005; Zambrano et al. 2006; Chen et al. 2007) but like deductive methods, they also have limitations to their application (Elith et al. 2006;

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Fitzpatrick et al. 2007). In particular, several studies have shown that niche models developed using native range occurrences may fail to predict the full extent of an invasion. This failure has often been attributed to changes in the niche of the invading species (Fitzpatrick and Hargrove 2008), biotic interactions and dispersal limitations that prevent the species from occupying potential suitable habitats (Anderson et al. 2002) and the choice of environmental variables used to train the models (Peterson and Nakazawa 2008; Rödder et al. 2009; Rödder and Lötters 2009, 2010). Despite these caveats, deductive and correlative approaches are widely applied as a screening tool to identify potential invasive species and prevent their transmission into novel river systems (e.g., Pheloung et al. 1999; Kolar and Lodge 2002; National Research Council 2002; Kolar 2004; Marchetti et al. 2004; Copp et al. 2005; Schleier et al. 2008).

Risk management involves the use of decision-support systems to estimate the risk of adverse ecological impacts associated with a given species establishment and spread to ecosystem structure and functioning in relation to environmental, social, and economic values of a given region (Copp et al. 2005). Risk management also enables concerned stake-holders to prioritise resource allocation for effective preventative and remediation efforts (Anderson et al. 2004; Copp et al. 2005).

This study investigates the ecological risk associated with the invasive Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) in the central sub-catchment of the Limpopo River basin, northern South Africa. 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 and feral populations are now established in most river catchments within the sub-region (van Schoor 1966; de Moor and Bruton 1988; Welcomme 1988; Schwank 1995; Chifamba 1998; Skelton 2001; Marshall 2006; Weyl 2008; Zengeya and Marshall 2007). These feral populations have been

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implicated in causing adverse effects on the recipient river systems such as decreased indigenous fish abundance and local extinction of indigenous congenerics 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 distributional range has since expanded to include the Limpopo River and other eastern rivers in South Africa and Mozambique where it is now established and spreading (van der Waal and Bills 1997, 2000; Weyl 2008). The advent of *O. niloticus* in the Limpopo river system is a cause for concern for the conservation of indigenous congenerics, especially for Mozambique tilapia *O. mossambicus* that 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 black tilapia *O. placidus*, redbreast tilapia *Tilapia rendalli* and banded tilapia *T. sparrmanii*. Greenhead tilapia *O. macrochir* is only known from one occurrence record (Kleynhans and Hoffman 1992) and might have failed to establish itself.

The ecology of seasonal rivers within the Limpopo river system is poorly understood and as a result of the lack of earlier information on the hydrology as well as biota, recent changes and environmental deterioration have not been recorded (van der Waal 1997; van der Mheen 1997; Davies and Wishart 2000). The impact of *O. niloticus* on indigenous fish communities in the Limpopo River system may be especially severe in rivers systems impacted by anthropogenic activities such a dam construction, pollution, siltation, invasive alien weeds and habitat destruction (Skelton 1990). It is therefore critical to identify areas within the Limpopo river basin where *O. niloticus* has been introduced, predict which river

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system(s) are vulnerable and possibly at risk of further Nile tilapia invasions, and more importantly, what can be done to stop its spread and reduce its impact.

In response to these knowledge gaps, this study developed a qualitative risk assessment method based on Schleier et al. (2008) and outlines its potential use as a screening tool for determining the risk of establishment and spread of *O. niloticus* within the central sub-catchment of the Limpopo river basin, northern South Africa. Ideally, ecological risk assessments should be quantitative but in cases where there are insufficient data on community structure and functioning, qualitative approaches have been successfully applied (Anderson et al. 2004; Colnar and Landis 2007; Schleier et al. 2008). This study considered the use of ecological risk assessment to predict the risk of establishment for *O. niloticus* in the central sub-catchment of the Limpopo River basin, northern South Africa and the major implications for the conservation of indigenous congenerics.

Methods

Problem formulation

Ecological risk assessment is defined herein as a process that evaluates the likelihood that adverse ecological effects may either occur or are occurring to indigenous congenerics in the Limpopo river basin, South Africa as a result of exposure to *O. niloticus*. The assessment was divided into four principal components according to Landis (2004) and Schleier et al. (2008). The assessment determined both the risk of *O. niloticus e*stablishment and spread, and the potential detrimental effects it may have on indigenous congenerics and other species of concern (hereafter referred to as SOC) within the Limpopo river basin.

The first component described the organism of interest, or stressor, as *O. niloticus* and outlined its known or potential adverse ecological impacts on receiving environments. The second component identified assessment end-points as indigenous congenerics, other

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indigenous species of concern (SOC), and rivers and streams that are at risk of an *O. niloticus* invasion within the Limpopo River basin. The third component consisted of an exposure analysis to estimate the likelihood of introduction, establishment and spread of *O. niloticus* within river systems in the Limpopo River basin by identifying the physiological tolerance of *O. niloticus* in relation to minimum water temperature in the receiving environment that would be suitable for the species establishment. The last component integrated the information from the second (assessment analysis) and third (exposure analysis) steps to generate a risk characterisation for *O. niloticus* establishment and potential impact to indigenous congenerics and species of concern.

Stressor description

Nile tilapia has been introduced worldwide for aquaculture, augmentation of capture fisheries, and sport fishing (Trewavas 1983; Welcomme 1988). It is well-suited for aquaculture because of its wide range of trophic and ecological adaptations, and its adaptive life history characteristics enable it to occupy many different tropical and sub-tropical freshwater niches (Trewavas 1983). These include a high reproductive rate and a remarkable physiological hardiness, adaptability and general level of tolerance to most potentially limiting environmental variables (Chervinski 1982; Philippart and Ruwet, 1982). Nile tilapia is eurythermal and tolerates a wide range of temperatures (8 - 42° C) with a preferred optimal range between 31 and 36° C (Philippart and Ruwet 1982; Sifa et al. 2002; Atwood et al. 2003; Charo-Karisa et al. 2005). Its salinity upper tolerance ranges from 20 - 30 $g.l^{-1}$ according to body size, age, and environmental factors such as water temperature (Watanabe et al. 1985; Villegas 1990; Likongwe et al. 1996; Lemarie et al. 2004). Optimal growth is achieved when salinity is < 5 $g.l^{-1}$ (Payne and Collinson 1983). *Oreochromis niloticus* is also a highly adept invader that is able to utilise degraded habitats in contrast to observed

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decreased abundance of indigenous congenerics in similar imperilled systems (Zengeya and Marshall 2007; Linde et al. 2008)

Oreochromis niloticus is a microphage that is known to feed selectively on phytoplankton (Moriarty and Moriarty 1973; Getabu 1994; Bwanika et al. 2004; Zengeya and Marshall 2007; Zengeya et al. 2011). Trophic distinctions for *O. niloticus* are, however, not always clearly defined and the species is known to exhibit opportunistic and versatile feeding strategies that reflect the abundance and composition of food sources in different environments, seasons and either the presence or absence of competing fish species and predators (Gophen et al. 1993; Balirwa 1998; Njiru et al. 2004; Njiru et al. 2007; Zengeya and Marshall 2007, Zengeya et al. 2011).

The reproductive biology of *O. niloticus* is characterised by fast growth rate, early sexual maturity (5 - 6 months), a high degree of parental care, ability to spawn multiple broods in a season and high fecundity associated with its large body size (Trewavas 1983; Ojuok et al. 2007). It is known to attain approximately 60 cm (standard length) and large males are often aggressive competitors that out-compete other species for spawning and mouth-brooding grounds, if these are limited (Lowe-McConnell 2000). These attributes have inherently predisposed it to be a successful invasive species, with established feral populations in most tropical and sub-tropical environments in which it has either been cultured or has otherwise gained access (Welcomme 1988; Pullin et al. 1997; Costa-Pierce 2003; Canonico et al. 2005).

Assessment of impacts

Invasion risk of *O. niloticus* to its indigenous congenerics is defined as the product of the likelihood of *O. niloticus* becoming successfully established in a given novel river system and the associated adverse ecological consequences (National Research Council 2002). The

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highest risk scenarios are likely to unfold when there is both a high probability of the establishment of *O. niloticus* in recipient river systems and associated adverse ecological impacts. In most tropical rivers the actual impact of introduced species is difficult to ascertain because data on the community structure and functioning before the introductions are often unavailable. Despite this, the well-documented success of *O. niloticus* in invading novel tropical river systems worldwide and associated adverse effects (see Canonico 2005 and references therein), provide strong circumstantial evidence to support the hypothesis of increased extinction rates and hybridisation risk to indigenous congenerics in recipient river systems as a result of *O. niloticus* invasions.

The mechanism of potential adverse ecological impact of *O. niloticus* include competition for food and the space necessary for spawning and mouth brooding. In areas where it has become established, *O. niloticus* has been shown to rapidly displace indigenous congenerics through competitive exclusion, to the extent that some populations have become locally extinct. For example, in Lake Kariba, Nile tilapia appeared in the mid-1990s after escaping from *in situ* cage-culture fish farms and has become abundant at the expense of Kariba tilapia *O. mortimeri* that has declined significantly in abundance (Chifamba 1998; Marshall 2006). As a result, Kariba tilapia is now listed as Critically Endangered (CR) on the IUCN Red List of threatened species (Marshall and Tweddle 2007). This has also been noted in Lake Victoria, where the introduced Nile tilapia has displaced the native *O. variabilis* and *O. esculentus* (de Vos et al. 1990; Goudswaard et al. 2002; Balirwa et al. 2003). The success of Nile tilapia has been attributed to its opportunistic feeding behaviour (Getabu 1994; Njiru et al. 2004), utilisation of a typically unoccupied phytoplanktonic trophic niche (Zengeya et al. 2011), parental care, high juvenile survival, fast growth rate (Balirwa 1998), and its ability to utilize a wide range of habitats for spawning and nursery purposes (Twongo 1995).

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Few studies have assessed the potential of *O. niloticus* to transmit diseases into novel aquatic systems and the only recent investigation was from Lake Nicaragua (Central America) (McCrary et al. 2007), where an out-break of trematodes that affected several cichlid species was linked to the dominance of both *O. mossambicus* and *O. niloticus* in the lake system. This notwithstanding, several bacteria and parasitic diseases are known to affect tilapias (Shoemaker et al. 2006) and studies of disease transmission by other invasive fish species elsewhere have demonstrated the potential of invasive fish species to spread pathogens into recipient aquatic systems (Gozlan et al. 2005). Another potential impact of *O. niloticus* is habitat alteration through increased nutrient loading from bio-turbation and nutrient recycling of ingested and excreted material, which can lead to accelerated eutrophication, with associated algal blooms and excessive growth of aquatic macrophytes (Starling et al. 2002; Figueredo and Giani 2005). *Oreochromis niloticus* can also alter aquatic habitats by the removal of underwater vegetation as reported in Nicaragua, where the decline of *Chara* sp. beds was associated with the spread and establishment of *O. niloticus* (McCrary et al. 2007) and a decline in indigenous species as a result of habitat loss and modification. It has also been implicated in hybridisation with other tilapiines such as *O. mossambicus* in the Limpopo River Basin (Moralee et al. 2000; van der Waal and Bills 2000; D'Amato et al. 2007). As with other cichlids, the tilapiines underwent a recent evolutionary radiation, and either recent or incomplete speciation processes allow them to hybridise readily, posing a threat to the integrity of local adaptation (D'Amato et al. 2007).

Despite the well-documented adverse ecological effects of *O. niloticus* on recipient river systems (see Canonico 2005 and references therein), it is among one of the most widely cultured species in aquaculture and stock enhancements (Suresh 2003). While aquaculture is perceived as a means of achieving protein security, poverty alleviation and economic development in many developing countries (NEPAD 2005), the decisions on exotic fish

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introductions are usually based on the trade-off between socio-economic benefits and potential adverse ecological effects (Cowx 1999). In most invaded aquatic systems, *O. niloticus* has had a pronounced impact on fisheries in terms of increased food production and poverty alleviation by creating alternative aquaculture and fisheries livelihoods (Wise et al. 2007). Interestingly, the establishment of *O. niloticus* in novel aquatic systems has not led to a decrease in overall yields, but rather the replacement of indigenous species (Ogutu-Ohwayo 1991; Twongo 1995; Balirwa et al. 2003; Shipton et al. 2008; Weyl 2008). In a few cases, *O. niloticus* has supplanted desirable species from fisheries such as in Lake Victoria, where it is often regarded as being of inferior quality in comparison to the various haplochromines that it supplanted and, therefore, commands lower market prices (Wise et al. 2007).

Assessment end-points

Species of concern

Species of concern (SOC) are defined herein as species within the Limpopo River basin (Table 1) that are either declining or appear to be in need of concerted conservation actions as a result of a combination of their restricted natural range and escalating anthropogenic activities such as pollution, habitat alteration, water abstraction, dam construction, inter-basin water transfer schemes and introduced species (Skelton 1990; Davies et al. 1992; Tweddle et al. 2009). The advent of *O. niloticus* in the Limpopo river system is a cause for concern for the conservation of indigenous congeneric species, especially *O. mossambicus* (Cambray and Swartz 2007). The other indigenous tilapiines in the Limpopo river system such as *Tilapia rendalli* and *T. sparrmanii* have low habitat and trophic overlaps with *O. niloticus* and will likely not be significantly affected by the establishment of *O. niloticus*. This study also included the southern barred minnow *Opsaridium peringueyi* that occurs naturally from the Save river system in Zimbabwe down to the Pongola river system in South Africa as a

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species of concern. It is listed as vulnerable because of its reduced distributional range through habitat alteration of flowing rivers by impoundments and excessive water abstraction (Skelton 2001). It is reported as possibly extinct in Zimbabwe as a result of severe drought and habitat alteration (Marshall and Gratwicke 1999).

Table 1. A list of species of concern (SOC) and introduced species (IS) in the Limpopo River basin, northern South Africa.

Common name	Scientific name	SOC or IS
Mozambique tilapia	*Oreochromis mossambicus*	SOC
Mosquito fish	*Gambusia affinis*	IS
Rainbow trout	*Oncorhynchus mykiss*	IS
Largemouth bass	*Micropterus salmoides*	IS
Nile tilapia	*Oreochromis niloticus*	IS

Other introduced species

The assessment of end-points in this study also included other introduced species (hereafter referred to as OIS). These include mosquito fish *Gambusia affinis*, bluegill sunfish *Lepomis macrochirus*, rainbow trout *Oncorhynchus mykiss* and largemouth bass *Micropterus salmoides*. The OIS were included as they are known to cause severe biological impacts on small riverine species and juveniles of large species elsewhere (Cambray 2003; Woodford and Impson 2004; Gratwicke and Marshall 2001).

Assessment of exposure

An assessment of exposure in this study was done to estimate the likelihood of introduction, establishment and spread of *O. niloticus* within river systems in the Limpopo River basin. The physiological tolerance limits of *O. niloticus* in relation to minimum water temperature were used to identify river systems that would be suitable for the species' establishment. Data layers summarising the main river systems and dams within the Limpopo River system were

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obtained from Resource Quality Services, Department of Water Affairs, South Africa (http://www.dwaf.gov.za/iwqs) and were analysed using ArcMap® 9.3. (ArcGIS™; ESRI®, Redlands, CA). Additional data summarising estimated annual predictions of mean monthly water temperature variables (maximum, median, minimum and range) were obtained from the African Water Resources Database (AWRD; Jenness et al. 2007; http://www.fao.org/geonetwork). A river segment was defined by first plotting a geographical grid of the main river systems within the Limpopo drainage basin. The grid was then superimposed onto a raster file of estimated mean monthly minimum water temperature (native pixel size of 30 arc seconds) from which the respective temperature values for each grid cell along a given river channel were extracted. Nile tilapia can tolerate a wide range of temperatures (8 – 42 °C) with a preferred optimal temperature range from 31 to 36 °C (Philippart and Ruwet 1982). However, the natural fitness of *O. niloticus* in terms of respiration, feeding, growth and reproduction is reduced at sub-optimal temperatures below 20 °C (Ross 2000). *Oreochromis niloticus* exhibits severe cold stress symptoms such as cessation of feeding, rapid and disoriented movement at temperatures below 15° C (Amoudi *et al.*, 1996; Atwood et al. 2003; Charo-Karisa et al. 2005). Its lower lethal temperature limit varies between 8-12° C (Likongwe et al. 1996, Sifa et al. 2002; Atwood et al. 2003; Charo-Karisa et al. 2005). River channels were therefore classified into three categories: 8 – 12 °C was characterised as of low risk (score = 1), 12 – 15 °C as of medium risk (score = 2), and > 15 °C as of high risk (score = 3).

Within southern Africa, *O. niloticus* has been extensively propagated by farmers and anglers for recreational and sport fishing into small and medium reservoirs around the sub-region. A positive spatial linkage between fish introductions for recreational and sport fishing and the presence of reservoirs within river catchments is well-documented elsewhere (Pringle et al. 2000; Marchetti et al. 2004; Han et al. 2008). For the purposes of this study, it was

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hypothesised that the successful establishment and spread of *O. niloticus* within the river system will likely have a strong spatial linkage with the presence of impoundments. Hence, the presence of a dam within a given river section was assigned a score of 2 and absence of impoundments was scored as 1.

The highly seasonal nature of river systems within the Limpopo river basin determines the availability of habitats for aquatic fauna (van der Waal 1997; Minshull 2008), hence, river channels were categorised either as perennial rivers and/or episodic/ephemeral rivers. Perennial rivers are defined as rivers with relatively regular, seasonally intermittent discharge (Davies et al. 1995) and were assigned a risk score of 2. Episodic/ephemeral rivers are defined as rivers that flow for short periods after high rainfall in their catchments (Uys and O'Keeffe 1997) and were assigned a risk score of 1.

The exposure of indigenous congenerics and SOC was herein defined as the presence of *O. niloticus* within a given river section of the river basin where the respective indigenous species naturally occur. Firstly, if *O. niloticus* was present within a given section of the river, the river section was assigned a score of 3 (high risk), and if *O. niloticus* was absent within a given section of the river but present in upper reaches of the river it was assigned a score of 2 (medium risk). If *O. niloticus* was absent from both upper and immediate reaches of a given river section, it was assigned a score of 1 (low risk). Secondly, if an SOC occurs within a given river section, the river segment was assigned a score of 2 and if no SOC are known to occur within a given river section it was assigned a score of 1. Lastly, if an OIS was known to be present within a given river segment, it was assigned a score of 2, and if a river section had no known record of introductions, it was given a score of 1.

Geo-referenced occurrence data for summarising species distributions were obtained from various sources including museum specimen records, biodiversity databases such as FishBase (http://www.fishbase.org), Global Biodiversity Information Facility (GBIF;

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http://www.gbif.org), the published literature, and fish survey data from various fisheries departments in southern African countries that included Botswana, Mozambique, Zambia, and Zimbabwe. A fish survey was also conducted from December 2008 – December 2009 on the Limpopo River and its associated tributaries within the Limpopo Province of South Africa to ascertain the extent of the current distribution of *O. niloticus* within the province from previously known introduction sites. The presence or absence of *O. niloticus* within a given river segment was confirmed through genetic and morphological identification of sampled populations in a parallel on-going study.

Characterisation of risk

Invasion vulnerability

The invasion vulnerability score (IVS) was derived as the sum of all physical variables (minimum temperature), dam score and river flow (either perennial or episodic/ephemeral) for each given river section. The minimum possible IVS was 3 and the maximum possible was 7. The IVS values were then divided into three risk categories using the natural break (Jenks), in Arc-Map® 9.3 where rivers with river segments with IVS values < 4 were characterised as of low risk, 4 - 5 as of medium risk, and 6 - 7 as of high risk.

Invasion impact

The invasion impact score (IIS) was calculated as the sum of *O. niloticus* exposure, SOC and OIS scores for each given river section. The minimum possible IIS was 5 and the maximum possible was 10. The IIS values were divided into two risk categories using the natural break (Jenks), in Arc-Map ® 9.3 where rivers segments with IIS values between 5 - 8 being characterised as of low risk and those between 9 - 10 as of high risk.

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Results

Invasion vulnerability

The river sections centred on the Limpopo main river channel and the immediate reaches of its associated tributaries east of the Limpopo/Lephalala river confluence along the Botswana-South Africa-Zimbabwe border recorded the highest possible IVS (6 - 7) for *O. niloticus* establishment (Fig. 1). This was mainly attributed to a suitable receiving environment in terms of minimum temperature (15-19° C), perennial availability of water and the presence of large numbers of reservoirs. In the upper Bushveld catchment, the Upper Limpopo, Mokolo, Matlabas and Crocodile rivers had IVS values between 4 and 5, which was categorised as medium risk. Headwater streams, especially in the Waterberg escarpment, recorded the lowest IVS of 3 relative to all other river sections. The decrease of the IVS values was mainly a function of low water temperature (8 - 12 °C) associated with increasing altitude and availability of water.

Invasion impact

A total of 92 of 290 (32%) river sections are at high risk of adverse impacts on indigenous riverine species from an *O. niloticus* invasion (Fig. 2). The Limpopo River's main river channel and its associated tributaries such as the Crocodile, Matlabas, Mokolo, and Luvhuvhu rivers recorded the highest possible IIS values (9 - 10) for *O. niloticus* establishment. The Limpopo River recorded high IIS scores mainly as a result of the presence of established *O. niloticus* feral populations east of the Shashe/Limpopo rivers confluence along the Botswana-South Africa- Zimbabwe border, while the remainder (Crocodile, Matlabas, Mokolo, and Luvhuvhu rivers), the high ORS scores can be attributed to the presence of other introduced species such as *M. salmoides* and *C. carpio* in the respective segments.

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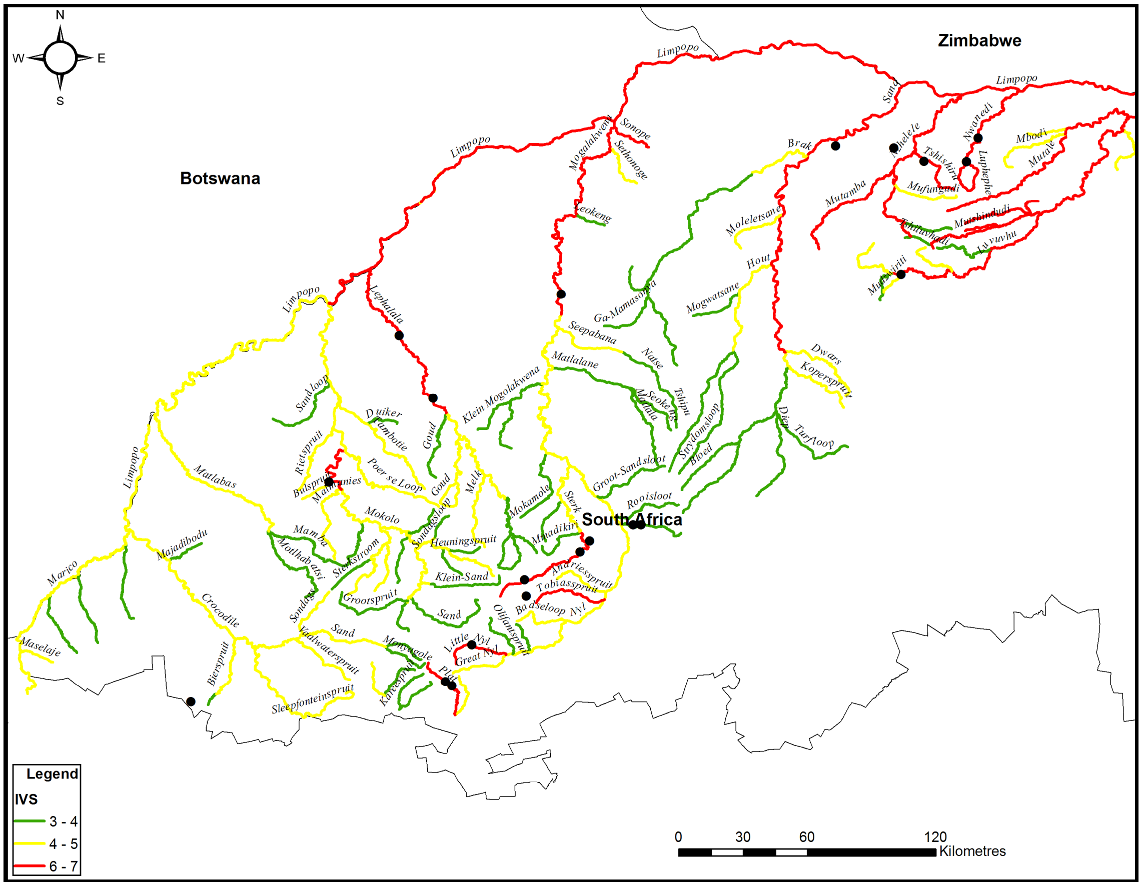

Figure 1. The invasion vulnerability scores (IVS) for the establishment and spread of Nile tilapia (*Oreochromis niloticus*) across the river systems in the Limpopo River basin, northern South Africa. Potential distribution is indicated by shaded areas, with red and green indicating high and low invasion vulnerability scores (IVS), respectively. Circles (●) indicate the presence of dams.

Uncertainty analysis

A major limitation for the application of ecological risk assessment to African freshwater systems is the general lack of ground-truthed aquatic environmental data (water quality variables, habitat availability and quality), and the scarcity of up-to-date, accurate and easily accessible species occurrence records. To circumvent the lack of aquatic environmental data, proxy estimates of annual water temperature trends derived from air temperature bio-climatic variables (Jenness et al. 2007) were used instead as they have been successfully applied to delimit areas where temperature might be a limiting factor to aquaculture production of *O. niloticus* and sharp tooth catfish, *Clarias gariepinus* within Africa (Kapetsky 1994). To ascertain the accuracy of the water temperature estimates, limited available data from 1950 to 2009 summarising annual temperature (mean, minimum and maximum) trends within the Limpopo river system was obtained from the Directorate of Resource Quality Services, Department of Water Affairs, South Africa (http://www.dwaf.gov.za/).

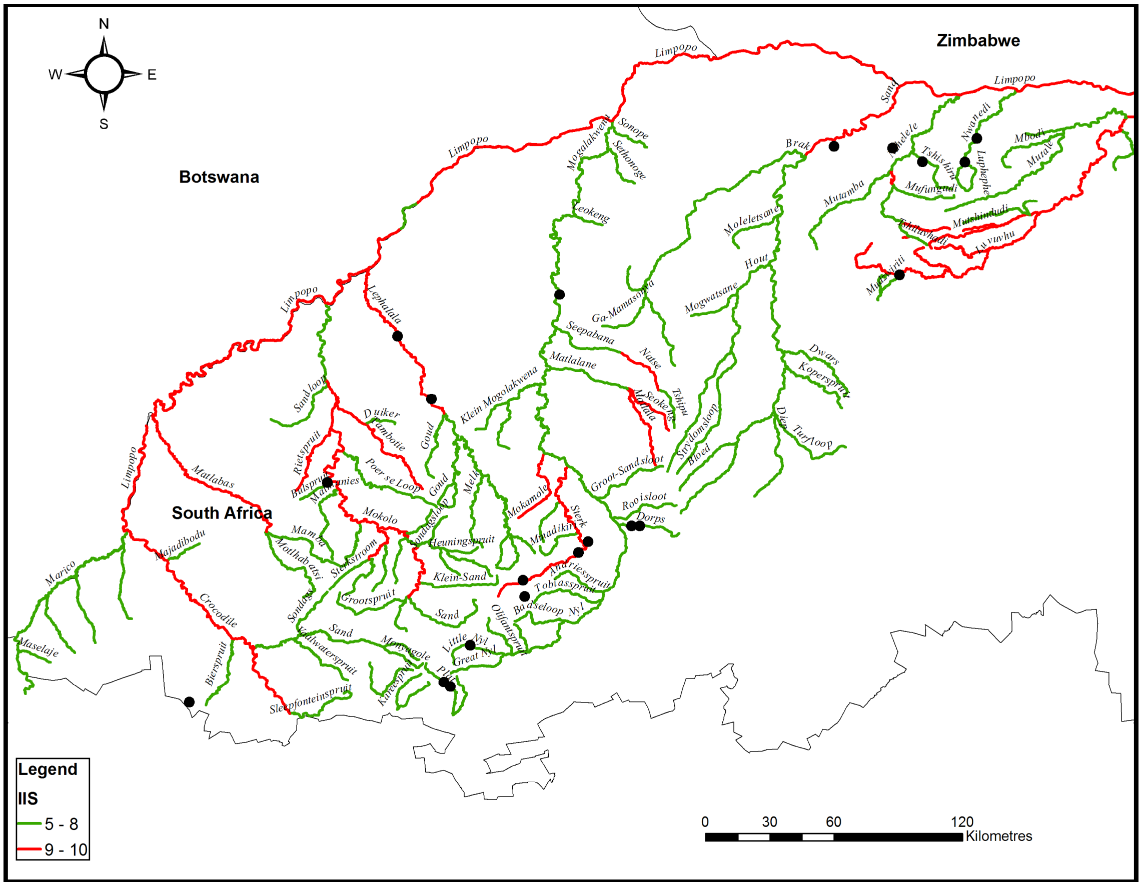

Figure 2. The invasion impact scores (IIS) for the establishment and spread of Nile tilapia (*Oreochromis niloticus*) across the river systems in the Limpopo River basin, northern South Africa. Potential ecological impact is indicated by shaded areas, with red and green indicating high and low invasion impact scores (IIS), respectively. Circles (●) indicate the presence of dams.

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Data summarising annual temperature trends between 1950 and 2009 were collated from 25 monitoring stations. Estimated temperature values were then extracted using Arc-Map® 9.3 for the selected monitoring stations and found to be significantly related to actual temperatures ($P < 0.05$). The regression (r^2) models only explained at most 33% of the variability of the observed temperature data. This indicates that estimated air temperatures are poor predictors of actual water temperatures. This disparity between actual and predicted air temperature could be partly due to the quality/accuracy of the available data. The available temperature data were patchy in spatial and temporal terms and were only available for certain years and for a small number of monitoring stations. These water temperature estimates are currently the best available data on thermal regimes within African river systems and must therefore be viewed as proxies when actual water temperature data are unavailable.

It was hypothesised that *O. niloticus* will not be able to establish in rivers that have a minimum temperature lower than 10° C (Likongwe et al. 1996; Sifa et al. 2002; Atwood et al. 2003; Charo-Karisa et al. 2005). It is however uncertain on how long and how frequently fish are exposed to this lethal limit. It was therefore prudent to analyse the mean and range of monthly water temperature to identify river systems that had favourable thermal regimes for the establishment of *O. niloticus*. In general, a decrease in mean water temperature and an increase in the amplitude of temperature fluctuations with increasing altitude were observed. River systems in the low-lying central river valley have mean monthly water temperatures of > 20 °C and a low range (< 12 °C) of temperature fluctuations. The mean monthly temperature is 20 °C in the middle reaches and 16 °C in the upper reaches. It is possible that *O. niloticus* might be able to over-winter in those environments where the amplitude of the annual thermal range is reduced by the presence of infrastructures such as dams and weirs.

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Discussion

The overall level of risk for the establishment of *O. niloticus* within the Limpopo basin was projected as high for the central river valley and moderate for river systems in the upper Bushveld catchment. The difference in overall risk score between the two areas was expected and is a composite of the three stages of invasion, namely, initial dispersal, establishment, and spread.

Initial dispersal

There are already established feral populations of *O. niloticus* along the channel of the Limpopo River and in the immediate reaches of its associated tributaries east of the Shashe/Limpopo rivers confluence, while it has yet to establish within river systems in the upper bushveld sub-catchment (van der Waal 2007; Zengeya et al. 2011). The presumed source of introduction of *O. niloticus* into the Limpopo system is from the Zimbabwean sub-catchment of the Limpopo river where *O. niloticus* has been extensively propagated by farmers and anglers for aquaculture, recreational and sport fishing (van der Waal and Bills 1997, 2000; Marshall 2000). It has inevitably spread down-stream into the Limpopo river system and its continued propagation in the upper catchments is likely to ensure a sustained influx of propagules into down-stream river systems.

The spread of *O. niloticus* into rivers and streams in the upper catchment may have been retarded by a limited natural dispersal pathway. As a result of the semi-arid climate and the unpredictable rainfall within the Limpopo River basin, water availability for human use has been secured through the construction of small- to medium-sized impoundments. This has led to a high degree of river fragmentation with 25 dams (> 15 m high) constructed within the river system. The physical barriers imposed by such dam and weir systems and the highly seasonal and episodic/ephemeral surface water flows are likely to restrict the natural

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up-stream migration of *O. niloticus* into the bushveld upper sub-catchment (van der Waal 2007). Although not integrated into the analysis of the model used in the present study, the idiosyncratic behaviour of humans as agents of spread of invasive fish species is likely to be an important driver of the spread of *O. niloticus* further up-stream of the Limpopo river catchment. In southern Africa, *O. niloticus* invasion seems to be highly correlated with human activities such as aquaculture and angling and the presence of impoundments. However, detailed studies on fish population dynamics within respective impoundments, their spatial linkages and correlation with land use patterns are needed to evaluate this hypothesis.

Establishment

The presence of large dams within the river system is also likely to promote *O. niloticus* invasion by increasing colonization opportunities through the provision of suitable habitats. Dams and impoundments greatly change the distribution of surface water and modify habitats (Havel et al. 2005). This is especially noticeable in water-scarce environments such as the Limpopo River basin where rivers recede into long stretches of dry sand, interspersed by a staggered series of residual pools, weirs and farm dams during the dry season (van der Waal 1997; Minshull 2008). These seasonal pools and small impoundments provide dry season refuges 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).

Impoundments are also likely to modulate the observed large monthly water temperature range from the extremes. In comparison to river systems, the relatively greater depth of water in a reservoir has a modulating effect on temperature extremes (Wetzel 2001). The thermal regimes of rivers in the upper Bushveld reveal that headwater streams, especially in the Waterberg escarpment, experience minimum water temperature below 10 °C and have higher amplitude of temperature fluctuations between the minimum and maximum monthly

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temperatures relative to the middle and lower reaches. It is, therefore, possible that *O. niloticus* might be able to over-winter in environments that are able to reduce the amplitude of the annual thermal range from extremes. *Oreochromis niloticus* is among the most cold-tolerant tilapia because the species can survive at elevations of between 1500 and 2000 m (Trewavas 1983). The water temperature profile for rivers becomes progressively warmer with decreasing elevation and the mean monthly temperature for most rivers in the lower catchment is above 20 °C. There is also a marked decrease in the amplitude of temperature fluctuations with decreasing altitude. Therefore, the Upper Limpopo, Mogalakwena, Lephalala, Mokolo, Matlabas and Crocodile rivers were categorised as of medium risk, where *O. niloticus* may be able to over-winter and establish provided other factors such as water availability are not limiting.

Potential impact

Oreochromis niloticus is a highly successful invader and this is attributed to its extreme hardiness, wide range of trophic and ecological adaptations, and its adaptive life history characteristics. We therefore consider that *O. niloticus* poses an unacceptable risk to its congenerics in the Limpopo River system. Of particular concern is that in systems within the Limpopo River basin where *O. niloticus* has already invaded and established feral populations, adverse ecological impacts such as reduced abundance of indigenous species and hybridisation with its congenerics have already been documented (D' Amato et al. 2007; Tweddle and Wise 2007; Weyl 2007).

Adverse ecological impacts of introduced fish in the Limpopo River system may be accentuated further by other anthropogenic ecosystem stressors such as pollution and habitat modification (Ashton 2007). For example, in Lake Victoria, anthropogenic eutrophication and the introduction of the Nile Perch *Lates niloticus* and *O. niloticus* led to a decline and

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local extinction of indigenous haplochromines through habitat modification, predation pressure from *L. niloticus* and competitive exclusion from *O. niloticus* (Witte et al. 1992; Seehausen et al. 1997; Goudswaard et al. 2002, Balirwa et al. 2003). In the Limpopo River basin, other invasive fish species such as *M. salmoides* and *C. carpio* have been widely introduced into most medium- to small-sized dams in the upper catchments of the Crocodile, Mokolo and Luvhuvhu rivers (Kleynhans et al. 2007). The projected impact of *O. niloticus* on indigenous fish communities is likely be severe in the Limpopo River system that is already imperilled by extreme environmental conditions associated with a seasonal and semi-arid climate (Davies and Wishart 2000) and effluent discharges from cities and towns in the upper catchments (Ashton 2007). Return flows from planned inter-basin water transfers are also likely to change the hydrology and biotic integrity of recipient river systems as observed in adjacent river catchments (Davies et al. 1992).

Are qualitative risk assessments useful?

The qualitative risk model presented in this study provides a preliminary logistic framework for assessing the probability of *O. niloticus* establishment within the Limpopo River basin. This was done by identifying the physiological tolerance of *O. niloticus* in relation to minimum water temperature in the receiving environment that would be suitable for the species' establishment. The probability of a successful *O. niloticus* invasion is inherently tied to other factors such as propagule pressure and biotic interactions. However, in the absence of quantitative data on population processes and inter-specific interactions, an ecological risk assessment based on the habitat suitability at least remains an objective method that is easy to implement, modify and can be improved on in a logical and systematic manner as new data become available. It also serves as a guide for future research by identifying areas of

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uncertainty within the model where additional data are either required or further research is needed to improve model efficiency.

Globally, there is a lack of real-time monitoring of physical and chemical data for most rivers systems. The use of real-time data loggers to collect data on basic physico-chemical variables should be encouraged because they save on cost related to manual real-time data acquisition. Data loggers are able to obtain data automatically on a 24-hour basis, and will help improve the understanding of daily thermal regimes that might affect fish populations in specific river systems. There is also a need to implement regular monitoring programmes in most river catchments for introduced species and also to educate farmers and anglers about the ecological impacts that invasive species such as *O. niloticus* have on indigenous congenerics. As is often the case in management of invasive species, resources for detailed field studies and quantitative risk assessment procedures tend to be limited. The risk assessment model presented here based largely on proxies of environmental data can be used to identify river segments that are highly vulnerable to the establishment of the invasive Nile tilapia. Concerted conservation efforts can then be directed in such areas to confirm establishment, direct remediation efforts and contain further spread. For example, in South Africa, *O. niloticus* is listed as a potential invasive species under the National Environmental Management (NEMA): Biodiversity Act (Number 10, 2004), and it’s stocking and utilisation is to be regulated through a zoning process. The delineation of high risk areas, as highlighted in this model can help stake-holders and managers to decide where in the river system indigenous congenerics are most vulnerable to *O. niloticus* invasion and where it is likely to spread.

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Chapter 6

General discussion

Introduction

The objective of this thesis was to investigate the nature, extent and impact that feral Nile tilapia has on indigenous congenerics within the Limpopo River basin. The research approach adopted was multidisciplinary and included molecular, morphometric, and ecological analyses. Overall, this thesis aimed at contributing towards a better understanding of those factors that facilitate Nile tilapia's success as an invasive species together with gaining additional insights into its invasion rate and its ecological impacts within southern Africa. To address this objective, the research was separated into two components. First, Chapters 2 and 3 explored those factors that enable Nile tilapia to be such a successful invader by investigating aspects such as its hybridisation and trophic ecology with indigenous congenerics within the Limpopo River Basin. Second, Chapters 4 and 5 applied modeling methods to evaluate the invasive potential of Nile tilapia to establish in novel systems in southern Africa by identifying those river system(s) that are either vulnerable or are at serious risk of invasion. Major concerns and implications for the conservation of indigenous congenerics were highlighted.

Hybridisation

A major constraint in the conservation of *Oreochromis* spp. within riverine environments within southern Africa is that their morphological identification is often difficult because of considerable variation and broad interspecific overlaps in both meristic and morphometric characters. Two complementary methods were utilised - molecular and morphometric analyses - to assess the variability between Nile tilapia and its indigenous congenerics. The following research questions were addressed: 1) In areas where Mozambique and Nile

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tilapias are sympatric, is it possible to identify hybrids from respective pure morpho-specimens based on either genetic analyses and/or morphometrics? and 2) What is the spatial extent of the hybrid zone of Nile tilapia and indigenous congenerics, 10 years after its introduction? No clear shape distinction was made between specimens of *O. niloticus* × *O. mossambicus* hybrids from morpho-specimens of Nile and Mozambique tilapia. Specimens of *O. niloticus* × *O. mossambicus* hybrids were initially detected by the presence of intermediate meristic characters and subsequently confirmed by mtDNA analysis. Disparities in morphology make the distinction of species and/or their hybrids difficult as backcrosses resemble both parental species and, as such, species identification would need to be confirmed using molecular analysis (Fitzpatrick et al. 2012).

Preliminary evidence for directional hybridisation within the river systems was also detected. The sexual selection hypothesis for unidirectional hybridization in hybrids zones (Wirtz 1999) may explain the proximate basis of heterospecific matings between Nile and Mozambique tilapia in the Limpopo River. A tentative hypothesis is proposed that bigger Nile tilapia males may have a competitive advantage over spawning grounds and in female mate choice. The spatial extent of Nile tilapia invasion into the Limpopo Province, a decade after its introduction, was determined and found to still be mainly restricted to the main channel of the Limpopo River and the immediate reaches of associated tributaries. Several reasons that could account for such a slow dispersal into the upper catchment are suggested and these include a limited natural dispersal pathway and lack of suitable habitats. The null hypothesis of no variation in meristic and morphometric between the two congeners and specimens of *O. mossambicus* × *O. niloticus* hybrids and that there is no evidence of establishment and further spread of Nile tilapia within the Limpopo river basin were therefore rejected.

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Trophic interrelationships

By comparing and contrasting stomach content analysis and the dual stable isotope tracers for carbon and nitrogen, changes in the diet of two indigenous tilapiines (*Oreochromis mossambicus* and *Tilapia rendalli*) and the introduced Nile tilapia was documented revealing evidence for resource partitioning. This part of the study confirmed the hypothesis that congeneric *Oreochromis* spp. in the Limpopo River system specialize on feeding on different food items. Resource partitioning has been reported elsewhere in tropical river systems (Winemiller and Winemiller 2003; Zengeya and Marshall 2007) where it plays an enabling role for sympatric species to co-exist through niche differentiation. It was also found that fish species within the system are highly adaptive to the highly seasonal patterns of precipitation and hydrology that exert a strong influence on the availability of habitats and food resources. 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 to perceive the dynamics that determine food resource availability. The null hypothesis that there are no patterns of food resource partitioning and that resource partitioning among tilapiines species is not influenced by ontogeny and seasonal changes were therefore rejected.

Realised and potential invasive range of Nile tilapia

An ecological niche model was developed to address the research questions - To what extent has Nile tilapia established in river systems in southern Africa and where is it likely to spread to? and 2) How do environmental factors affect the realised and potential distributional ranges within African River systems? It was found that the predicted potential geographical range of Nile tilapia was extensive and revealed a broad invasive potential over most of

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central and southern Africa. Nile tilapia is prevalent in most river systems within southern Africa as a result of subsequent escapement from aquaculture facilities together with its continued intentional propagation into novel river systems. Within southern Africa, it is widely distributed and common in river systems such as the Kafue, Middle Zambezi, Nata (Makgadikgadi/Okavango), Runde-Save, Buzi and Limpopo (Schwank 1995; van der Waal and Bills 1997, 2000; Marshall 2006; Tweddle and Wise 2007; Weyl 2008; Zengeya and Marshall 2008). Of particular concern are those areas that have been free of non-native species but were predicted to be potentially suitable for Nile tilapia's establishment. These systems include the Cunene, Upper Zambezi, Okavango, Lake Malawi and associated affluent rivers, Lower Zambezi River and other eastern river systems such as the Buzi, Save-Runde and the Limpopo river systems.

The ecological niche model's performance and the predicted degree of niche conservatism varied significantly with those variables selected together with the spatial extent of the study area. These results indicated that the spatial distribution of suitable and unsuitable environmental variables varied between the two regions. This was expected as the two populations are allopatric and few disjunct areas globally have sufficiently similar climates to produce a signal of niche equivalency (Warren et al. 2008). Furthermore, the results indicate the ability of Nile tilapia to survive in conditions not congruent with its native range. Nile tilapia is considered to be an extremely hardy and eurytopic invader, with a wide range of trophic and ecological adaptations and adaptive life history characteristics (Welcomme 1988; Getabu 1994; Balirwa 1998; Njiru et al. 2004). These adaptive life history characteristics predispose it to be a highly successful invader, and may partly explain, among other factors, its successful establishment in novel river systems in southern Africa. The null hypothesis that 1) there are no differences in the predictive power of models trained with different environmental variables, 2) Nile tilapia occupies the same environments (niche

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space) in its native and introduced ranges, and 3) The invasive potential of Nile tilapia within river systems in southern Africa is localised and restricted to river systems with known introductions from aquaculture and recreational fishing activities were therefore rejected.

Ecological risk assessment

The qualitative risk assessment model presented in Chapter 5 extended the ecological niche model that found that the environmental variable that explained the most variation in model performance was minimum water temperature where the probability of Nile tilapia establishment was low at temperatures of < 20 °C but increased exponentially with temperature up to maximum of ≥ 30 °C. The river systems that are highly suitable for the establishment of Nile tilapia were, therefore, associated with this threshold minimum monthly temperature. The effects of water temperature regimes on the habitat suitability for a successful Nile tilapia invasion was further explored within the risk assessment model by including known physiological tolerance limits of Nile tilapia in relation to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern. This analysis was restricted to the Limpopo River and associated tributaries in South Africa, the study area, as a result of lack of species occurrence records and necessary environmental data from other sub-catchments in southern Africa. In this assessment, both the risk of Nile tilapia establishment and spread, and the potential detrimental effects it may have on indigenous congenerics and other species of concern within the Limpopo river basin, was determined.

The model predicted that river sections with suitable receiving habitats in terms of minimum water temperature (15-19 °C), perennial availability of water and the presence of large numbers of reservoirs are highly vulnerable to a Nile tilapia invasion. A decrease in invasion vulnerability was observed with decreasing temperature that was associated with

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increasing altitude. It was also found that the spread of Nile tilapia into the upper catchment river streams may have also been retarded by limited natural dispersal pathways as a result of high river fragmentation from dam construction together with the seasonal and intermittent river flows common to the area (FAO 2004). Consequently, river segments in the middle reaches (Upper Limpopo, Mogalakwena, Lephalala, Mokolo, Matlabas and Crocodile rivers) were categorised as of medium ecological risk, while headwater streams were considered to be of low ecological risk. A similar pattern is also evident for the projected impact of Nile tilapia on recipient systems. River systems that recorded the highest impact scores were characterized by a suitable receiving environment for Nile tilapia, presence of already established feral Nile tilapia populations and other introduced species such as largemouth bass, *M. salmoides* and common carp, *C. carpio*. The null hypothesis that the use of habitat suitability measures such as physiological tolerance to minimum water temperature, presence or absence of dams, seasonality of river flows and the presence of indigenous fish species of concern is not adequate to identify river systems that would be suitable and vulnerable to Nile tilapia establishment was therefore rejected.

As with the predictions from any ecological risk assessment model, it is acknowledged that the apparent restricted invaded range of Nile tilapia might only be an artefact of inadequate sampling. Nile tilapia has been extensively propagated by farmers and released by anglers for recreational and sport fishing into small and medium reservoirs around the sub-region, often circumventing the necessary permitting processes. As a consequence, these introductions are usually undocumented and not monitored. This paucity of information inherently makes it difficult to ascertain exactly those areas where Nile tilapia has been introduced and to predict those areas where it is likely to spread to. While it was possible to source geo-referenced occurrence data for Nile tilapia from museum specimen records, biodiversity databases, the published literature, and fish survey data, only 173

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occurrence points were obtained for the species (92 points in its native range and 81 in its introduced range) and it is assumed that these data represent the most up-to-date compilation of known occurrences of Nile tilapia within Africa.

Conservation implications

The major conservation implication from this study is that the receiving environment (Limpopo River basin) offers suitable habitats/conditions for the establishment of Nile tilapia. This is implied from the broad overlap in resource requirements between Nile tilapia and its indigenous congenerics. Nile tilapia utilised a broad and versatile feeding strategy that reflected the abundance and composition of food sources in different environments, seasons and ontogeny. The broad food niche of Nile tilapia also encompassed all the food resources utilised by indigenous congenerics plus an additional unoccupied phytoplanktonic trophic niche. Being trophically versatile is a major advantage for any potential invader as it allows it to adapt to the extreme environmental conditions associated with the seasonal and semi-arid Limpopo River system.

Nile tilapia also exhibited a broad invasive potential over most of southern Africa that overlaps the natural range of all six endemic congenerics. Of particular concern is that the localised distribution of all six endemic congenerics predisposes them to high invasion risk as the introduction of Nile tilapia into a given catchment is likely to have a significant impact on species with limited natural ranges. For example, in the middle Zambezi, Nile tilapia is already established and appears to be displacing the endemic Kariba tilapia, *O. mortimeri* which has subsequently been listed as Critically Endangered (CR) on the IUCN Red List of threatened species (Marshall and Tweddle 2007; IUCN 2011). Further introductions of Nile tilapia into adjacent catchments have also put other indigenous congenerics under high risk of extirpation. Of special concern are those areas that have been free of exotic species but are

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now vulnerable due to the ardent promotion of aquaculture and sport fishing. These areas currently act as “reserves” for the conservation of indigenous congenerics and concerted conservation efforts should be implemented to keep them free of invasive species.

The impacts of invasive species are most insidious when they affect the genetic integrity of indigenous congenerics (Lockwood et al. 2007). In the Limpopo river system, the detection of hybridisation between indigenous congenerics and Nile tilapia raises important questions about its dynamics and consequences. In general, tilapiines are known to hybridise readily and in the case of sympatric congenerics, hybridisation may pose no significant threat to the integrity of local adaptation and genetic diversity (D’Amato et al. 2007). However, the extensive movement of formerly allopatric *Oreochromis* spp. into artificial sympatry within most river systems in southern Africa may result in different consequences as noted elsewhere. For example, in Lake Victoria and associated satellite lakes, indigenous *Oreochromis* congenerics have been replaced by an admixture/introgressed genotypes as a result of hybridisation with Nile tilapia (Tweddle and Wise 2007; Angienda et al. 2011).

It is recognised that 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. Despite the lack of data, the well-documented success of Nile tilapia in invading novel tropical river systems worldwide and associated adverse effects (see Canonico 2005 and references therein) provides strong circumstantial evidence to support the hypothesis of increased extirpation rates and hybridisation risk to indigenous congenerics in recipient river systems as a result of Nile tilapia invasions.

Potential applications, future research and recommendations

The highly significant prediction of the occurrence of Nile tilapia in novel freshwater systems in southern Africa suggests that niche models can, in future, be used to assess potential

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threats before an introduced species becomes invasive and as such preventive measures can be implemented. However, those methods used to develop niche models remain variable and unstandardised (Jiménez-Valverde et al. 2011). In agreement with recent studies that have criticised the use of the Area Under the Curve (AUC) statistic to evaluate potential distribution of species (Lobo et al. 2007; Peterson et al. 2008; Jiménez-Valverde et al. 2012) this study also found some major limitations in its application. The AUC statistic was found to be sensitive and positively related to the spatial extent of the study area. These caveats are especially true when dealing with invasive species, particularly when evaluating model performance that are trained with different variable sets and in different geographical regions as this often leads to equivocal conclusions (Peterson 2011). An information criterion approach was included in this study for comparative purposes and it appears to be subject to similar problems with the spatial extent of the study area as AUC, as both parameters produced identical model performance assessments. To reduce such discrepancy in model evaluation, novel methods have been suggested such as the removal of spatial sorting bias (Hijmans 2011) and the use of precision measurements such as standard error and confidence intervals for each model evaluation test statistic in order to statistically compare its accuracy between alternate models (Liu et al. 2011). These suggested methods have, however, not been thoroughly applied and still need to be assessed further.

The qualitative risk model presented in this study provides a preliminary logistic framework for assessing the probability of Nile tilapia establishment within the Limpopo River basin. It is recognised that the probability of a successful Nile tilapia invasion is inherently tied to other factors such as propagule pressure and biotic interactions, but in the absence of quantitative data on ecosystem structure and functioning, an ecological risk assessment based on the habitat suitability at least remains an objective method that is easy to implement, modify and improve on in a logical and systematic manner as new data become

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available. It also serves as a guide for future research by identifying areas of uncertainty within the model where additional data are either required or further research is needed to improve model efficiency.

The use of integrated analytical methods, as demonstrated in this study, should be encouraged when investigating the impact of invasive species on community structure and functioning. For example, the dual use of stable isotopes and stomach content analysis, aids in our understanding of trophic dynamics in the river system, as the two methods give differential temporal perspectives of changes occurring in food web structure and functioning. Molecular and morphometrics techniques can be used in conjunction to assess levels of gene transfer among conspecific species within a community and this has implications in fish conservation through the assessment of species diversity, their distribution, translocation/introduction into novel areas and hence identification of areas and/or species at risk of hybridisation.

In southern Africa, Nile tilapia's invasion is highly correlated with human activities such as aquaculture, angling and the presence of impoundments. However, there are no quantitative studies integrating population dynamics of Nile tilapia within recipient impoundments, their spatial linkages and correlation with land-use patterns. There is also a need to investigate the possible synergistic effects caused by the dual introduction of Nile tilapia and other invasive species in a given river system. In the Limpopo River basin, other invasive fish species such as largemouth bass, *M. salmoides* and common carp, *C. carpio* have been widely introduced but interactive impact of Nile tilapia with other introduced species is largely unknown. There is also a need to implement regular monitoring programmes in most river catchments for introduced species and also to educate farmers and anglers about the ecological impacts that invasive species such as Nile tilapia have on indigenous congenerics.

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One major limitation of mtDNA analysis is that it can only demonstrate that genetic material of a species is present but cannot provide definite evidence that it is absent because the paternal component is not considered. As a result of this limitation of mtDNA analysis, recent studies on hybridisation in cichlids have used mtDNA in combination with either microsatellite markers (e.g. D' Amato et al. 2007) or with nuclear DNA (Angienda et al. 2011). This was not done in this study, but morphometric analysis was used instead to try to morphological identify morpho-specimens of *O. niloticus* and *O. mossambicus* to determine if morphological species assignment was congruent with mitochondrial lineages. The hypothesis that an *Oreochromis* specimen was not a hybrid could only be proved if they was no conflict between mitochondrial lineages and morphological species assignment. However, morphology may not have been the ideal deterrent either, because there was strong evidence of hybridisation among the three congenerics in the system. This therefore places into doubt the genetic integrity of the comparative material and the Limpopo *Oreochromis* cichlid population may already comprise a hybrid swarm.

Furthermore, the *Oreochromis* specimens for morphological analysis were limited to the Limpopo river system. Ideal the *Oreochromis* specimens from the Limpopo system should be compared with specimens from regions where there is clear evidence of pure genetic material such as the Eastern Cape for *O. mossambicus* and Nile region for *O. niloticus*. There is therefore a need to characterise the genetic diversity of indigenous *Oreochromis* spp. within the Limpopo river system. These data can assist stake-holders and conservation managers to focus future conservation and management efforts on river systems where indigenous *Oreochromis* spp. are most vulnerable invasion. Furthermore, better knowledge of extant variability among indigenous congenerics may help shed light on their adaptive life history traits.

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Preliminary evidence points to directional hybridisation among *Oreochromis* congeners within the Limpopo River system. Additional reproductive biology studies including behavioural and genetic paternity studies are, therefore, required to evaluate this hypothesis. Unidirectional hybridisation will likely have a major impact on the conservation of indigenous congenerics as they are likely to be extirpated from the river system and replaced by an admixture of introgressed hybrids.

In conclusion, the impact of Nile tilapia on recipient novel river systems highlights problems associated with fish introductions for aquaculture. This study, therefore, recommend that in areas that are predicted to be highly suitable for the establishment of Nile tilapia the precautionary approach to capture fisheries and species introductions should be applied when deciding where and what species should be used for aquaculture. In southern Africa, Nile tilapia should be restricted to catchments where it has already established and prohibited in pristine areas that are still free of invasion. In addition, and if possible, potential point sources of Nile tilapia should be eradicated in non-invaded river systems. Alternatively, the use of indigenous species should be promoted and enhanced through stock improvement and better farming methods. It should, however, be noted that the alternative species should also not be introduced to novel river systems outside their native range as they would possibly pose the same invasion-related problems as encountered with Nile tilapia.

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