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INVASION NOTE

Using a unified invasion framework to characterize Africa's first loricariid catfish invasion

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Abstract This paper presents evidence of establishment of a loricariid population in the Nseleni River in South Africa and uses a unified framework to determine its invasion stage. Specimens were identified morphologically as *Pterygioplichthys disjunctivus* (Weber 1991), but genetic barcoding results indicated close association with specimens that may have a hybrid history. The species was introduced into South Africa via the pet trade and the first record of introduction into the wild was in 2004. Samples collected in 2011 and 2012 demonstrated that there were multiple length cohorts in the population including juveniles (12–130 mm total length TL) and large (>300 mm TL) adult fish. Gonadal assessment of adults demonstrated the presence of reproduction capable specimens. The concurrent occurrence of mature adults and juvenile fish demonstrated establishment. Locality records indicate that *P. disjunctivus* has already spread between two rivers through an inter basin water transfer. Using a unified framework for invasions this invasion was categorized as a self-sustaining population in the wild with individuals surviving and reproducing a significant distance from their original point of introduction. Containment is suggested as potential management strategy.

Keywords Invasion stage · Barrier · Pet trade · Gonad · Reproduction · Establishment · Catfish · Vermiculated sailfin

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Introduction

The South American catfish family Loricariidae is the largest family of catfishes and includes more than 700 nominal species (Ferraris 2007). Some are popular aquarium fishes (Nico et al. 2009a, b), which as a result of releases by aquarists and escape from aquaculture facilities have become invasive in the Americas and Asia (Bunkley-Williams et al. 1994; Page and Robins 2006; Hossain et al. 2008; Levin et al. 2008; Pound et al. 2011). Reported impacts of their invasions include the potential effects of burrowing on river bank stability (Nico et al. 2009a, b) and concerns about competition with native biota (Nico et al. 2012). In South Africa the discovery of loricariid catfish from the Mhlathuze River (2004) and then from a Nature Reserve in the adjacent Nseleni River (2007) was cause for concern because alien invasive species management is a high priority in national legislation [National Environmental Management: *Biodiversity Act* (*Act 10 of 2004*) and the National Environmental Management: *Protected Areas Act* (*Act 57 of 2003*)].

An important step in developing alien invasive species management strategies in protected areas is determining their extent and traits (Spear et al. 2011). A unified framework such as that proposed by Blackburn et al. (2011), which combines previous stage-based and barrier models, and provides a terminology and categorisation for populations at different points in the invasion process, is therefore a potentially valuable tool. According to the framework, the invasion process can be divided into a series of four stages (transport, introduction, establishment and spread). Each of these stages is confounded by barriers (geography, captivity, survival, reproduction, dispersal and environmental) that need to be overcome before passing on to the next invasion stage. Blackburn et al. (2011) also suggest management actions for each invasion stage. These include preventing introductions, containment once introduced, mitigating spread once established in the wild and eradication when feasible. The aim of the current study was to assess the loricariid population in the Nseleni River and use the Blackburn et al. (2011) unified framework to determine invasion stage and to identify appropriate management actions.

Assessing invasion stage

This study was conducted in the Richards Bay area of the subtropical KwaZulu-Natal Province of South Africa (Fig. 1). Direct sampling was focused in the lower 16 km of the Nseleni River above its inflow into the 286 ha Lake Nsezi. In the study area, the river is approximately 100 m wide and 3 m deep. The substrate is muddy and riparian vegetation is characterized by large stands of powder puff *Barringtonia racemosa* and fig *Ficus* spp. trees. Aquatic vegetation includes the submerged macrophytes *Ceratophyllum demersum* and *Stuckenia pectinata* and stands of emergent papyrus *Cyperus papyrus* and the floating alien, water hyacynth *Eichhornia crassipes*. Lake Nsezi is a source of water for the towns of Richards Bay and Empangeni. To meet water demands the level of the lake is maintained by a dam wall that has been constructed at its outlet and by water that is pumped into it through an intra-basin transfer from the Mhlathuze River (Cyrus 2001).

Fish surveys were conducted in May 2010, January 2011 and January 2012. During each survey, 10 valve traps (10 cm diameter valve, 5 mm mesh diameter), 6 double-ended fyke nets (8 mm mesh) and 4 multi-mesh (25, 38, 50, 60, 75 and 100 mm mesh diameter) monofilament gill nets, were set at multiple locations over four nights. During these surveys electrofishing was also conducted opportunistically at multiple locations using a backpack electrofisher. Habitat traps constructed from one meter lengths of 50, 75 and 110 mm diameter PVC piping were set at nine locations from January 2011 to January 2012 and serviced monthly. We also conducted interviews with a local ornamental fish wholesaler/distributor and with local community members who were encouraged to report and donate specimens. Information from interviews and specimens collected during surveys and from donations were then used to identify the loricariid species and its invasion stage according to the criteria presented in Table 1.

To determine the introduction source, specimens were identified to species level. Identification of loricariid fishes obtained from outside of their native range proved difficult because taxonomic keys (e.g. Armbuster and Page 2006) require that the natural distribution of the specimen is known. Identification was further complicated because invading loricariids have been subjected to selection for desirable traits by the pet trade and their colour patterns are often no longer representative of natural populations (Wu et al. 2011; Nico et al. 2012). Specimens were therefore identified using both taxonomic keys (Armbuster and Page 2006) and by DNA barcoding (as part of the FISH-BOL project, described by Swartz et al. 2008). For DNA-Barcoding, six loricariid individuals from the introduced population were sequenced for the part of the mitochondrial cytochrome oxidase subunit I (COI) gene used in DNA barcoding, following the standard protocols implemented for fish barcoding by the Canadian Centre for DNA Barcoding at the University of Guelph (methods summarised by Ward et al. 2005).

To assess for establishment, we investigated the population structure to determine whether there was evidence of juveniles and adults in the population and to determine whether spawning capable fish were present in the population. A total of 346 sampled fish

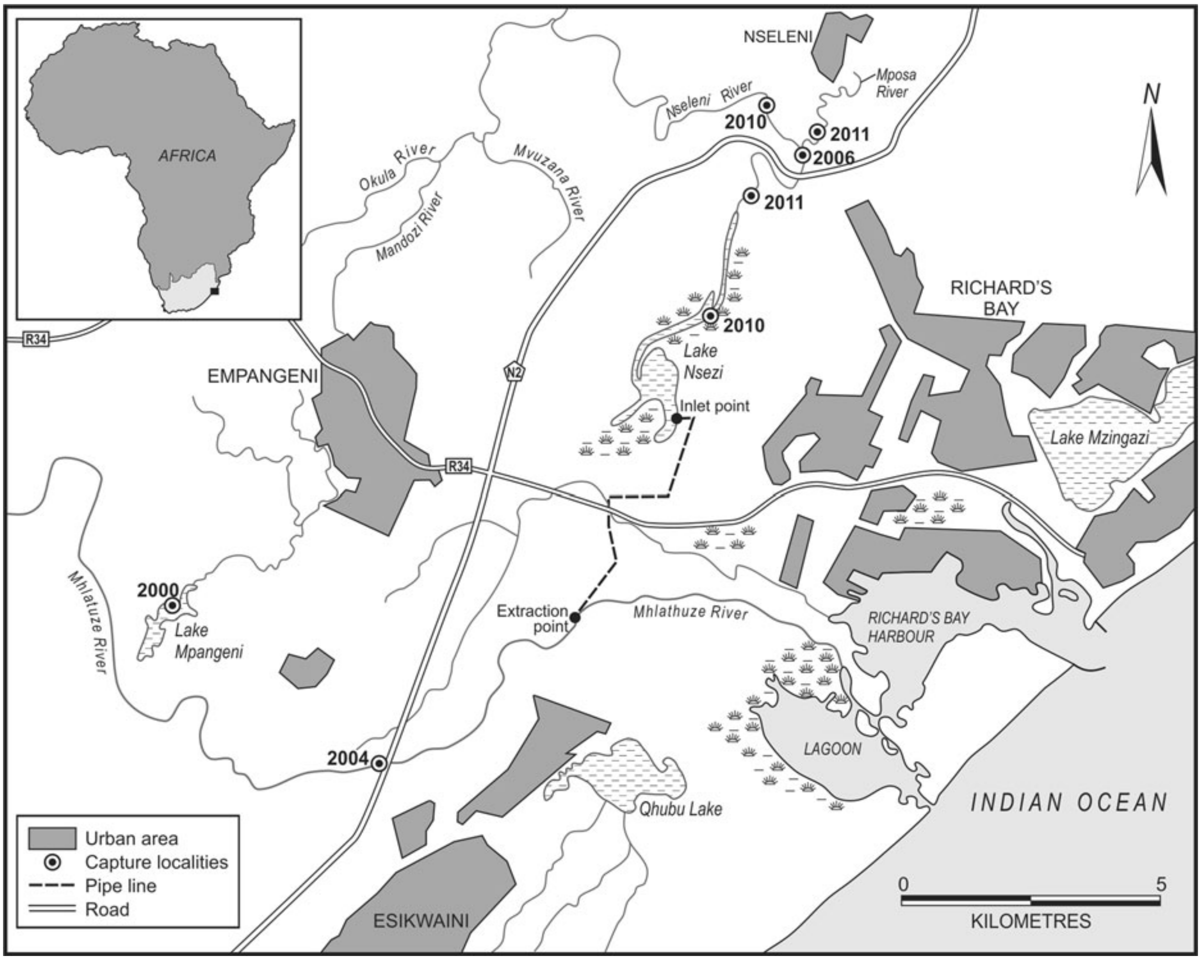

Fig. 1 Invasion history of *P. disjunctivus* in the Richards Bay area of KwaZulu-Natal, South Africa. Dates next to capture localities indicate first records

were dissected and their gonads were examined and visually categorized as: immature (no ova visible and testes small and tubular in shape) or spawning capable (yolky ova visible and testes distended and lobular) using established criteria to determine maturity (Gibbs et al. 2008).

Stage 1: Transport

The first stage in the invasion process is the breaching of a geographical barrier (Table 1). The pet trade is a major pathway for introducing alien fishes into South Africa and more than 1,200 alien fishes are permitted for importation and trade (Anonymous 1994). According to Mr N. Stallard owner of Fish Designs and ornamental fish wholesale business, loricariid catfishes have been imported and traded in South Africa at least since the 1970s (N. Stallard, e-mail interview 02/11/2012).

Stage 2: Introduction

The history of loricariid site locality records is illustrated in Fig. 1. The first report was from Lake Mpangeni in 2000 (Skelton and Weyl 2011), but the first specimen-linked evidence of introduction into the wild was from the Mhlathuze River in 2004 (South African Institute for Aquatic Biodiversity, National Fish Collection voucher specimen: SAIAB83643). In 2007 a specimen was collected from the Mposa River tributary of the Nseleni River (SAIAB83178) and then in 2009 two specimens (SAIAB85934 and SAIAB85934) were obtained from

Table 1 Categorisation of the *P. disjunctivus* invasion in South Africa using the Blackburn et al. (2011) unified framework

Stage	Category	Management action	*Pterygioplichthys disjunctivus* in South Africa
Transport	A. Not transported beyond limits of native range	Prevention	Native to South America, Loricariid catfishes have been introduced into South Africa via the pet trade since at least since the late 1970s
Geograhical barrier			
Introduction	B1. Individuals transported beyond limits of native range and explicit measures of containment are in place	Prevention and/or eradication	Wild specimens identified as *P. disjunctivus*, a native to the Madeira River drainage of the Amazon basin in Brazil and Bolivia and invasive in Mexico, the USA, the Philippines and Taiwan
Introduction	B2. Individuals transported beyond limits of native range but measures to prevent dispersal are limited at best		
Captivity barrier			
Introduction	B3. Individuals transported beyond limits of native range, and directly released into the novel environment	Prevention and/or eradication	First reported from Lake Mpangeni in the year 2000. Introduction into the wild was either intentional release by aquarists of unwanted pets or by escape from an urban pond
Introduction	C0. Individuals released into the wild in the location where introduced but are incapable of surviving for a significant period	Eradication	
Survival barrier			
Establishment	C1. Individuals surviving in the wild in the location where introduced but no reproduction	Containment and/or eradication	Interviews with fishers in 2011 indicated continued survival in Lake Mpangeni
Establishment	C2. Individuals surviving in the wild in location where introduced, reproduction occurring, but population is not self-sustaining		
Reproduction barrier			
Establishment	C3. Individuals surviving in the wild in location where introduced, reproduction occurring, and the population is self-sustaining	Containment and/or eradication	In 2004, two juvenile specimens were collected from the Mhlathuze River downstream of Lake Mpangeni
Dispersal barrier			
Spread	D1. Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction	Mitigation and/or eradication	The collection of an adult specimen from the Nseleni River in 2007 demonstrated spread using an inter basin water transfer to breach the dispersal barrier
Spread	D2. Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction		Juvenile and spawning capable adult fish are present in the Nseleni River population
Environmental barrier			
Spread	E. Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence	Mitigation and/or eradication	Further dispersal is likely if natural dispersal barriers between catchments are breached

The invasion process is divided into four stages, each of which is confounded by barriers that need to be overcome before passing onto the next invasion stage. Invasions are categorised (A–E) according to criteria within each invasion stage and management actions for each invasion stage are suggested the Nseleni River. Subsequent directed sampling from and donations from the local community resulted in the collection of a further 365 specimens from the Nseleni River system including its Mposa River tributary from May 2010 to April 2012 (Fig. 1).

On the basis of morphology and ventral surface coloration (Fig. 2) specimens were identified as *Pterygioplichthys disjunctivus* (Weber 1991) using Armbuster and Page (2006). BLAST searches done in Genbank and subsequent phylogenetic analysis revealed that sequences from the six Nseleni River specimens (612 base pairs; Genbank numbers KC170030 - KC170035) matched 100 % (same haplotype) with individuals referred to as *Pterygioplichthys disjunctivus* (JF498722 - JF498724 and JF769355 - JF769356 from Jumawan et al. 2011 and AP012021 and NC_015747 from Nakatani et al. 2011) and *Pterygioplichthys pardalis* (JF498752, JF769358 and JF769360 from Jumawan et al. 2011). The genetic results could therefore neither reject nor confirm the morphological identification.

The morphological and genetic identifications are consistent with the findings of Wu et al. (2011) and suggest that there could be confusion with the identification of introduced loricariid populations and/or that hybridisation has occurred between closely related species (especially considering potential mixing in the ornamental trade). Positively identified specimens from the type localities of *P. pardalis* and *P. disjunctivus* are required to resolve the taxonomic confusion surrounding introduced loricariid populations. Given that the genetic analysis could neither reject or refute the morphological identification, the most likely identity of the South African invasion is *P. disjunctivus*, but the possibility remains that the population has a hybrid history (see Wu et al. 2011).

Fig. 2 Lateral and ventral view of a 170 mm juvenile *P. disjunctivus* collected from the Nseleni River, South Africa in April 2010 (South African Institute for Aquatic Biodiversity, National Fish Collection voucher specimen: SAIAB96602)

Pterygioplichthys disjunctivus is native to the Madeira River drainage of the Amazon basin in Brazil and Bolivia and is the main nuisance species in Mexico (Capps et al. 2011), the USA (Nico et al. 2009a, b), the Philippines (Vallejo and Soriano 2011) and Taiwan (Wu et al. 2011). Interestingly, *P. disjunctivus* is not on South Africa's permitted fish list that includes three species of the closely related genus *Hypostomus*. It is therefore likely that *P. disjunctivus* was erroneously imported as one of these species. Such misidentifications are common in the pet trade and often result in the inadvertent importation of invasive organisms (Keller and Lodge 2007).

Although the exact introduction pathway from captivity into the Mthlathuze and Nseleni Rivers is not known, the most likely source of introduction was through release by aquarists or escape from an ornamental pond into Lake Mpangeni; from the lake into the Mthlathuze River and then via the inter basin water transfer pipe line into Lake Nsezi and upstream into the Nseleni and Mposa Rivers.

Stage 3: Establishment

A strong indicator of establishment was the sampling of specimens throughout the 15-month sampling period and the presence of multiple length cohorts in the population including small juveniles (12–130 mm TL) and large (>300 mm TL) adults (Fig. 3). Of the 346 fish examined for maturity, 64 were juveniles, 91 were mature males and 191 spawning capable females. The smallest mature female fish was 270 mm TL and the largest was 490 mm TL. The smallest mature male fish measured 305 mm TL and the largest was 480 mm TL. Female spawning capability was also inferred from a gonadosomatic index $I_G > 1$ $(I_G = 3.9 \pm 2.4)$ calculated $(I_G = gonad\ mass\,(g)/whole\ body\ mass\,(g) \times 100)$ from a sample of 54 female fish collected during austral summer (December 2011–January 2012). Estimates of

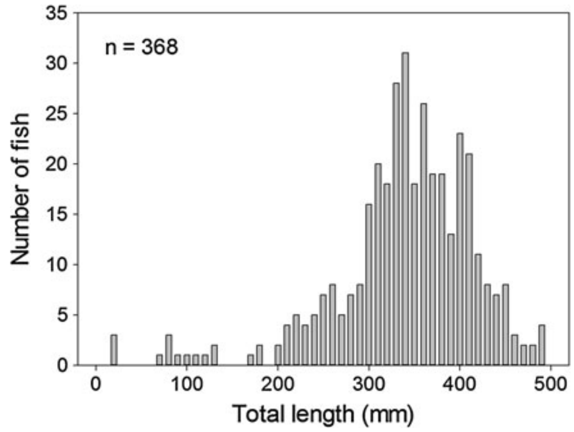

Fig. 3 Length frequency of *P. disjunctivus* collected (January 2011–May 2012) from the Nseleni River, South Africa

maturity and GSI were consistent with those determined for the invasive population in Florida (Gibbs et al. 2008). In the Nseleni River, the large number of mature specimens and the concurrent occurrence of both adult and juvenile fishes demonstrated establishment.

Stage 4: Spread

Collection records indicate that *P. disjunctivus* is in a stage of spread having dispersed from the Mthlathuze River into the Nseleni River system. This spread was most likely facilitated by the inter basin water transfer pipe which transports water from the Mthlathuze weir into Lake Nsezi. Using the unified invasion framework proposed by Blackburn et al. (2011), Africa's first loricariid invasion could thus be categorized as a stage "D2" invasion: "*self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from their original point of introduction*" (Blackburn et al. 2011. pg 337, Table 1). The only barrier to it becoming a fully invasive species (stage "E") is its inability to cross catchments without human assistance.

Management

It is obvious that legislative prevention mechanisms (Anonymous 1994) failed to stop the importation of *P. disjunctivus* and its eventual escape from captivity was inevitable given its proven establishment ability on several continents. The consequences of this invasion in the recipient ecosystem are not yet known, but due to negative impacts reported elsewhere (Nico et al. 2012) further spread is undesirable and management action is necessary. The Nseleni and Mthlathuze Rivers are relatively large rivers in a South African context, which makes the eradication of *P. disjunctivus* neither logistically nor economically feasible. The management measures of containment and mitigation suggested by Blackburn et al. (2011) for stage D invasions are therefore the most appropriate response strategy. To contain the *P. disjunctivus* population in the Mthlathuze and Nseleni rivers and mitigate against further spread, we suggest an education campaign to inform the general public on the dangers associated with fish introductions. Given the taxonomic complexity of this group of fishes, we suggest that the trade of *P. disjunctivus* be prohibited and that the trade in at least closely related loricariid species with proven invasion histories elsewhere be discouraged.

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