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Reconstructing the introduction history of an invasive fish predator in South Africa

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Abstract The Largemouth Bass (*Micropterus salmoides*) is a global invader with demonstrated ecological impacts on native fish communities. Introductions of fishes in freshwater ecosystems are often characterized as complex processes, yet an understanding of the nature of the introduction can inform management and conservation actions. Early in the twentieth century, two introductions of Largemouth Bass were made into South Africa for the establishment of a recreational fishery, and subsequent translocations have expanded their distribution to include much of southern Africa. In this study we quantified neutral genetic variation, modeled potential introduction scenarios, and identified potential source regions from within the native range. We documented limited levels of genetic diversity in nuclear microsatellite genotypes across populations (mean allelic richness = 1.80 and mean observed heterozygosity = 0.16) and observed low levels of genetic differentiation among four of the five focal populations (mean pairwise fixation index = 0.09), with a fifth population displaying greater levels of genetic divergence (mean pairwise fixation index = 0.27). A total of three cytochrome *b* haplotypes were recovered from South Africa samples and the single most common haplotype (93% of individuals) was identical to a haplotype from a population of Largemouth Bass in Maryland, USA. Using limited available stocking data along with outputs from Principal Component Analysis and approximate Bayesian evaluation of competing introduction scenarios we confirm the presence of multiple introductions. Despite evidence for multiple introductions, Largemouth Bass in South African water bodies harbor extremely low neutral genetic diversity, suggesting that even a very limited number of propagules can experience a high likelihood of success in invading nonnative waters.

Keywords Invasion reconstruction · Microsatellite · Freshwater fish · Centrarchidae · DIYABC

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Introduction

Invasive species commonly encounter biotic and abiotic conditions that differ greatly from their native habitats, and as a result, natural selection and adaptive potential may be key factors in determining the fate of introduced populations during the initial stages (Dlugosch and Parker 2008). Levels of genetic variation present in invasive populations, and by extension their ability to adapt to novel environments, are determined by a series of factors including the number of introduction events, their sources of origin, and the number individuals that were transferred (Roman and Darling 2007; Dlugosch and Parker 2008; Uller and Leimu 2011; Dlugosch et al. 2015). For example, populations established from a single introduction involving few propagules are expected to display significant reductions in genetic diversity relative to scenarios involving more sources, individuals, or repeated introductions. Patterns of genetic diversity inferred using neutral genetic markers (e.g., mtDNA, microsatellites) can play an integral role in reconstructing invasion histories (Estoup and Guillemaud 2010; Cristescu 2015) including demographic changes (Kalinowski et al. 2010), and identifying sources of origin (Muirhead et al. 2008; Rius et al. 2012). Knowledge of such factors can play an important role in invasive species management (Darling 2015); for example, by identifying vectors of spread enabling the design of measures to prevent additional introductions (Chapple et al. 2012) and in the selection of appropriate biocontrol strategies (Gaskin et al. 2011).

Native to eastern regions of the United States, the Largemouth Bass (*Micropterus salmoides*) is a freshwater fish species that supports a multibillion dollar recreational sport fishery in the United States (U.S. Department of Interior 2011) and has been introduced worldwide to create angling opportunities (Heidinger 1975; Welcomme 1988; Jackson 2002; Quinn and Paukert 2009; Ellender et al. 2014). As an opportunistic top carnivore, Largemouth Bass are capable of altering community structure and species abundance (Cambray 2003, Weyl et al. 2010) and are considered among one of the world's worst invaders (Lowe et al. 2000). Despite the global distribution of Largemouth Bass (Welcomme 1988) and extensive work that has characterized the details of alien populations (e.g., age and growth, diet) relative to native ones (Weyl and Hecht 1999; Lorenzoni et al. 2002; Takamura 2007; Britton et al. 2010; Riberio and Collares-Pereira 2010), no work has been conducted to characterize genetic variation or reconstruct invasion routes in Largemouth Bass populations.

In 1928, two separate shipments of Largemouth Bass fingerlings totaling 88 individuals were made from one or more hatcheries in Europe to South Africa. The first shipment contained 45 juveniles that were placed into the Jonkershoek Inland Fish Hatchery in southwest South Africa, near Cape Town, while the second shipment of 43 fingerlings were brought to the Pirie Hatchery near King William's Town in the Eastern Cape (Harrison 1936). Government funded stockings and angler-mediated translocations since this time have expanded the distribution of Largemouth Bass to include much of South Africa (DeMoor and Bruton 1988; Ellender et al. 2014) and portions of southern Africa (Hargrove et al. 2015). Location data from Black Bass tournament angling competitions were used by Hargrove et al. (2015) to identify 66 extant populations of Largemouth Bass present in Namibia, Botswana, Zimbabwe, and Mozambique as well as eight of the nine South African provinces. The widespread distribution of Largemouth Bass across southern Africa in conjunction with their role as an apex predator raises concerns about the potential for deleterious consequences on native invertebrate and fish communities.

Southern Africa is a region characterized by high levels of aquatic endemism and imperilment (Skelton et al. 1995), and Largemouth Bass are a source of concern given their detrimental impacts on the distribution and abundance of native animal communities (Gratwicke and Marshall 2001; Weyl and Lewis 2006; Lowe et al. 2008; Weyl et al. 2010, 2013; Ellender and Weyl 2014; Weyl et al. 2014). For example, significant decreases in Cyprinid minnow (*Barbus* spp.) abundance and diversity were noted in Zimbabwean streams containing Largemouth Bass, which raises concerns given the threatened status of many of these fishes throughout southern Africa (Cambray and Stuart 1985; Gratwicke and Marshall 2001). Furthermore, Weyl et al. (2010) showed significant differences in community structure of aquatic invertebrates found in marginal vegetation in stretches of the Wit River with and without Largemouth Bass. Lastly, Weyl and Lewis (2006) demonstrated predation on migrating estuarine fishes by Largemouth Bass

sampled in a tidal river in southern South Africa. Combined, these studies illustrate the role of Largemouth Bass as apex predators that opportunistically forage on available prey to such an extent that community structure at multiple trophic levels may be altered.

In the present study, we examined contemporary population genetic patterns of variation of Largemouth Bass sampled in a series of South Africa water bodies, and using a combination of microsatellite and DNA sequence data we evaluated competing demographic models to explain observed patterns of genetic variation. Specifically, our goals were to test for evidence of multiple introductions and establish likely introduction pathways associated with different introduced populations. We hypothesized that neutral genetic markers would reveal evidence of historical founder events in the form of limited genetic diversity and that coalescent based Bayesian models would identify a multiple introduction scenario as the best supported model given our observed microsatellite data.

Methods

Sample collection and study sites

Largemouth Bass were collected via hook-and-line angling from four localities in the Eastern Cape and one locality in the Western Cape of South Africa (Fig. 1). A total of 50 individuals per water body were collected with a 1 cm^2 portion of pectoral fin removed from each individual and stored in 95% non-denatured ethanol. Water bodies ranged in surface area from <1 to 260 ha at altitudes from 3 to 660 m above sea level with mean depths ranging from 1 to 14.2 m. The Kowie Weir population occurs on the lower stretches of the Kowie River and consists of a pooled river section behind a 3 m high weir. Groenvlei is a naturally formed lake, whereas Binfield, Mankazana, and Settlers were formed via dams constructed between 23 and 54 years ago. The oldest known population of Largemouth Bass in this study was Groenvlei which was stocked in 1934 (Harrison 1936). One formal stocking record exists for the Kowie River, nine fish planted in 1934 from the Pirie Fish Hatchery, but we hypothesize that it may have received Largemouth Bass from stocked farm dams during spillover events within the river catchment (Harrison 1936). Settlers, Binfield, and Mankazana Dams were built in 1962, 1980, and 1982, respectively. Studies of Largemouth Bass in Mankazana suggest a recent (2006) date of establishment (Taylor et al. 2012).

DNA extraction

DNA was extracted from a 1 mm^2 portion of fin clip using a 96-well silica-based plate extraction method (Ivanova et al. 2006) and a positive and negative control was included in each plate. Template DNA was quantified using a ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) and all samples were brought to a standardized concentration of 20 ng/μL prior to PCR amplification.

Microsatellite amplification

All samples were genotyped at 10 previously developed polymorphic microsatellite loci (Seyoum et al. 2013) in multiplex polymerase chain reactions (PCR; see Trippel et al. (In Press) and Supplemental Table 1 for multiplex conditions and primer details). PCR reactions were performed in 15 μL reactions containing 20 ng template DNA, 7.5 μL 1 × Qiagen Multiplex PCR Master Mix (Qiagen, Valencia, CA), 1.64–5.03 μL ddH20, 0.02–0.2 μM forward primer, and 0.2 μM reverse primer. Thermal cycling conditions for all multiplex combinations were: 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 1 min 30 s, 72 °C for 1 min 30 s, followed by a final extension step at 72 °C for 10 min.

Electrophoresis of PCR products was performed on an ABI 3130 xl (Applied Biosystems, Foster City, CA) using a ROX 500 size standard (Applied Biosystems). Automated allele calls were made in GeneMarker software (SoftGenetics, LLC, State College, PA) and then manually confirmed.

DNA sequencing

Gene sequences were generated for 5–7 fish per population. We PCR amplified a 702 base pair segment of the mitochondrial cytochrome *b* (cyt *b*) gene region using the HA (5′-CAACGATCTCCGG TTTACAAGAC-3′) and LA (5′-GTGACTTGAAA AACCACCGTTG-3′) primer pair (Kassler et al. 2002). Polymerase chain reactions were performed in 25 μL volumes containing ~20 ng/μL DNA

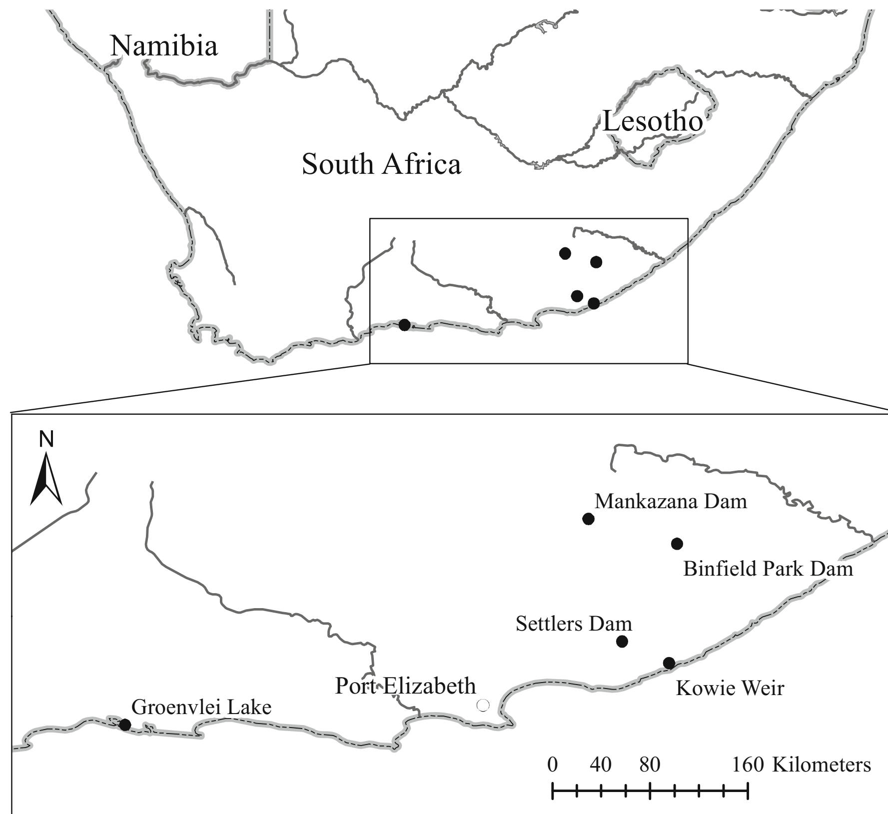

Fig. 1 The distribution of sampling sites (*black dots*) for Largemouth Bass (*Micropterus salmoides*) collections made in South Africa

template, 1× Qiagen Multiplex PCR Master Mix, 0.2 µM forward and reverse primers, and ddH_2O to final volume. Thermal cycling conditions were: 94 °C for 15 min; 35 cycles of 94 °C for 30 s, 53 °C for 90 s, 72 °C for 90 s; with a final extension of 72 °C for 10 min. PCRs contained both positive and negative controls to check for reaction success and contamination, respectively. PCR products were examined under UV light on a 2% agarose gel stained with ethidium-bromide. Positive PCR products with appropriate banding sizes were cleaned using Exo-SAP-IT chemistry (Applied Biosystems, Foster City, CA, USA).

Bidirectional sequences were generated using BigDye terminator sequencing chemistry (Applied Biosystems) in 6.75 µL reactions, containing 0.83 µL Terminator v3.1 Ready Reaction mix, 1.67 µL 5× reaction buffer, 0.22 µL of primer, 1 µL of PCR product, and 3.92 µL ddH_2O. Products were cleaned using 0.2 µm Sephadex columns (Princeton Separations, Freehold, NJ, USA) prior to electrophoresis on a 3130 ×1 Genetic Analyzer (Applied Biosystems). Chromatograms were edited and assembled using Geneious v 6.1.2 (http://www.geneious.com; Kearse et al. 2012).

Population genetic diversity: microsatellite data

The number of alleles per locus, observed (H_O) and expected (H_E) heterozygosity, and the number of private alleles per population were calculated using GenAlEx v 6.5 (Peakall and Smouse 2006). Allelic richness was calculated using rarefaction techniques implemented in HP-Rare (Kalinowski 2005). Both deviations from Hardy–Weinberg Equilibrium (HWE) and the presence of linkage disequilibrium (LD) were tested for using Genepop (Rousset 2008) with the following parameters: dememorization = 5000, batches = 5000, iterations per batch = 1000. Corrections for multiple comparison tests were performed using the False Discovery Rate (FDR; Benjamini and Hochberg 1995). We tested for the presence of null alleles using MICROCHECKER software version 2.2.3 (Van Oosterhout et al. 2004).

To assess patterns in genetic differentiation, we calculated F_{ST}, G'_{ST} (Hedrick 2005), and Jost's D (Jost 2008) using GenAlEx with 9999 permutations and 9999 bootstrap iterations. Multiple metrics of genetic differentiation were included to account for the potential influences of variation in mutation rates and heterozygosity levels that may bias individual metrics (Leng and Zhang 2011). An analysis of molecular variance (AMOVA) was performed using ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) to determine sources of genetic variation among populations, among individuals within populations, and within individuals. Patterns in genetic diversity across populations were visualized via Principal Component Analysis (PCA) using the *ade4* and *adegenet* packages (Thioulouse et al. 1997; Jombart 2008) in R v 3.3 (R Development Core Team 2016). The PCA was performed using microsatellite allele frequencies with missing data replaced by mean frequency values (corresponding to 215 of 7657 values or 2% of all genotypes). Principal component analysis was performed on a correlation matrix of scaled (i.e., normalized) allele frequencies. Our graphical output displayed the absolute variance (i.e., eigenvalues) explained by each of the principal components and X and Y labels represent the percentage of total variance explained by the first and second components, respectively. An unrooted dendrogram was constructed based on the Edward's genetic distance (Edwards 1971) between population pairs using 10,000 bootstrap iterations as implemented in the R package *poppr* (Kamvar et al. 2014).

We tested for a genetic signature associated with a recent population bottleneck using the software program BOTTLECK (Piry et al. 1999) using the 'heterozygosity-excess' test developed by Cornuet and Luikart (1996). Specifically, we tested if the focal populations experienced a contemporary bottleneck based on interpretation of Wilcoxon's Test assuming a two-phase model (TPM). We assumed mutations were comprised of 95% single-step and 5% multiple-step and a variance among multiple steps of 12 following the author's recommendation (Piry et al. 1999). Outputs were based on a total of 5000 iterations.

Population genetic diversity: DNA sequence data

Gene sequences generated from South Africa Largemouth Bass samples were combined with cytochrome *b* sequences for both Largemouth and Florida Bass downloaded from GenBank (Accessed: 10 Aug 2016; Supplemental Table 2). Sequences downloaded from GenBank included 14 *M. floridanus* and 17 *M. salmoides* gene sequences from their native and introduced range. All sequences were aligned using the ClustalW algorithm using default parameters within Mega v 5.2.2 (Tamura et al. 2011). Metrics of population genetic diversity including the number of haplotypes (n_H) number of private haplotypes (n_{PH}), haplotype diversity (H), and nucleotide diversity (π; Nei 1987) were generated using ARLEQUIN 3.5.1.2. A minimum spanning tree based on unique cyt *b* haplotypes from North America and South Africa was created using Population Analysis with Reticulate Trees (PopART; http://popart.otago.ac.nz).

Invasion reconstruction

The probabilities associated with different introduction scenarios were estimated using microsatellite data in the software program DIYABC v2.1.0 (Cornuet et al. 2014). We sought to answer if multiple introductions occurred and establish what the most likely routes of establishment were among introduced populations. We generated likelihoods associated with four different models, all of which included an unsampled ancestral source population (Fig. 2). In scenario 1, all populations were independently derived from the same, common ancestral source at the same point in time. In scenarios 2, 3, and 4, we postulated that Kowie Weir was introduced independently from all other populations. The justification for a 'separate introduction' scenario was based on historical records that identified Kowie Weir was stocked with fish from a different hatchery relative to the other populations, and that the two separate fish hatcheries (Pirie and Jonkershoek) received distinct, independent shipments of Largemouth Bass from Europe (Harrison 1936). In scenario 2, Settlers, Mankazana, and Binfield populations were independently introduced (at different times in history) from the oldest known population, Groenvlei. This differed from scenario 3, in which Settlers, Mankazana, and Binfield were derived independently from Groenvlei at the same time. A fourth scenario involved an admixture event between Groenvlei and Settlers giving rise to Mankazana and Binfield with rates of exchange between populations being equal. This scenario was designed to model the

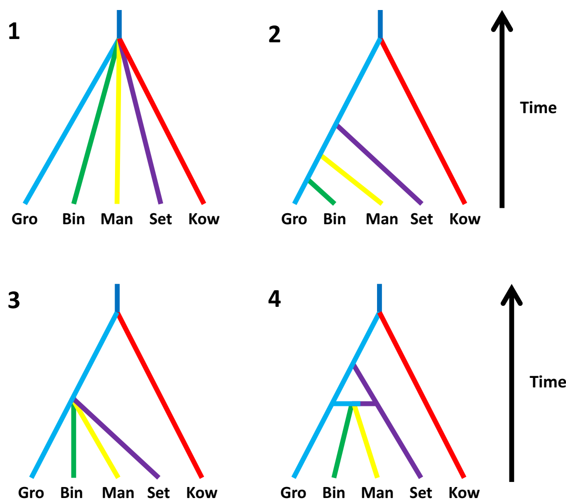

Fig. 2 Introduction scenarios tested using Approximate Bayesian Computation analysis of population histories based on microsatellite data collected from *Micropterus salmoides* sampled in South Africa: **1** All populations were independently derived from a common ancestral population; **2** Independent introductions with Binfield, Mankazana, and Settlers being independently introduced from the oldest introduced population (Groenvlei); **3** Independent introductions with Binfield, Mankazana, and Settlers populations being derived from Groenvlei; **4** Independent introductions Binfield and Mankazana arising from an admixture event between Groenvlei and Settlers populations. Abbreviations of individual populations are Groenvlei (Gro), Binfield (Bin), Mankazana (Man), Settlers (Set), and Kowie Weir (Kow). Population bottlenecks were modeled to have occurred at each establishment event. The unsampled populations in all scenarios are represented by the *dark blue line*

prospect of fish transfers between pre-existing populations (Groenvlei and Settlers) being used to establish new populations; as illegal, angler-mediated translocations are known to occur in South Africa (Ellender et al. 2014). Population bottlenecks were modeled to have occurred at the establishment of each new population.

For all analyses, prior parameters were drawn from a uniform distribution and defined as follows: $100 < N < 10{,}000$; $10 < t_1 \leq t_2 \leq t_3 \leq t_4 \leq 100$; $1 \leq Nb \leq 100$, $1 \leq db \leq 100$, $0.001 < r < 0.999$, where N is the current effective population size, t denotes time in generations, Nb is the number of founders used to establish each new population, db is the duration of bottleneck in generations, and r is the population admixture rate. Mutation model priors for microsatellite markers were set to default values as were the selected distributions, minimum, and maximum values. Summary statistics for model comparison included the mean number of alleles, mean genic diversity, and mean size variance for the '1-sample' statistics and F_{ST} for '2-sample' statistics. A total of one million simulated datasets were generated for each scenario, for a total of four million simulations. Posterior probabilities of scenarios were calculated using linear discriminant analysis on summary statistics via the direct and logistic regression approach. The error rates associated with specific scenarios were evaluated by analyzing 100 simulated pseudo-observed data sets (pods) using parameter values drawn from one individual scenario (e.g., scenario 1). The number of times the selected scenario was falsely rejected (i.e., posterior probabilities were higher for one of the other scenarios) was then divided by 100 (i.e., the total number of pseudo-observed data sets), and this was considered the type I error rate (Cornuet et al. 2014). The type II error rate, or the percentage of the 100 pods in which an untrue scenario received the highest posterior support, was also calculated for each individual scenario.

Results

Population genetic diversity: microsatellite data

We omitted one microsatellite locus (Msa 28) from down-stream analyses due to inconsistent peak morphology leading to scoring error. Deviations from Hardy–Weinberg Equilibrium, specifically deficiencies in heterozygotes, were detected at one locus in the Kowie Weir population (Msa 22), and at a singular locus in both Groenvlei and Mankazana (Msa 6; adjusted P value $= 0.002$; see Supplemental Table 3 for genetic characteristics by locus and population). MICROCHECKER identified the presence of null alleles at locus Msa 6 in Mankazana and Groenvlei populations. No single locus or population departed consistently from Hardy–Weinberg expectations which suggested locus- and population-specific factors were unlikely sources for the observed deviations. Three pairs of loci were in genotypic disequilibrium in the Kowie Weir population only (loci combinations: Msa 6 $\times$ Msa 24, Msa 6 $\times$ Msa 5, Msa 5 $\times$ Msa 24; adjusted P value $= 0.0007$).

The average proportion of polymorphic loci per population ranged from 0.22 to 1 (mean $\pm$ SE,

0.47 ± 0.15) and only two populations contained private alleles (Binfield—1, Kowie Weir—15). The number of alleles per locus ranged from 1 to 4 (mean ± SE, 1.80 ± 0.40) with average multilocus observed heterozygosity ranging from 0.05 to 0.49 (mean ± SE, 0.16 ± 0.08; Table 1). The total number of alleles detected across all loci was highest for Kowie Weir (30), followed by Binfield (16), Settlers (12), Mankazana (12), and Groenvlei (11). In Mankazana, Settlers, Binfield, and Groenvlei populations, allele frequencies were typically dominated by a singular allele that was common to all populations (Supplemental Table 4), and when more than one allele was present those alleles typically occurred at low frequencies.

Tests of genetic differentiation (F_{ST}, G_{ST} or Jost's D), were highly correlated and revealed lower levels of distinction among the Settlers, Mankazana, Binfield, and Groenvlei populations (mean pairwise F_{ST} ± SE, 0.09 ± 0.02; range = 0.03–0.19) relative to comparisons that involved Kowie Weir (mean pairwise F_{ST} 0.27 ± 0.01; range = 0.24–0.30). Across all loci, pairwise estimates of F_{ST} were all highly significant (max $P = 0.006$; Table 2). Global estimates of G_{ST} ranged from 0.05 to 0.46 and Jost's D from <0.01 to 0.34, all of which comparisons were significant (G_{ST}, max $P = 0.006$; Jost's D, max $P = 0.007$). Similar to the patterns observed with F_{ST}, both G_{ST} and Jost's D displayed lower levels of differentiation among pairwise comparisons of Groenvlei, Binfield, Settlers Dam, and Mankazana (mean G_{ST} ± SE = 0.15 ± 0.01; Jost's $D = 0.02 \pm 0.02$) relative to those involving Kowie Weir (mean G_{ST} ± SE = 0.42 ± 0.03; Jost's $D = 0.33 \pm 0.01$). A dendrogram of genetic distances strongly supported the division between Kowie Weir relative to all other populations (bootstrap value = 100), and groupings within the

Table 1 Summary statistics by population for microsatellite loci and mitochondrial sequence data from invasive populations of Largemouth Bass (*Micropterus salmoides*) collected from five South African water bodies

Population	ID	Microsatellite DNA							Mitochondrial DNA					
		n	P	A_p	A_R	N_A (±SD)	H_O (±SD)	H_E (±SD)	n	n_H	n_{PH}	H_F	h	π
Binfield	Bin	49	0.56	1	1.74	1.78 (0.28)	0.14 (0.07)	0.14 (0.07)	5	2	1	A (0.80) B (0.20)	0.40	0.0005
Groenvlei	Gro	50	0.22	0	1.22	1.22 (0.15)	0.04 (0.04)	0.06 (0.05)	6	2	1	A (0.83) C (0.17)	0.33	0.0004
Kowie River	Kow	50	1.00	15	3.32	3.33 (0.24)	0.49 (0.06)	0.52 (0.07)	5	1	0	A (1.00)	0.00	0.0000
Mankazana	Man	49	0.33	0	1.32	1.33 (0.17)	0.05 (0.04)	0.10 (0.06)	7	1	0	A (1.00)	0.00	0.0000
Settlers	Set	49	0.22	0	1.33	1.33 (0.24)	0.10 (0.06)	0.12 (0.08)	7	1	0	A (1.00)	0.00	0.0000

Listed are the population name, sample ID, microsatellite results [sample size (n), proportion of polymorphic loci (P), number of private alleles (A_P), allelic richness (A_R), average number of alleles per locus (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E)] and results from mitochondrial sequence data [sample size (n), number of haplotypes (n_H), number of private haplotypes (n_{PH}), haplotypes and their frequencies within populations (H_F), haplotype diversity (h), and nucleotide diversity (π)

Table 2 Results from pairwise F_{ST} calculations (below diagonal) based on microsatellite genotypes collected from *Micropterus salmoides* introduced into South Africa

	Binfield	Groenvlei	Kowie Weir	Mankazana	Settlers
Binfield	–	*<0.001*	*<0.001*	*<0.001*	*0.003*
Groenvlei	0.130	–	*<0.001*	*0.007*	*<0.001*
Kowie Weir	0.240	0.300	–	*<0.001*	*<0.001*
Mankazana	0.072	0.042	0.272	–	*<0.001*
Settlers	0.030	0.185	0.259	0.072	–

P values are listed above diagonal and italicized P values indicate significant at the 0.05 level

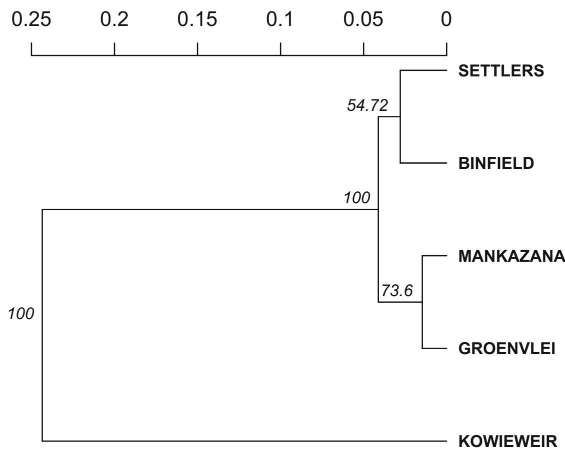

Fig. 3 Unrooted dendrogram displaying Edward's genetic distances between introduced populations of *Micropterus salmoides* from South Africa. Bootstrap values are displayed on nodes based on 10,000 iterations

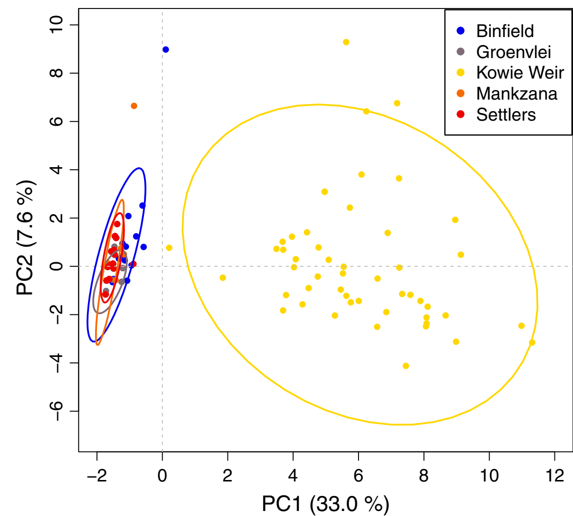

Fig. 4 Results from Principal Component Analysis of microsatellite genotypes generated from five populations of Largemouth Bass (*Micropterus salmoides*) sampled from South Africa. *Ellipses* represent 95% confidence intervals and values on the X- and Y-axes represent the absolute variances explained by the first and second eigenvalues respectively. Percentage values in parentheses correspond to the percentage of total variation explained by the first two components

Binfield, Groenvlei, Mankazana, and Settlers populations were less well supported (Fig. 3).

Analysis of Molecular Variance (AMOVA) indicated that a large and significant proportion of the genetic variation (34.5%, $P < 0.001$) was distributed among Largemouth Bass populations, while the largest proportion of variance was distributed within individuals (57.6%, $P < 0.001$), and 7.9% of divergence ($P < 0.001$) was explained by variation among individuals within populations (Table 3). Principal Component Analysis (PCA) revealed that the among-population variance was due to differentiation between Kowie Weir and to all other South Africa Largemouth Bass populations examined (Fig. 4). The first two principal components (PC) explained 33.0 and 7.6% of the total variation present in allele frequencies, and 95% confidence clusters surrounding individuals sampled from Binfield, Groenvlei, Mankazana, and Settlers exhibited significant overlap. The wider and non-overlapping spread of Kowie Weir individuals among PC1 and PC2 may be reflective of the greater variance in allele frequencies associated with greater allelic diversity and alleles private to that specific population.

Based on outputs from tests for population bottlenecks we were unable to reject the null hypotheses of 'no heterozygosity excess' in any population (Binfield, $P = 0.95$; Groenvlei, $P = 0.25$; Kowie, $P = 0.21$; Mankazana, $P = 0.19$, Settlers, $P = 0.13$). Mode shifts in allele distributions were however observed in Mankazana and Settlers populations, suggesting evidence of a recent population bottleneck (Luikart et al. 1998). It is recommended that 8–10 polymoprhic loci be used in bottleneck tests in order to avoid unreasonably high type I error rates

Table 3 Analysis of molecular variance (AMOVA) results performed on *Micropterus salmoides* microsatellite data collected from introduced population in South Africa

Source of variation	Sum of squares	Variance components	Percentage variation	Fixation Index	*P* value
Among populations	177.73	0.45	34.5	$F_{IS} = 0.11$	<0.001
Among individuals	224.74	0.10	7.9	$F_{ST} = 0.35$	<0.001
Within individuals	182.00	0.76	57.6	$F_{IT} = 0.42$	<0.001
Total	584.463	1.32			

(Luikart et al. 1998; Piry et al. 1999). All loci were polymorphic in the Kowie River population (9), however no other population had more than five variable loci suggesting caution when interpreting test results.

Population genetic diversity: DNA sequence data

The final cytochrome *b* alignment consisted of 702 base pairs from 30 individuals representing 5 populations (Binfield, $n = 5$; Groenvlei, $n = 6$; Kowie Weir, $n = 5$; Mankazana, $n = 7$; Settlers, $n = 7$). A total of three haplotypes were identified, with a singular haplotype being present in all populations and occurring at a high frequency (28 of 30 individuals). Only two private haplotypes were recovered, one each in Groenvlei and Binfield. Mankazana, Settlers and the Kowie Weir populations shared a single haplotype. Haplotype diversity for Binfield was 0.40 and 0.33 for Groenvlei. Values of nucleotide diversity were 0.0005 and 0.0004 for Binfield and Groenvlei, respectively (Table 1).

A total of 31 sequences were retrieved for *Micropterus salmoides* and *M. floridanus* from GenBank. The minimum spanning network nested South African haplotypes within a cluster of Largemouth Bass haplotypes primarily from the northern United States (Wisconsin, Maryland) and Ontario (Fig. 5). The most common haplotype from South Africa (93% of samples; denoted A in Table 1 and in Fig. 5) was identical to a sequence generated for an individual collected in the Zekiah Swamp Run, Maryland. The haplotypes B and C (Table 1; Fig. 5) from South Africa were placed among *M. salmoides* sequences from across their native and non-native range (i.e., Alabama, Louisiana, New Mexico, North Carolina, Texas). In particular, haplotypes B and C were most similar (i.e., were separated by the fewest number of mutational differences between sequences) to Largemouth Bass sampled in Ontario and Wisconsin.

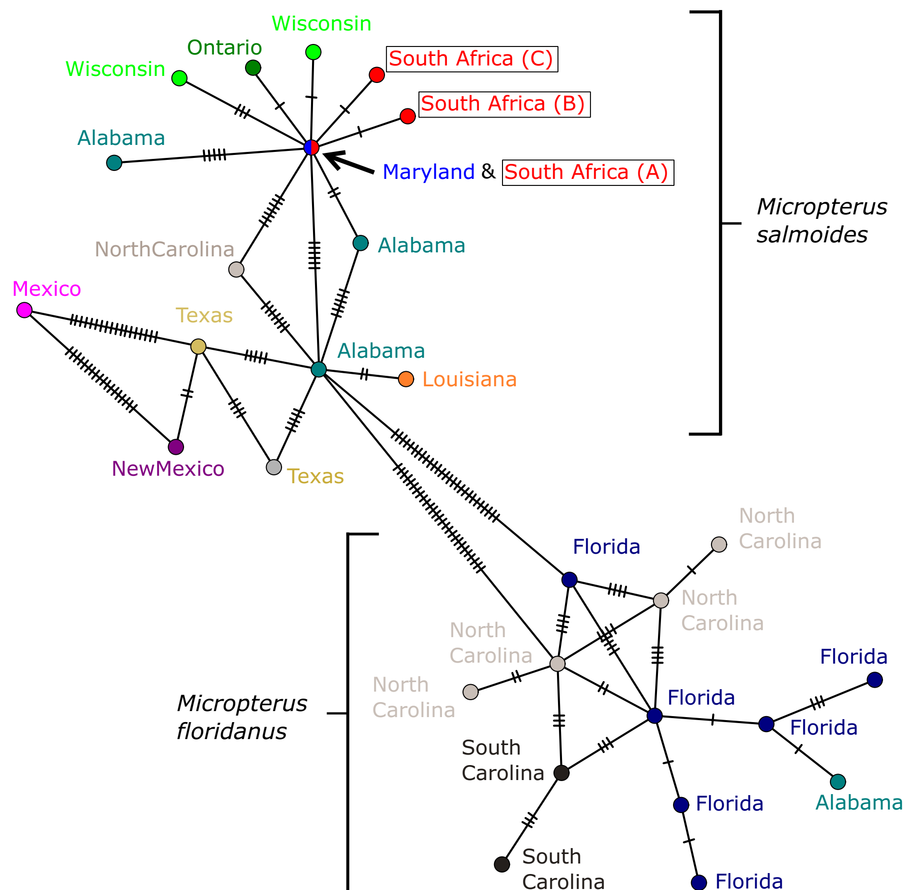

Fig. 5 Minimum spanning network of Largemouth Bass (*Micropterus salmoides*) and Florida Bass (*M. floridanus*) cytochrome *b* haplotypes sampled from the United States (native) and South Africa (introduced). Individual *tick marks* denote one base pair difference between haplotypes. *Letters* in hypotheses represent haplotypes referenced in Table 1. Note Maryland and South African haplotype A are identical

Table 4 Confidence in scenario selection based on outputs from DIYABC for proposed introduction pathways of Largemouth Bass (*Micropterus salmoides*) into South Africa

Scenario	Posterior probability	95% Credibility interval	Type I error	Type II error
1	0.0000	0.0000–0.0000	0.11	0.47
2	0.8966	0.8922–0.9010	0.06	0.07
3	0.0414	0.0385–0.0444	0.49	0.22
4	0.0620	0.0586–0.0654	0.25	0.15

See Fig. 2 for visual description of introduction scenarios

Invasion reconstruction

Output from Approximate Bayesian Computation consistently provided higher posterior probabilities for introduction scenarios involving multiple independent introductions, relative to a single source event (Table 4). Scenario 2, in which individuals from Binfield, Mankazana, and Settlers were independently introduced from Groenvlei, while the Kowie Weir population received propagules from a separate introduction had the highest posterior probability (0.8966, 95% CI 0.8922–0.9010). Scenario 1, in which all populations were independently derived from a common ancestral population through a single introduction received the lowest posterior probability support. Scenario 4 in which multiple introduction events occurred with an admixture event occurring between Groenvlei and Setters which then gave rise to Binfield and Mankazana received higher posterior support than Settlers, Mankazana, and Binfield being derived independently from the most ancestral lake population, Groenvlei (Scenario 3). Type I and II error rates for the best supported model were 0.06 and 0.07 respectively which indicated modest accuracy in model selection and a somewhat limited ability to confidently differentiate between competing models, perhaps due to the low levels of variability contained in our microsatellite dataset.

Discussion

Despite the global distribution (Welcomme 1988) and profound economic importance of Largemouth Bass as a sport fish (Schramm and Hunt 2007; U.S. Department of Interior 2011; Driscoll et al. 2012), almost no studies have examined the processes and patterns associated with the establishment of non-native Largemouth Bass populations. Our study has revealed that low levels of genetic diversity associated with documented introductions of small number of propagules is not universal, with Kowie Weir being highly differentiated and highly diverse. Bai et al. (2008) quantified levels of microsatellite heterozygosity and allelic richness in Largemouth Bass introduced into China for aquaculture purposes, and recorded significant reductions in both allelic richness (39–64%, depending on comparison) and heterozygosity (19–45%) relative to native (but not source) populations. They proposed that reductions in effective population size explained the low number of alleles per locus and levels of heterozygosity (mean: $N_A = 2.17$; $H_O = 0.37$), and mode shifts in allele frequencies indicated evidence of a founder event. In South Africa bass, levels of heterozygosity and the number of alleles per locus were substantially lower than those observed in China (mean: $N_A = 1.80$; $H_O = 0.16$) and we too attribute the observed levels of genetic diversity to a founder event. A limited number of bass were imported into South Africa (88), and in instances where records of fish transfers within South Africa exist, we know that small numbers of propagules were typically used (e.g., Groenvlei, 18 fingerlings; Harrison 1936).

We considered a number of potential scenarios that might explain the low levels of nuclear genetic diversity observed in Largemouth Bass sampled in South Africa. First we considered the prospect that reduced diversity was an artifact of locus selection (i.e., that our selected genetic markers displayed inherently low levels of polymorphism). However, the same markers applied to naturally outbreeding populations of *Micropterus* spp. displayed significantly higher levels of variability (Barthel et al. 2010;

Seyoum et al. 2013; Hargrove and Austin 2016; Trippel et al. (In Press); JS Hargrove unpublished data). Two Largemouth Bass populations in Wisconsin and four in Oklahoma assayed using the same nine markers as in this study displayed higher average (i.e., grand mean across all loci and populations) number of alleles (Oklahoma: $N_A = 5.82$, Wisconsin: $N_A = 3.88$, South Africa: $N_A = 1.80$), observed heterozygosity (Oklahoma: $H_O = 0.54$, Wisconsin: $H_O = 0.31$, South Africa: $H_O = 0.16$), and expected heterozygosity (Oklahoma: $H_E = 0.55$, Wisconsin: $H_E = 0.33$, South Africa: $H_E = 0.19$) relative to South African populations (Seyoum et al. 2013; JS Hargrove unpublished data). We also considered population size as a potential explanation for observed patterns in genetic diversity; however, formal estimates of population size were not available. Kowie Weir boasted the highest observed levels of nuclear genetic diversity but was potentially the smallest population examined. This was based on the fact that Largemouth Bass in the Kowie River inhabit a pooled river section that is only 10 m wide $\times$ 2 m deep, which contrasts with Binfield that has a surface area of 260 hectares and a large littoral zone. Thus greater amounts of available habitat (and by extension potential population size) alone may poorly explain observed levels of genetic diversity but instead may be explained by historical demographic events such as founder events.

Based on the available evidence, we argue the low levels of genetic diversity observed in our study populations were the result of a historical (i.e., at the introduction of bass to South Africa) and not contemporary founder event (i.e., at the establishment of individual populations). A limited number of alleles (16) were recovered from Binfield, Groenvlei, Mankazana, and Settlers populations, and of these alleles only one was private (i.e., found in one but not other populations). That so few private alleles were detected suggests a common source harboring limited genetic diversity. Tests for the presence of contemporary genetic bottlenecks were not statistically significant, further corroborating the potential for a historical founder event rather than multiple recent bottlenecks. The statistical power to detect bottleneck however may be limited when sample sizes are modest (<100) and few markers are assayed (<16 microsatellite loci; Peery et al. 2012). If individual populations had historically contained higher allelic diversity than what we have measured, the expectation is that genetic drift would have resulted in a random, and not systematic, pattern of allele frequencies. Drift should lead to different alleles being fixed across population replicates, thus the fact that monomorphic loci were fixed for the same variants in each population likely reflects a single founder event occurring at the time of introduction in 1928.

The overall pattern of limited genetic diversity observed in our microsatellite data was also reflected in our mitochondrial sequence data. In particular, we recovered only a small number of unique cytochrome *b* haplotypes (3), and a single haplotype was common to all populations and almost all individuals. Low haplotype and nucleotide diversity in introduced fish species are not necessarily rare; for example, PCR–RFLP analysis of the ND1 and D-loop regions of 1783 Bluegill Sunfish (*Lepomis macrochirus*) introduced into 72 populations across Japan and Korea revealed only five distinct haplotypes (Kawamura et al. 2006). Interestingly, Kawamura et al. (2006) noted that haplotype diversity was negatively correlated with establishment date such that more recently established populations displayed lower levels of diversity. In South Africa populations we failed to observe changes in genetic diversity as a function of establishment date; specifically, the number of haplotypes recovered from Groenvlei and Binfield were highest (2 each), which corresponds to the oldest (Groenvlei 1934) and second youngest (Binfield: since 1980s) Largemouth Bass populations examined (Harrison 1936). One potential limitation of inferences made using our mitochondrial dataset was the modest number of sample sizes; although, other studies of introduced fishes have revealed similar, low levels of haplotype diversity despite high numbers of sample sizes (e.g., Kinziger et al. 2014).

The movement of Black Basses (a collective for members of the genus *Micropterus*) within the United States has a long history dating back to the mid-1800's (Long et al. 2015), and many modern populations of Largemouth Bass in parts of the United States are the result of extra-limital translocations (MacCrimmon and Robbins 1975). A Largemouth Bass haplotype recovered from the Zekiah Swamp Run (Maryland) by Near et al. (2003) was identical to the most common sequence recovered from South Africa, suggesting this region as a potential source of origin. However, no records exist of Largemouth Bass in Maryland prior to

1874 (Truitt et al. 1929; Powell 1967), yet by 1889 bass were well established as a sport fish (Powell 1967) and are currently widespread throughout the state (Lee et al. 1981). Sequences from Lake Opinicon in Ontario and Lipsett Lake in Wisconsin were also highly similar (although not identical) to haplotypes recovered from South Africa, both of which occur within their native range (Becker 1983; MacCrimmon and Robbins 1975). Ultimately, the identification of a specific source of origin for South African Largemouth Bass may remain an elusive goal as hatchery propagation of black bass occurs in at least 31 U.S. states (Noble 2002), millions of juvenile bass are stocked annually (Heidinger 2000), and many stocking programs intentionally introduce non-native alleles (e.g., Florida Bass alleles) for specific objectives such as increasing the potential maximum growth (Philipp et al. 1983; Myers and Allen 2005). If Largemouth Bass in South Africa originated from a hatchery strain stocked in Maryland, this two-stage founder event may also serve to explain the limited levels of genetic diversity observed.

Evidence from principal component analysis and tests of genetic differentiation identified two genetic groups within South African Largemouth Bass populations, supporting the hypothesis of multiple independent introductions. Genetic differentiation among sample sites consistently identified Kowie Weir as being highly differentiated relative to all other populations, regardless of metric (F_{ST}, G_{ST} or Jost's D), and Kowie Weir formed a non-overlapping cluster in our PCA. Further support for a scenario involving multiple introductions was provided by ABC modeling of population history, which returned the highest posterior support for a scenario in which Kowie Weir was independently derived relative to Binfield, Settlers, Mankazana, and Groenvlei populations. These observed differences may be explained with several possible scenarios with several possible scenarios; first, the two known stocks of Largemouth Bass imported into South Africa were genetically distinct and placed into discrete sets of lakes, second, that Kowie Weir is a combination of both stocks, third, or that the elevated levels of genetic diversity in Kowie Weir were a byproduct of introgression with congeneric species.

In the first two scenarios, the founding stock of bass used to seed Binfield, Mankazana, Groenvlei, and Settlers was derived from one of the two imported shipments while Kowie Weir was either a product of the other source or a combination of both sources. The latter of these two scenarios, Kowie Weir possessing alleles from both hatcheries, is feasible given records that indicate shipments of fingerlings were made from both hatcheries to areas near Kowie Weir in the early 1930's (42 fingerlings from the Pirie Fish Hatchery to the Albany Angling Association of Grahamstown in 1933, and 24 fingerlings from the Jonkershoek Fish Hatcheries to a Grahamstown resident in 1934) and that a single stocking of nine Largemouth Bass from Pirie Hatchery were placed into to the Kowie Weir (Harrison 1936). Although the specifics of where the Jonkershoek fish were planted are unknown, distributions of hatchery fingerlings during this period were typically made to private dam owners. The Kowie River has a 580 km^2 catchment that begins just south of Grahamstown (Watling and Watling 1983) and fish from Jonkershoek Hatchery could have washed down from private dams into the Kowie River. That all but one of the microsatellite alleles present in Binfield, Groenvlei, Mankazana, and Settles populations was also detected in Kowie Weir supports this scenario. Records of distribution to the other water bodies are scarce, but 18 fingerlings were stocked in December 1934 into Groenvlei from the Jonkershoek hatchery (Harrison 1936). Early reports suggest fingerling production at the Jonkershoek Fish Hatchery was significantly higher than Pirie with over 2000 black bass distributed to 25 districts across southern South Africa from Jonkershoek over a 4 year period (1930–1933). Pirie Hatchery on the other hand, distributed just 183 fingerlings to 9 locations over a 2-year period (1933–1934; Harrison 1936). If variation in hatchery production persisted through time, dams such as Binfield, Mankazana, and Settlers would likely have received fish from the more prolific Jonkershoek Hatchery.

An alternative explanation for the differentiation among populations involves Kowie Weir being an admixed population of *M. salmoides* and another *Micropterus* species such as Florida Bass, *M. floridanus*, or Spotted Bass, *M. punctulatus*. Florida Bass were introduced into Southern Africa in 1980 and subsequent informal angler stockings have expanded their distribution to include many provinces in the country (DeMoor and Bruton 1988; McCafferty et al. 2012). Spotted Bass were first introduced into South Africa in 1939 (DeMoor and Bruton 1988), and

although their distribution is known to be limited, they have been recorded in the Kowie River (OLFW unpublished data) and from freshwater systems adjacent to the Kowie River (James et al. 2008). Mitochondrial haplotypes recovered from Kowie Weir were, however, not reflective of introgression between closely related species; suggesting introgression as an unlikely source of the observed elevated levels of genetic diversity in the Kowie Weir population.

A second result gleaned from coalescent based models of introduction history was that the best supported scenario involved repeated introductions from Groenvlei into Binfield, Mankazana, and Settlers water bodies. This scenario was generated based on the historical records which indicate Groenvlei was established decades in advance of the construction of Binfield, Mankazana, or Settlers Dams (Harrison 1936; Taylor et al. 2012). We did not observe substantial differences in levels of genetic diversity (Supplemental Table 3) between these populations and allele frequencies were highly similar (Supplemental Table 4). This may have resulted from sufficient numbers of individuals being transferred from Groenvlei to other water bodies to transfer significant quantities of available genetic diversity (Allendorf and Lundquist 2003). Interestingly, Groenvlei harbored the lowest levels of allelic diversity of all populations, and there appears no clear explanation for this other than potential genetic drift or population bottlenecks subsequent to fish transfers.

Conclusion

Results from this study provide a detailed scenario of the historical events explaining the genetic characteristics of modern populations of the invasive apex predator, the Largemouth Bass, inhabiting select South African waters. These data add to the growing body of knowledge concerning the molecular patterns in successful species invasions, and highlight the ability of freshwater fish populations to persist for over 80 years despite being founded with highly limited number of propagules and possessing limited levels of neutral genetic diversity caused by genetic drift. Our study provides insightful descriptions of molecular patterns in a globally significant invasive species and can serve as a baseline for future comparisons. Furthermore, in the absence of detailed stocking records we were able to reconstruct the likely hatcheries used to seed a series of water bodies across southern South Africa. Although there are examples of members of the genus *Micropterus* being successfully eradicated from invaded environments (Weyl et al. 2014), efforts to eradicate established populations are unlikely to be successful except in small or isolated systems (Loppnow et al. 2013). Future studies should consider combining cutting-edge molecular methods (e.g., Genotyping-By-Sequencing such as RAD-seq; Baird et al. 2008) with environmental and biological data to identify genomic adaptations that have contributed to the success of Largemouth Bass as an aquatic invader.

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References

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17:24–30

Bagley J, Mayden R, Roe K et al (2011) Congeneric phylogeographical sampling reveals polyphyly and novel biodiversity within black basses (Centrarchidae: Micropterus). Biol J Linn Soc 104:346–363

Bai J, Lutz-Carrillo DJ, Quan Y et al (2008) Taxonomic status and genetic diversity of cultured largemouth bass *Micropterus salmoides* in China. Aquaculture 278:27–30

Baird NA, Etter PD, Atwood TS et al (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3:e3376

Barthel BL, Lutz-Carrillo DJ, Norberg KE et al (2010) Genetic relationships among populations of Florida Bass. Trans Am Fish Soc 139:1615–1641

Becker GC (1983) The fishes of Wisconsin. University of Wisconsin Press, Madison

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Britton JR, Harper DM, Oyugi DO (2010) Is the fast growth of an equatorial *Micropterus salmoides* population explained by high water temperature? Ecol Freshw Fish 19:228–238

Cambray JA (2003) Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. Hydrobiologia 500:217–230

Cambray JA, Stuart CT (1985) Aspects of the biology of a rare redfin minnow, *Barbus burchelli* (Pisces, Cyprinidae), from South Africa. S Afr J Zool 20:155–165

Chapple DG, Whitaker AH, Chapple SNJ et al (2012) Biosecurity interceptions of an invasive lizard: origin of stowaways and human-assisted spread within New Zealand. Evol Appl 6:324–339

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Cornuet J-M, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC Bioinform 11:401

Cornuet J-M, Pudlo P, Veyssier J et al (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics 8:1187–1189

Cristescu ME (2015) Genetic reconstructions of invasion history. Mol Ecol 24:2212–2225

Darling JA (2015) Genetic studies of aquatic biological invasions: closing the gap between research and management. Biol Invasions 17:951–971

DeMoor I, Bruton M (1988) Atlas of Alien and translocated indigenous aquatic animals in Southern Africa. CSIR, National Scientific Programmes Unit, Delhi, p 317

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449

Dlugosch KM, Anderson SR, Braasch J et al (2015) The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Mol Ecol 24:2095–2111

Driscoll MT, Schramm H, Hunt KM (2012) Trends in fishery agency assessments of black bass tournaments in the southeastern United States. In: Proceedings of the annual conference of Southeastern association of fisheries and wildlife agencies, pp 25–32

Edwards AWF (1971) Distances between populations on the basis of gene frequencies. Biometrics 27:873–881

Ellender BR, Weyl OL (2014) A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquat Invasions 9:117–132

Ellender BR, Woodford DJ, Weyl OLF et al (2014) Managing conflicts arising from fisheries enhancements based on non-native fishes in southern Africa. J Fish Biol 85:1890–1906

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: Why, how and so what? Mol Ecol 19:4113–4130

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Gaskin JF, Bon M-C, Cock MJW et al (2011) Applying molecular-based approaches to classical biological control of weeds. Biol Control 58:1–21

Gratwicke B, Marshall BE (2001) The relationship between the exotic predators *Micropterus salmoides* and *Serranochromis robustus* and native stream fishes in Zimbabwe. J Fish Biol 58:68–75

Hargrove JS, Austin JD (2016) Parentage and mating patterns in a Florida Largemouth Bass (*Micropterus salmoides floridanus*) hatchery. Aquac Res. doi:10.1111/are.13051

Hargrove JS, Weyl OLF, Allen MS et al (2015) Using tournament angler data to rapidly assess the invasion status of alien sport fishes (*Micropterus* spp.) in Southern Africa. PLoS ONE 10:e0130056

Harrison AC (1936) Black bass in the Cape Province: second report on the progress of American largemouth black bass (*Micropterus salmoides,* Lacepede). South Africa Division of Fisheries Investigational Report, Pretoria, p 119

Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633–1638

Heidinger RC (1975) Life history and biology of the largemouth bass. In: Stroud RH, Clepper H (eds) Black bass biology and management. Sport Fishing Institute, Washington, pp 11–20

Heidinger RC (2000) A White paper on the status and needs of Largemouth bass culture in the North Central Region. North Central Region Aquaculture Center, East Lansing

Ivanova NV, Dewaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Mol Ecol Notes 6:998–1002

Jackson DA (2002) Ecological effects of *Micropterus* introductions: the dark side of black bass. In: Phillip DP, Ridgway MS (eds) Black bass: ecology, conservation, and management. American fisheries society symposium 31. Bethesda, Rockville, pp 221–232

James NC, Whitfield AK, Cowley PD (2008) Long-term stability of the fish assemblages in a warm-temperate South African estuary. Estuar Coast Shelf Sci 76:723–738

Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jost LOU (2008) GST and its relatives do not measure differentiation. Mol Ecol 17:4015–4026

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Kalinowski ST, Muhlfeld CC, Guy CS et al (2010) Founding population size of an aquatic invasive species. Conserv Genet 11:2049–2053

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

Kassler T, Koppelman J, Near T et al (2002) Molecular and morphological analyses of the black basses: implications for taxonomy and conservation. American Fisheries Society Symposium. American Fisheries Society, Bethesda, pp 291–322

Kawamura K, Yonekura R, Katano O et al (2006) Origin and dispersal of bluegill sunfish, *Lepomis macrochirus*, in Japan and Korea. Mol Ecol 15:613–621

Kearse M, Moir R, Wilson A et al (2012) Geneious basic: an integrated and extendable desktop software platform for

the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Kinziger A, Nakamoto R, Harvey B (2014) Local-scale invasion pathways and small founder numbers in introduced Sacramento pikeminnow (*Ptychocheilus grandis*). Conserv Genet 15:1–9

Lee DS, Norden A, Gilbert CR (1981) A revised list of the freshwater fishes of Maryland and Delaware. In: Southeastern fishes council proceedings, pp 205–211

Leng L, Zhang D (2011) Measuring population differentiation using G_{ST} or D? A simulation study with microsatellite DNA markers under a finite island model and nonequilibrium conditions. Mol Ecol Resour 20:2494–2509

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Long J, Allen M, Porak W et al. (2015) A historical perspective of black bass management in the United States. In: American Fisheries Society Symposium, pp 99–122

Loppnow GL, Vascotto K, Venturelli PA (2013) Invasive smallmouth bass (*Micropterus dolomieu*): history, impacts, and control. Manag Biol Invasions 4:191–206

Lorenzoni M, Martin Dörr AJ, Erra R et al (2002) Growth and reproduction of largemouth bass (*Micropterus salmoides* Lacépède, 1802) in Lake Trasimeno (Umbria, Italy). Fish Res 56:89–95

Lowe S, Browne M, Boudjelas S et al (2000) 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group, Auckland

Lowe SR, Woodford DJ, Impson DN et al (2008) The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. Afr J Aquatic Sci 33:51–62

Luikart G, Allendorf FW, Cornuet JM et al (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238–247

MacCrimmon HR, Robbins WH (1975) Distribution of the black basses in North America. In: Stroud RH, Clepper H (eds) Black bass biology and management. Sport Fishing Institute, Washington, pp 56–66

McCafferty J, Ellender B, Weyl O et al (2012) The use of water resources for inland fisheries in South Africa. Water SA 38:327–344

Muirhead JR, Gray DK, Kelly DW et al (2008) Identifying the source of species invasions: sampling intensity vs. genetic diversity. Mol Ecol 17:1020–1035

Myers RA, Allen MS (2005) Factors related to angler catch of trophy largemouth bass in Texas reservoirs. Lake Reserv Manag 21:309–315

Near TJ, Kassler TW, Koppelman JB et al (2003) Speciation in North American black basses, *Micropterus* (Actinopterygii: Centrarchidae). Evolution 57:1610–1621

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, NewYork

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, Oxford

Noble RL (2002) Reflections on 25 years of progress in black bass management. In: Philipp D, Ridgway M (eds) Black bass: ecology, conservation, and management. American Fisheries Society, Bethesda, pp 419–431

Peakall R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peery MZ, Kirby R, Reid BN et al (2012) Reliability of genetic bottleneck tests for detecting recent population declines. Mol Ecol 21:3403–3418

Philipp DP, Childers WF, Whitt GS (1983) A biochemical genetic evaluation of the northern and Florida subspecies of largemouth bass. Trans Am Fish Soc 112:1–20

Piry S, Luikart G, Cornuet J-M (1999) Computer note. BOTTLENECK: a computer program for detecting recent reductions in the effective size using allele frequency data. J Hered 90:502–503

Powell AM (1967) Historical information of Maryland commission of fisheries: with some notes on game. Maryland Department of Natural Resources, Annapolis

Quinn S, Paukert C (2009) Centrarchid fisheries. In: Cooke S, Philipp D (eds) Centrarchid fishes diversity, biology and conservation. Wiley, West Sussex, pp 312–339

R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ribeiro F, Collares-Pereira MJ (2010) Life-history variability of non-native centrarchids in regulated river systems of the lower River Guadiana drainage (south-west Iberian Peninsula). J Fish Biol 76:522–537

Rius M, Turon X, Ordóñez V et al (2012) Tracking invasion histories in the sea: facing complex scenarios using multilocus data. PLoS ONE 7:e35815

Roe KJ, Harris PM, Mayden RL et al (2002) Phylogenetic relationships of the genera of North American sunfishes and basses (Percoidei: Centrarchidae) as evidenced by the mitochondrial cytochrome b gene. Copeia 2002:897–905

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106

Schramm HL, Hunt KM (2007) Issues, benefits, and problems associated with fishing tournaments in inland waters of the United States: a survey of fishery agency administrators. Fisheries 32:234–243

Seyoum S, Barthel B, Tringali M et al (2013) Isolation and characterization of eighteen microsatellite loci for the largemouth bass, *Micropterus salmoides*, and cross amplification in congeneric species. Conserv Genetic Resour 5:697–701

Skelton PH, Cambray JA, Lombard A et al (1995) Patterns of distribution and conservation status of freshwater fishes in South Africa. S Afr J Zool 30:71–81

Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshw Biol 55:152–174

Takamura K (2007) Performance as a fish predator of largemouth bass [*Micropterus salmoides* (Lacepède)] invading Japanese freshwaters: a review. Ecol Res 22:940–946

Tamura K, Peterson D, Peterson N et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taylor GC, Weyl OL, van Wyk J (2012) Otoliths versus scales: evaluating the most suitable structure for ageing largemouth bass, *Micropterus salmoides*, in South Africa. Afr Zool 47:358–362

Thioulouse J, Chessel D, Dolédec S et al (1997) ADE-4: a multivariate analysis and graphical display software. Stat Comput 7:75–83

Trippel NA, Hargrove JS, Leone EH, et al. (In Press) Angling-induced impacts on recruitment and contribution to reproduction in Florida Bass, *Micropterus floridanus*. Transactions of the American Fisheries Society

Truitt RV, Bean BA, Fowler HW (1929) The fishes of Maryland. State of Maryland Conservation Department, Annapolis

Uller T, Leimu R (2011) Founder events predict changes in genetic diversity during human-mediated range expansions. Glob Change Biol 17:3478–3485

U.S. Department of Interior (2011) National survey of fishing, hunting, and wildlife-associated recreation. United States Government Printing Office, Washington

Van Oosterhout C, Hutchinson WF, Wills DPM et al (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Watling R, Watling H (1983) Metal surveys in South African estuaries VII Bushmans, Kariega, Kowie and Great Fish Rivers. Water SA 9:66–70

Welcomme R (1988) International introduction of aquatic species. FAO Fisheries Technical Paper 294. Food and Agriculture Organisation of the United Nations, Rome

Weyl OL, Hecht T (1999) A successful population of largemouth bass, *Micropterus salmoides*, in a subtropical lake in Mozambique. Environ Biol Fishes 54:53–66

Weyl OL, Lewis H (2006) First record of predation by the alien invasive freshwater fish Micropterus salmoides L. (Centrarchidae) on migrating estuarine fishes in South Africa. Afr Zool 41:294–296

Weyl PS, De Moor F, Hill M et al (2010) The effect of largemouth bass *Micropterus salmoides* on aquatic macro-invertebrate communities in the Wit River, Eastern Cape, South Africa. Afr J Aquat Sci 35:273–281

Weyl OLF, Ellender BR, Woodford DJ et al (2013) Fish distributions in the Rondegat River, Cape Floristic Region, South Africa, and the immediate impact of rotenone treatment in an invaded reach. Afr J Aquat Sci 38:201–209

Weyl OLF, Finlayson B, Impson ND et al (2014) Threatened endemic fishes in South Africa's cape floristic region: a new beginning for the Rondegat River. Fisheries 39:270–279