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Improving the containment of a freshwater invader using environmental DNA (eDNA) based monitoring

Jonas Bylemans · Elise M. Furlan · Luke Pearce · Trevor Daly · Dianne M. Gleeson

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Abstract On-ground management actions targeting invasive and/or native species are often undertaken based on incomplete and biased distribution data for the species of interest. Once an invasive species becomes established, containment can provide an effective management option to conserve native biodiversity only if it is implemented beyond the outer distribution limits of the species of interest. Determining these outer distribution limits is currently difficult for freshwater fish species because of the low sensitivity and biases associated with conventional monitoring methods. The improved sensitivity of environmental DNA-based surveys makes them particularly useful to determine these outer distribution limits. In this study, we used conventional monitoring methods and eDNA-based monitoring using real-time PCR to determine the spread of the invasive redfin perch (*Perca fluviatilis*) in an intermittent river system. This voracious predatory fish is responsible for the continued decline of several threatened and vulnerable species within Australia. We found that eDNA detection rates were high in our study system, when redfin perch presence was confirmed by conventional monitoring, compared to previously published works. Additionally we describe how the combination of conventional and eDNA-based monitoring can improve redfin perch distribution data compared to conventional monitoring alone. This improvement has subsequently been used to inform management and determine the optimal location for the construction of an exclusion barrier.

Keywords Environmental DNA · Invasive alien species · Endangered species · Management · Containment · Real-time PCR

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J. Bylemans (✉) · E. M. Furlan · D. M. Gleeson
Institute for Applied Ecology, University of Canberra, Canberra, ACT 2617, Australia
e-mail: Jonas.Bylemans@canberra.edu.au

J. Bylemans · E. M. Furlan · D. M. Gleeson
Invasive Animals Cooperative Research Centre, University of Canberra, Canberra, ACT 2617, Australia

L. Pearce
New-South Wales Department of Primary Industries, Albury, NSW 2640, Australia

T. Daly
New-South Wales Department of Primary Industries, Batemans Bay, NSW 2536, Australia

Introduction

Freshwater ecosystems and their associated biodiversity are considered to be highly threatened on a worldwide scale (Geist 2011). One of the stressors responsible for this threatened status is the widespread

occurrence of freshwater Invasive Alien Species (IAS) and their impacts on native biodiversity (Hulme 2009). Although it is recognized that the prevention of new incursions is most effective in protecting native biodiversity (Caffrey et al. 2014; Hulme 2009; Vilizzi and Copp 2013), containment of already established IAS is important to reduce their further spread and additional impacts on native species (Britton et al. 2011a; Gozlan et al. 2010). The ultimate effectiveness of species containment actions however, depends on the quality of the species distribution data used to inform management decisions (Campbell et al. 2009; Gormley et al. 2011). Improvements to monitoring surveys will ultimately result in the implementation of more effective management actions for IAS which in turn will benefit the conservation of native species (Campbell et al. 2009; Gozlan et al. 2010).

Conventional monitoring methods for freshwater fish species (i.e. electrofishing, fyke netting, bait trapping) suffer from low sensitivity and detection biases (Britton et al. 2011b; Lintermans 2015; Maxwell and Jennings 2005; Porreca et al. 2013). Consequently, the ability to make well informed management decisions based on species distribution data obtained from these surveys is challenging (Britton et al. 2011b; Campbell et al. 2009). In recent years, advances in molecular techniques like real-time PCR, High Throughput Sequencing and Target Capture have significantly improved our ability to detect aquatic species at low densities using environmental DNA (eDNA) (Dowle et al. 2015; Jerde et al. 2011; Thomsen et al. 2012; Valentini et al. 2016). The increased sensitivity and efficiency of eDNA-based surveys make it a very useful tool to accurately determine the distribution limits of freshwater fish species (Jerde et al. 2011; Sigsgaard et al. 2015). However, eDNA-based species monitoring is technically challenging and continued research is needed to ensure the implementation of this technology in standard monitoring surveys (Rees et al. 2014a, b; Roussel et al. 2015). Publications to date have focussed on improving our understanding of; eDNA production and degradation rates (Klymus et al. 2014; Maruyama et al. 2014; Strickler et al. 2014), the fate of eDNA within the environment (Jane et al. 2014; Turner et al. 2014a, b), the relationship between species biomass and eDNA quantities (Doi et al. 2015; Takahara et al. 2012) and the collection and processing methods (McKee et al. 2014; Minamoto et al. 2016; Renshaw et al. 2014; Wilson et al. 2016). While eDNA-based monitoring has been successfully used in both lentic water bodies and perennially flowing rivers, no study to date has used eDNA-based monitoring in rivers with intermittent flow. Furthermore, the potential conservation applications of eDNA surveys have been widely recognized but examples in which eDNA data have been used to inform and influence management actions are scarce (Goldberg et al. 2014; Herder et al. 2012; Jerde et al. 2013; Rees et al. 2014a).

Within Australia, 74 % of the freshwater fish fauna is endemic and new or cryptic species are continued to be described (Lintermans 2013a). Within the Murray–Darling Basin (MDB); Australia's largest river catchment spanning the states of the Australian Capital Territory, South Australia, New South Wales (NSW), Queensland and Victoria; approximately 56 % of all native fishes are considered rare or threatened with a majority of these being negatively impacted by IAS (Koehn and MacKenzie 2004; Lintermans 2007; Olden et al. 2007). One of these species is the Southern pygmy perch (SPP) (*Nannoperca australis*). Populations of this small bodied (<85 mm) fish, with a historically widespread distribution, have suffered greatly from predation by alien fish species such as redfin perch (*Perca fluviatilis*) (Pearce 2015). The NSW populations in particular have suffered extensive declines with only three self-sustaining populations remaining (i.e. Blakney Creek, Coppabella Creek and Upper Billabong Creek) (Lintermans 2007; Pearce 2015). The survival of these populations remains under threat as new redfin perch incursions continue to occur and already established redfin perch populations are spreading into valuable SPP habitat.

Within Blakney Creek, a small intermittent lotic system of the Upper Lachlan catchment, redfin perch were first recorded in 2005 (Gilligan et al. 2010). The continued upstream spread of redfin perch in this system is pushing the already fragmented SPP population to the brink of extinction (Gilligan et al. 2010; Pearce 2015). Management actions for SPP (i.e. establishment of a captive breeding population and translocations) have been undertaken by the New South Wales Department of Primary Industries (NSW DPI) (Pearce 2015). In order to further protect the remnant SPP population in Blakney Creek, a redfin perch exclusion barrier was planned to be installed during 2015. The aims of this study are to: (1)

determine the optimal placement for the exclusion barrier by determining the extent of the redfin perch invasion front using conventional and eDNA-based surveys and (2) evaluate the performance of species-specific eDNA monitoring surveys using real-time PCR in an intermittent river systems.

Materials and methods

Both conventional and eDNA-based sampling was conducted in Blakney Creek (BC) and the adjoining Urumwalla Creek (UC) during the autumn of 2015 (Fig. 1 and Table S1). At the time of sampling, water flow in the system was heavily reduced and all sampling sites were remnant pools (mean length = 48.2 m and mean width = 9.9 m) which were completely or partially (i.e. separated by shallow and stagnant water) isolated from each other. Sampling sites were selected based on the most recent distribution data for redfin perch with the aim of sampling across the upstream limits of the invasion front (Pearce 2015).

Conventional monitoring

Conventional fish monitoring was conducted using a combination of active and passive methods to reduce biases associated with individual methods (Fischer and Quist 2014; Neebling and Quist 2011). Four selected sites within BC were sampled using the standard Sustainable Rivers Audit (SRA) protocol which combines backpack electrofishing with the deployment of unbaited traps (Pearce 2015). Backpack electrofishing consisted of eight operations of 150 s power on per site. A single operation consisted of a stationary operator fishing an accessible area of approximately 1.5–2 m radius before moving upstream in a zig-zag fashion and

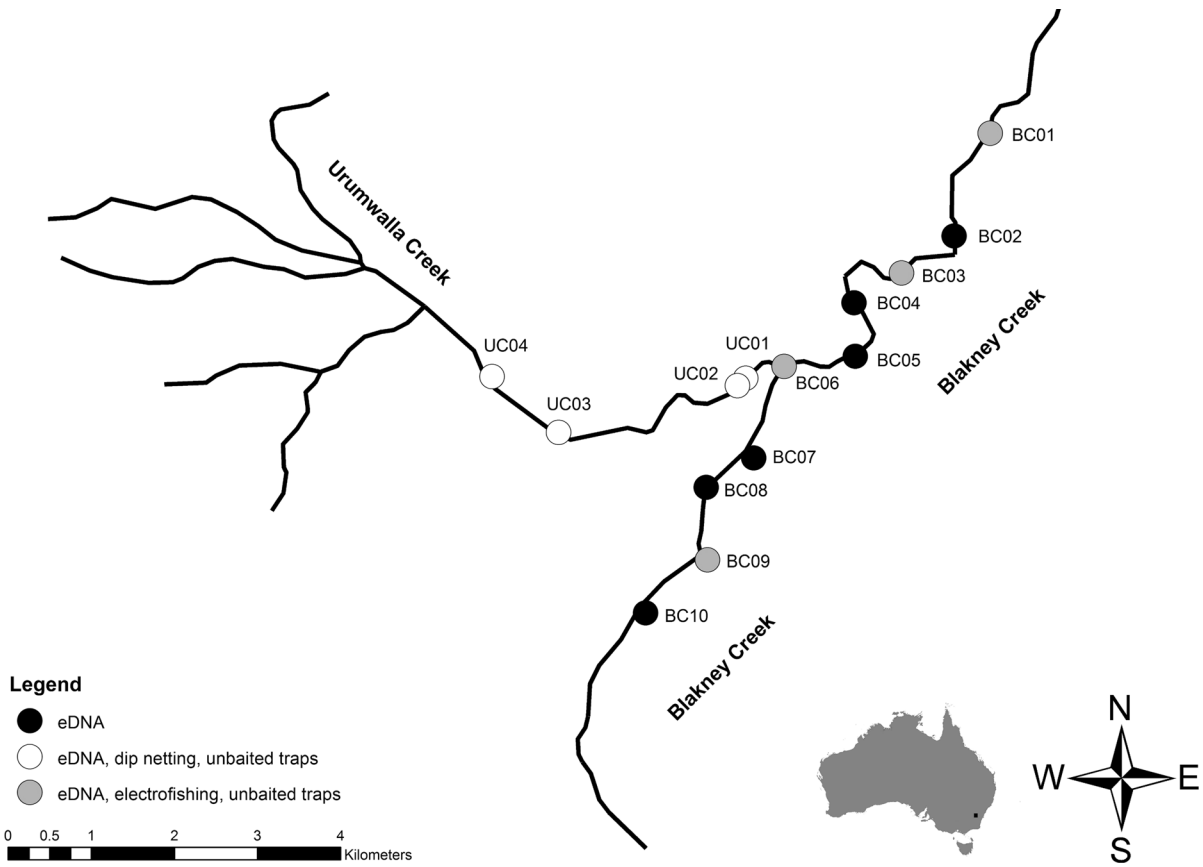

Fig. 1 Map of all sampling sites within Blakney Creek (BC) and Urumwalla Creek (UC). *Shading* indicates the sampling methods employed at the different sampling sites

repeating the protocol. Ten unbaited concertina-style bait traps (measurements: 450 mm × 250 mm × 250 mm, opening: 40 mm, mesh size: 2 mm) were deployed prior to electrofishing and collected after a minimum period of two hours (Pearce 2015). For each sampling site, all fish caught were identified to species level and their length was measured. Within UC a rapid assessment monitoring technique was used to determine the presence/absence of both SPP and redfin perch. Ten unbaited traps were deployed per sampling location and left overnight for a period of 12 h. Additionally, dip netting (net size: 380 mm × 320 mm, mesh size: 3 mm) was conducted by sweeping all available habitat (i.e. macrophyte beds) in ten pools per location for a minimum period of ten minutes. Species abundance and length measurements were not recorded for the UC sampling sites.

eDNA-based monitoring

Environmental DNA-based monitoring was conducted across 14 sites in BC and UC. This included the eight sites surveyed with conventional methods (as described in the conventional monitoring section) and an additional six sites (Fig. 1). An additional site in nearby Pudman Creek (not shown) was sampled for eDNA analyses exclusively and served as a negative control site given that previous surveys were unable to detect redfin perch in this system (Pearce 2015). Samples were first collected at sites where redfin perch are expected to be absent or present at low densities (i.e. the most upstream sites), followed by consecutive sampling of the downstream sites. Before sampling, all field equipment (i.e. waders and 2 L plastic sampling containers) was sterilized using a 10 % bleach solution and thoroughly rinsed with UV-sterilized tap water. At each sampling site, eight 2 L surface water samples were collected along the banks over the entire length of the pool and stored on ice. For each sampling day a negative field control (NFC) was included and consisted of a sampling container filled with 2 L of UV-sterilized tap water which was opened on-site for approximately one minute, closed, submerged in the water and stored with all other samples. Further processing of all samples was conducted at the University of Canberra (ACT, Australia). All filtration equipment (i.e. magnetic filter funnels and forceps) was sterilized as described above. Water samples were filtered within 12 h on a 1.2 µm glass fibre filter (MicroScience®) and stored in a 5 mL tube at −20 °C (Furlan et al. 2016). Prior to filtering the field samples, 500 mL of UV-sterilized tap water was filtered through the sterilized equipment and filters were stored in a 5 mL tube at −20 °C to serve as a negative equipment control (NEC).

DNA extractions, using the PowerWater® DNA Isolation Kit (Mo-Bio Laboratories Inc., Carlsbad, CA), and PCR set-ups were performed at the University of Canberra's trace DNA laboratory (ACT, Australia). To reduce the risk of contamination from laboratory procedures, the entire laboratory is UV-radiated nightly and eDNA extractions and PCR set-ups are conducted in spatially separated rooms with the latter having a positive air pressure. The presence of redfin perch eDNA was determined using a redfin perch-specific TaqMan® real-time PCR assay targeting a short fragment of the 12S rRNA gene region (Table 1). The specificity of the redfin perch assay has been confirmed in silico (PrimerBLAST) and in vitro (i.e. performing real-time PCR reactions on genomic DNA extracts and field samples and subsequent sequencing of amplicons) (Furlan and Gleeson 2016b). A generic fish TaqMan® real-time PCR assay, developed to amplify a short fragment of the 16S rRNA gene region of freshwater fish occurring in Australia, was included to serve as a positive control (Table 1) (Furlan and Gleeson 2016a). Real-time PCR replicates (referred to as PCR replicates in future sections) were performed using 10 µL of TaqMan® Environmental Master Mix 2.0 (Life Technologies), 1X redfin perch-specific TaqMan® assay (Life Technologies), 0.75X generic fish TaqMan® assay (Life Technologies), 8 µL of template DNA and DEPC-Treated water (Life Technologies) in a total volume of 20 µL. All reactions were run on 96-well plates using the ViiA™ 7 Real-Time PCR system (Life Technologies) with fluorescent thresholds (ΔRn) set at 0.1 and 0.05 for the redfin perch and generic fish assay respectively. Cycling conditions were 2 min at 50 °C, 10 min at 95 °C, 55 cycles of 15 s at 95 °C and 30 s at 60 °C. All runs included positive (i.e. redfin perch DNA added) and negative controls (i.e. no template added) run in triplicate.

Within our study system the use of the generic fish assay as a positive control is appropriate given that fishes are known to be abundant throughout both creeks (Pearce 2015). Failed amplification in either assay can thus be attributed to failed sample processing (i.e. collection, capture and extraction) and/or the

Table 1 Primer and probe details designed to amplify a short fragment of the 12S mitochondrial gene region in redfin perch (*Perca fluviatilis*) and a short fragment of the 16S mitochondrial gene region in multiple fish species (Furlan and Gleeson 2016a; Furlan and Gleeson 2016b)

Assay target	Label	Sequence (5′–3′)	Fragment (bp)
Redfin perch	Pflu_12S_F	GGGATTAGATACCCCACTATGCCT	92
	Pflu_12S_R	GGTTTCAAGCTGATGCTCGTAGTT	
	Pflu_12S_probe	(FAM)-CCATAAACATTGGTAGCACACT-(MGB)	
Generic fish	Fish_16S_F	GACCTCGATGTTGGATCA	87–88
	Fish_16S_R	CTCAGATCACGTAGGACTTTA	
	Fish_16S_probe	(VIC)-ACATCCTAWTGGTGC-(MGB)	

presence of PCR inhibition (Furlan and Gleeson 2016a). Consequently, PCR replicates were only considered valid if a clear amplification curve could be observed from one of the assays. For each field sample three initial PCR replicates were performed and when, for a given site, no positive redfin perch detections (i.e. amplification of the redfin perch assay) were observed an additional three PCR replicates were performed. For each sampling site the number of valid and positive PCR replicates was recorded. The means and standard deviations of the Ct-values were calculated for each site using the positive replicates. Contamination within the NFCs and a selection of NECs (a minimum of one for each sample location) was monitored by running six PCR replicates for each control.

Results

Conventional monitoring

The results of the conventional fish monitoring survey detected redfin perch in all but the most upstream sampling site within BC (Table 2). Sampling efforts conducted in UC failed to detect redfin perch, suggesting that the species is currently absent from this tributary. Although the rapid assessment monitoring methods used within UC are potentially less sensitive then the SRA protocol, the relative small size of the sampled pools makes dip netting a suitable method which was able to sample multiple species (Table S2).

eDNA-based monitoring

The generic or species-specific assay amplified in 99.5 % of all the PCR replicates indicating that the sampling processing procedures used were appropriate and/or PCR inhibition did not affect our ability to detect redfin perch eDNA in the majority of the replicates. Analyses of all valid PCR replicates shows that within our study system high eDNA detection rates (i.e. $\approx$100 % positive PCR replicates) were obtained for all sites in which the presence of redfin perch could be confirmed with conventional monitoring (Table 2). While all sampling locations within BC tested positive for redfin perch eDNA, the samples collected from the most upstream site in UC did not return any positive eDNA detections (Table 2).

All NFC, NEC, negative PCR controls and samples collected from the negative control site yielded no positive amplification for redfin perch DNA (Table S3). Although a positive amplification of the generic fish assay was observed in two PCR replicates from one of the NFC (Ct-value: 46.82 $\pm$ 2.84), the samples collected that day (i.e. 13th March 2015) yielded no positive redfin perch detections. Although the observed levels of contamination would not affect the general conclusions from the eDNA survey; we strengthened our confidence in our low eDNA detection rates observed in sites BC10, UC01, UC02 and UC03 by performed an addition six PCR replicates on all NEC associated with samples that tested positive for redfin perch eDNA. These additional analyses did not produce any positive amplification in either assay.

Discussion

The combined results of both surveys suggest that redfin perch are currently widespread throughout BC but appear to be limited to the downstream section of UC. Compared to the conventional monitoring, the eDNA survey was able to detect redfin perch DNA 3.4

Table 2 Results of the conventional and eDNA-based monitoring for all sampling sites within Blakney Creek (BC) and Urumwalla Creek (UC) (NSW, Australia)

Site	Conventional monitoring	eDNA-based monitoring (8 × 2L samples per site)				
	Redfin perch caught	Total PCRs	Valid PCRs	Positive PCRs	Mean Ct-value	SD Ct-value
BC01	10	24	24	24	32.84	0.47
BC02	na	24	24	24	32.55	0.50
BC03	2	24	24	24	34.47	0.67
BC04	na	24	22	22	37.25	0.69
BC05	na	24	24	24	33.29	0.50
BC06	21	24	24	23	37.08	0.74
BC07	na	24	24	24	37.72	1.08
BC08	na	24	24	24	34.89	0.49
BC09	0	24	24	14	37.88	1.74
BC10	na	48	48	1	38.57	na
UC01	0	24	20	1	40.29	na
UC02	0	24	24	3	42.33	0.70
UC03	0	24	24	7	39.57	0.99
UC04	0	48	48	0	na	na

For the conventional monitoring results the numbers of redfin perch caught per sampling site are given. eDNA-based monitoring results are shown as the total number of PCR replicates performed per site and the number of valid and positive PCR replicates per site. In addition, the mean and the standard deviation (SD) of the Ct-values obtained from the positive PCR replicates are given

and 2.8 km further upstream in BC and UC respectively. Although the detection of redfin perch eDNA can be seen as a proxy for the recent presence of this species, other vectors might transport eDNA beyond the actual redfin perch distribution range. Despite these inaccuracies, eDNA-based monitoring provides a conservative indication of the upstream distribution limits of a species. Considering the results of both surveys would thus place the optimal location for the redfin perch exclusion barrier significantly further upstream then conventional monitoring alone, which is likely to improve the success of containment actions.

Besides the immediate implication of the presented results for management, the data also provide important insights into the efficiency of eDNA-based monitoring in an intermittent stream. Previous studies investigating the feasibility of using eDNA-based species detections in streams have focussed on perennial systems. The current literature suggests that eDNA detection rates observed in perennial streams are lower compared to those observed in lentic systems (Thomsen et al. 2012) and depend strongly on environmental and sampling conditions (Jane et al. 2014; Laramie et al. 2014; Pilliod et al. 2014). The results from this study suggest that within intermittent streams, high eDNA detection rates can be obtained when sampling is conducted during dry periods. This is apparent from the high detection rate observed in sampling site BC03 (100 %) while conventional methods were only able to catch two redfin perch (Table 2). Although water temperature and microbial activity is likely to be elevated in intermittent streams during dry periods and these factors are known to impact on eDNA persistence (Barnes et al. 2014; Strickler et al. 2014), the most likely reasons for the high eDNA detection rates observed here are the lack of a significant water flow and the increased species densities in the remnant pools at the time of sampling.

Distribution data obtained through eDNA-based surveys can be controversial due to the occurrence of type I (false positive) and type II (false negative) errors (Lahoz-Monfort et al. 2016; Roussel et al. 2015; Sarre et al. 2014). False negative and false positive (i.e. due to misidentification of species) are a common concern in traditional monitoring surveys and can have serious consequences for management (Britton et al. 2011b; Lintermans 2015; Valentini et al. 2009). In the case of

invasive species containment, type II errors will underestimate the true species distribution and may lead to containment actions being inappropriately implemented within the distribution range of the species of interest. Although the increased sensitivity of eDNA surveys reduces the occurrence of type II errors, they remain susceptible to type I errors (i.e. the detection of the species DNA while the species itself is absent). In eDNA-based surveys, type I errors can originate from contamination during sample handling or the transport of eDNA beyond the actual species distribution range by other vectors. The occurrence and impacts of type I errors can however be monitored and excluded by rigorously testing of the PCR protocol and the inclusion of controls at every stage in sample handling (Sarre et al. 2014). The methodology used in the current survey has been tested extensively for its specificity and sufficient negative controls were incorporated and analysed to ensure the integrity of the data (Furlan and Gleeson 2016b).

In addition to understanding the limitations of the monitoring methods employed it is important to formally evaluate the effects of management actions before conclusions can be made as to whether or not their goal has been achieved. Consequently, continued monitoring of the redfin perch distribution within the Blakney Creek catchment and evaluating the effect of the redfin perch exclusion barrier on both the target and non-target species will be required (Campbell et al. 2009; Lintermans 2013b). The construction of the redfin perch exclusion barrier has been completed in December 2015 and continued monitoring of the system will be conducted using convention methods and eDNA-based community assessments (i.e. using High Throughput Sequencing) to evaluate the outcomes of management actions.

Conclusion

Although eDNA detection has been used for monitoring surveys of elusive aquatic vertebrates, these survey results have not been translated to direct management actions (Herder et al. 2012; Rees et al. 2014a; Sigsgaard et al. 2015). In this study we have shown that the improved sensitivity of eDNA-based monitoring can be used to inform species management and improve the likely success of containment actions for aquatic IAS.

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