

## Research Article

## Environmental DNA monitoring and management of invasive fish: comparison of eDNA and fyke netting

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### Abstract

The potential of environmental DNA (eDNA) methods to enhance the detection of invasive species during routine monitoring is of interest to management agencies. Here we applied the eDNA methodology concurrent with conventional detection techniques during two routine monitoring seasons to detect the presence of three invasive fish in Australia with contrasting spatial distributions (benthopelagic, pelagic and benthic): common carp (*Cyprinus carpio*), redfin perch (*Perca fluviatilis*) and Oriental weatherloach (*Misgurnus anguillicaudatus*). Our objectives were to compare the seasonal detection of the target species using eDNA and conventional detection (fyke nets), determine the relationship between catch per unit effort (CPUE) and DNA copy number and ascertain the best water location (surface vs. subsurface) for eDNA detection. Our results show that eDNA had a higher detection rate than fyke nets for Oriental weatherloach and redfin perch during both the autumn and spring surveys. Common carp was detected at all sites for both seasons using fyke nets and eDNA with the exception of one site during the autumn survey where common carp was captured using fyke nets but no carp eDNA was detected. Season had a significant effect on DNA concentration for common carp ( $P < .005$ ) and Oriental weatherloach ( $P = .002$ ) but sampling location (surface vs. subsurface) had no significant effect on DNA concentration for all three species. We found a positive correlation between CPUE and DNA copy number for Oriental weatherloach ( $r_s = .718$ ,  $\alpha = .045$ ) and redfin perch ( $r_s = .756$ ,  $\alpha = .030$ ) during spring but a non-significant, negative trend was observed for common carp in both seasons ( $r_s = -.357$ ,  $\alpha = .385$  spring;  $\rho = -.539$ ,  $\alpha = .168$  autumn). Our results show that eDNA is an effective tool for the detection of single or multiple species to complement the traditional approaches using physical capture. As with all survey methods, the eDNA approach suffers from imperfect detection. We conclude that eDNA survey results are more powerful when used in conjunction with other survey methods as a way to enhance detection rates and increase confidence in the monitoring results.

**Key words:** invasive species, carp, redfin perch, Oriental weatherloach, species detection

### Introduction

The detection of invasive species during routine monitoring and surveillance is important to management agencies. Data generated by such programs must be robust to support reliable and timely interventions (Yoccoz et al. 2001). However, it is often difficult to detect all individuals or species present during monitoring, especially for elusive or cryptic species and those present at low density (Mills et al. 2000). Rates of non-detection are typically higher for rare

species, particularly aquatic species where most are concealed under the water's surface (Jerde et al. 2011). Survey methods can also bias sampling, allowing differential probability of detection for particular species or size classes. For example, several methods exist for sampling fish (e.g. electrofishing, fyke nets, gill nets, bait traps) but the efficiency of each technique depends on the species targeted as well as their size and developmental stage (Jackson and Harvey 1997; Lintermans 2015).

Recently, environmental DNA (eDNA) has been used to detect a number of invasive fish including

the bluegill sunfish, Mozambique tilapia, and Asian carp (Dejean et al. 2012; Jerde et al. 2013; Robson et al. 2016; Takahara et al. 2013). The eDNA method detects DNA released into the environment through skin sloughing or bodily discharges and genetic sequence matching can then be used to indicate the presence of the target organism (Pilliod et al. 2013). eDNA surveys used in the Great Lakes in the USA to detect Asian carp were found to be more sensitive compared to traditional methods such as fyke net sampling (Turner et al. 2012) or electrofishing (Jerde et al. 2011). The advantages of DNA-based detection, such as sensitivity, ease of sample collection, and ability to discern species presence regardless of size or life stage, makes it a useful detection tool (Darling and Blum 2007; Dejean et al. 2012).

The potential of eDNA to infer abundance has also been investigated. Earlier eDNA experiments using discrete static mesocosms (aquariums or ponds) showed an association between density and amplification rate (Ficetola et al. 2008), density and DNA concentration (Thomsen et al. 2012) and biomass and DNA concentration (Takahara et al. 2012). A more recent study in natural lakes in Canada found an association between eDNA concentration and catch per unit effort (CPUE: number of catch per overnight set with the number of stations sampled being proportional to the lake size) (Lacoursière-Roussel et al. 2015). A similar positive association occurs in lotic ecosystems between amphibian density (measured by conventional field methods) and eDNA concentration (Pilliod et al. 2013). In contrast, one study found common carp eDNA concentration were not associated with abundance using fyke net capture data in coastal wetlands (Turner et al. 2012). These variable results suggest caution in inferring abundance through eDNA concentration, particularly in natural flowing bodies of water.

Most studies of eDNA detection in aqueous systems involve sampling from the water surface, perhaps as a matter of convenience, despite the fact that DNA occurs in various environmental compartments such as the water column and sediments (Everhart et al. 2013; Santas et al. 2013; Turner et al. 2015). Only a few studies investigated eDNA concentrations between surface and subsurface water samples, and most of these studies have been in enclosed systems such as ponds and small lakes. Moyer et al. (2014) found that surface water was the optimal location in the water column in ponds for eDNA detection of African jewel fish (*Hemichromis letourneuxi*) whereas no difference in common carp eDNA concentration was found between surface and subsurface samples (Eichmiller et al. 2014). Species-specific differences, such as habitat preference, may affect the dispersal

of eDNA in the water column, and thus, success in eDNA detection.

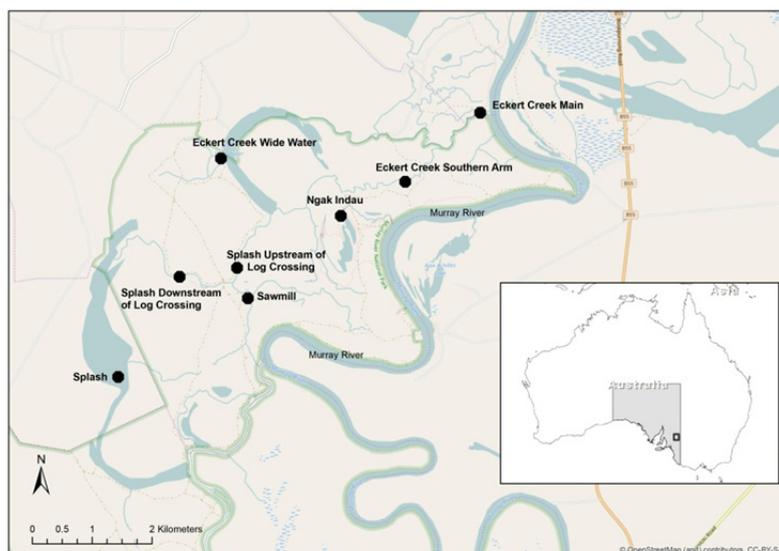
Recent publications have suggested that temporal factors such as breeding and migratory seasons should also be considered in eDNA surveys to increase the likelihood of detection (Barnes and Turner 2016; Thomsen and Willerslev 2015). For example, Spear et al. (2015) found a strong increase in Eastern hellbender eDNA during its breeding season compared to other survey windows. In addition, Goldberg et al. (2011) found seasonal variation in eDNA detection of two stream amphibians and they attributed this to species-specific seasonal changes in abundance. Future research on the temporal as well as the spatial aspects of eDNA across various species and habitats have been advocated to increase the eDNA method's application to conservation (Furlan et al. 2015; Thomsen and Willerslev 2015).

The use of eDNA as a detection tool shows enormous promise, but because it entails additional cost, effort, and altered practice, studies comparing the performance of eDNA with conventional tools during routine monitoring are necessary for management uptake. In this paper, we compared detection of three invasive fish species using eDNA and fyke nets in the Katarapko/Eckert Creek anabranch system of the Lower Murray River, South Australia. The objectives of the study were to i) compare the seasonal detection of the target species using traditional fish monitoring methods (fyke nets) and eDNA, ii) determine the relationship between CPUE and DNA copy numbers in a natural flowing system, and iii) determine the best water stratum for eDNA detection for the three study species with contrasting spatial distribution. We show that the eDNA method is an effective tool for targeted species detection to complement traditional approaches.

## Materials and methods

### Study area

The study was carried out at eight sites in the Katarapko/Eckert Creek anabranch system located near the town of Berri in South Australia (Figure 1 and Supplementary material Figure S1). The Katarapko/Eckert anabranch and floodplain system is one of three large anabranch systems located within the lower Murray River, South Australia. This system encompasses a range of diverse aquatic habitats incorporating permanent fast-flowing and slow-flowing creeks, and backwaters (Bice et al. 2015). Such hydraulic diversity is now scarce in the lower River Murray main channel during low flow conditions.



**Figure 1.** Survey site locations along the Katarapko/Eckert Anabranch in Katarapko, South Australia.

### *Study species*

The common carp (*Cyprinus carpio* Linnaeus, 1758), redfin perch (*Perca fluviatilis*, Linnaeus, 1758) and Oriental weatherloach (*Misgurnus anguillicaudatus* Cantor, 1842) are three invasive fish species previously recorded in the area (Leigh et al. 2009; Wegener and Suitor 2013). The species were chosen due to management requirements and different habitat preferences including a pelagic (redfin perch), benthopelagic (common carp) and benthic species (Oriental weatherloach). These species were selected to test whether DNA concentration in surface and subsurface water samples vary among the species with different vertical distribution.

The common carp is the most abundant freshwater alien fish in Southeast Australia (Koehn 2004). Introduced to the country in the mid-1800's, it now represents the largest biomass in Australia's largest catchment—the Murray-Darling Basin (Gehrke et al. 1995). The redfin perch was introduced early in the 1900's (Coy 1979) for recreational angling and has established in six states across Australia (Koehn and MacKenzie 2004). The Oriental weatherloach is a more recent invader, having been detected at low densities along the South Australian (SA) region of the Murray-Darling Basin since 2011. Its dispersal in the region is of particular interest to management authorities and has been tracked annually through monitoring (Wegener and Suitor 2013).

Carp are habitat generalists and have been known to congregate in deeper river waters during winter and move to off-stream spawning habitats prior to spawning season in spring (Butler and Wahl 2010;

Stuart and Jones 2002). The peak spawning months for common carp in Australia have been reported to be between September and April (spring to mid-autumn) (Sivakumaran et al. 2003; Smith and Walker 2004), with gonadal changes indicating spawning peaks during spring (Brown et al. 2005). For Oriental weatherloach, the spawning season in Japan has been reported to span from mid-May to August (late spring to summer) (Fujimoto et al. 2008). There is limited information on the ecology and precise distribution of the Oriental weatherloach in Australia owing to its cryptic behaviour (Lintermans et al. 2014). It is also a habitat generalist and has been found in a wide variety of habitats ranging from clear upland streams to turbid degraded habitats, with a particular preference for habitats with a sandy or silty substrate (Lintermans et al. 2014). Redfin perch is known to spawn in late winter to early Spring (July to September) (Morgan et al. 2003). It has been found in lakes and rivers but prefers still or slow flowing water with rich vegetation (Lintermans et al. 2014; Morgan et al. 2003).

### *Water sampling for eDNA analysis*

Surveys were carried out in November 2014 (spring) and March 2015 (autumn). Water samples for eDNA analysis were obtained concurrent with the spring and autumn fish monitoring conducted by wetland staff from the Department of Environment, Water and Natural Resources (DEWNR). Water samples were collected either immediately before fyke net deployment (in spring) or 4–7 days before fish sampling (in autumn).

Six 2-L water samples (three surface, three sub-surface) were taken per site and placed inside plastic wide-mouthed bottles previously decontaminated with 10% bleach solution and rinsed with UV-sterilised water. The water samples were taken approximately 0.5m away from the stream bank. Sub-surface water samples were taken ~0.3m below the water surface by submerging the sampling bottles and opening them underwater. Samples were transported and kept inside ice chests prior to filtration.

At each site, a 2-L bottle filled with UV-sterilised water served as a field control. The control bottles were opened, the contents exposed to the air, re-sealed and submerged in the water. The controls were placed in the same ice chest and handled the same way as the rest of the samples in the field and the laboratory.

Water samples and field controls were filtered within 12–36 h of sampling through 42mm, 1.2  $\mu$ m pore size glass fibre filter papers (Microscience<sup>®</sup>) using a peristaltic pump (Geopump Series II, Geotech Environmental Equipment Inc., Denver, Colorado). Prior to filtering each sample, 500 ml of UV-sterilised water was passed through the filter equipment and on to a 1.2  $\mu$ m glass fibre filter paper to serve as equipment blank. Filter papers were stored in a –20 °C freezer prior to extraction. All filtering equipment was bleached for a minimum of 10 minutes and rinsed with UV sterilised water between samples.

#### *Conventional monitoring using fyke nets*

A total of nine fyke nets (7 m wing length, 0.7 m entry diameter and 6 mm mesh, three sets of fyke nets set ~10m apart) per site were set in the afternoon, left overnight and pulled the morning after. The nets were positioned perpendicular or parallel to the bank to capture the habitat utilization by different fish species. Catch per unit effort (CPUE) per fyke net was calculated by dividing the number of fish caught in the fyke net by the total soak time and then multiplying by 24 to determine number of fish entering a net per hour over a 24 hour time period. Site CPUE was expressed as the mean CPUE of all fyke nets in a site.

#### *DNA extraction and amplification*

DNA was extracted from the filter papers using the PowerWater<sup>®</sup> DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). DNA extraction and PCR preparation took place in separate rooms in a designated trace DNA laboratory. We tested for DNA in the environmental samples using common carp, Oriental weatherloach and redbfin perch qPCR primers and probes previously developed for the three species (Furlan and Gleeson 2016a; Furlan and

Gleeson 2016b; Furlan et al. 2015). Quantitative PCR reactions were performed in a separate laboratory using the Vii<sup>™</sup> 7 Real-Time PCR System (Applied Biosystems<sup>®</sup>, Vic., Australia). PCR reaction mixes consisted of 4  $\mu$ l of DNA template, 1  $\mu$ l of TaqMan<sup>®</sup> assay, 10  $\mu$ l of TaqMan<sup>®</sup> Environmental Master Mix (Life Technologies, Carlsbad, CA, USA), 1  $\mu$ l of Exogenous Internal Positive Control (IPC) Reagent (Applied Biosystems<sup>®</sup>), 0.2  $\mu$ l of IPC DNA, and 3.8  $\mu$ l of PCR water to make a total volume of 20  $\mu$ l. Real-time PCR cycling conditions were set at 50 °C (2 min), 95 °C (10 min), followed by 55 cycles of 95 °C (15 s), 60 °C (60 s). Six PCR replicates were done for each environmental sample and positive and negative controls (including IPC negatives) were included in each run. Three replicates of synthetic oligonucleotides of the target sequence in a series of 10-fold dilutions with concentrations ranging from 10<sup>6</sup> to 10<sup>2</sup> copies per  $\mu$ l were included in each plate and the amount of eDNA present in each sample was quantified by comparison to these known concentrations (details of the synthetic oligonucleotides published in Furlan et al. (2015)). We considered a positive detection if there was an exponential phase at any point during the 55 reaction cycles. Presence of inhibitors were deemed the likely cause of delayed or non-IPC amplification. A Ct shift of  $\geq 3$  cycles beyond the blank was regarded as significant inhibition (Hartman et al. 2005). Representative samples from positive detections for each of the target species from each site were taken and Sanger sequenced to confirm sequence identity (10% of positive samples for common carp; all samples for Oriental weatherloach and redbfin perch).

#### *Data analysis*

We checked the data for normality using the Shapiro-Wilk test and assessed the distribution of DNA copy number and CPUE between seasons through visual inspection of histograms. PCR replicates with no amplifications were given a copy number of zero (0) and the eDNA concentration in each site was calculated by averaging PCR replicates. We included all outliers in our analysis as these represented valid detections. For example, only one sample was positive for Oriental weatherloach eDNA during spring in the Eckert Creek Main site and the sample contained 18DNA copies/4 $\mu$ L of DNA extract. This was flagged as an outlier simply because the rest of the replicates had 0 copies (no detection). Removing this outlier would be erroneous because it would mean that we have taken out the only detection in that site.

Generalized linear mixed modelling (GLMM) was used to examine the effect of season (spring vs.

autumn) and sampling location (surface vs. subsurface) on eDNA copy number. GLMMs are the most appropriate analytical tool for non-normal data with random effects (Bolker et al. 2009). A negative binomial regression with a log link function was used to account for overdispersion (Gardner et al. 1995; Ver Hoef and Boveng 2007). Site was included in the model as a random effect while sample location and season were fixed effects. A Mann-Whitney U test was used to compare the difference in CPUE between seasons. Spearman's rank correlation was used to determine the relationship between CPUE and DNA copy numbers ( $H_0$  = There is no monotonic association between CPUE and DNA copy numbers). All statistical analyses were performed using IBM SPSS Statistics 21.

## Results

The seasonal distributions of copy number and CPUE for all three species did not follow a normal distribution (positively skewed with overdispersion). DNA copy numbers and CPUE varied widely among sites and between seasons (Figure 2, Table S2). No DNA amplification occurred in field controls and no inhibition was observed in any sample. All internal positive controls also amplified. However, low-level contamination (1 copy/4 $\mu$ l) was detected in two equipment blanks (one each from the spring and autumn surveys). The two samples filtered through the contaminated equipment were discarded and were not included in the analysis. The  $R^2$  values of the qPCR standard curves ranged from 0.992–0.999 while efficiency ranged from 0.86–0.99. Sequenced reads correspond accordingly to the DNA sequences of the target species.

### *Seasonal detection using fyke nets and eDNA*

The eDNA method detected Oriental weatherloach and redbfin perch in more sites during the two seasons compared to fyke nets (Table 1). Oriental weatherloach DNA was detected in seven out of eight sites during the spring survey (compared to two sites using fyke nets) and in all eight sites during autumn (zero sites using fyke nets). Redfin perch eDNA was detected at two sites during spring (only one site using fyke nets) and three sites in autumn (zero sites using fyke nets). Common carp was detected at all sites for both seasons using fyke nets and eDNA with the exception of the wetland site Ngak Indau during the autumn survey where common carp was captured using fyke nets (CPUE = 1.4), but no carp eDNA was detected (Table S2). A seasonal difference in CPUE was seen only in carp, with higher

CPUE recorded in spring compared to autumn (spring mdn = 5.1, autumn mdn = 1),  $U = 6$ ,  $p < .005$ .

### *Effect of season and sampling location on DNA copy number*

Season had a significant effect on DNA copy number for common carp ( $P < .005$ ) and Oriental weatherloach ( $P = .002$ ) based on the fitted models, with estimated means significantly higher in spring compared to autumn (Table 2). Sampling location (surface vs. subsurface) did not have a significant effect on copy numbers for all three species. Note that we first fitted a model with the random effect for all three species, but found the variance of the random effect was effectively zero for Oriental weatherloach and redbfin perch. Thus, we refitted the model without the random effect for the two species and reported those results in Table 2.

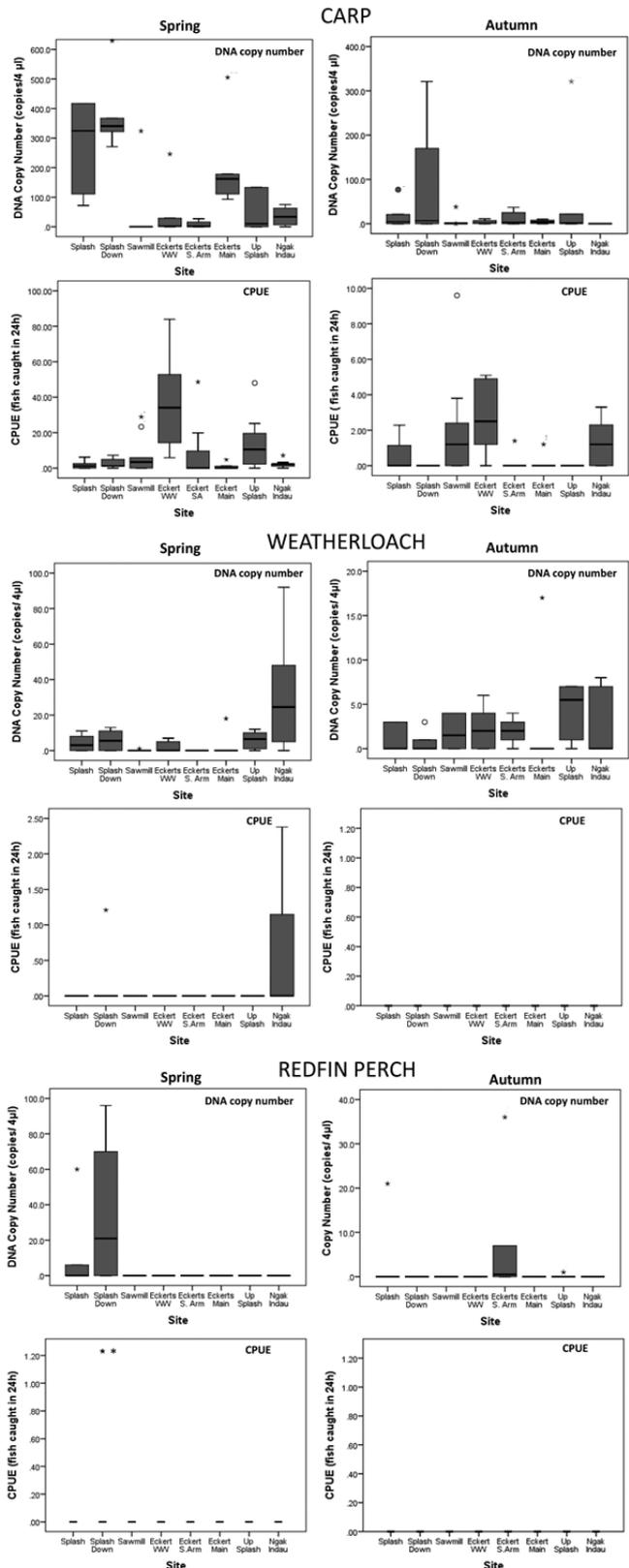
### *Relationship between CPUE vs. DNA copy number*

The results demonstrate a strong, positive correlation between CPUE and DNA copy number for Oriental weatherloach ( $r_s = .718$ ,  $\alpha = .045$ ) and redbfin perch ( $r_s = .756$ ,  $\alpha = .030$ ) during the spring season. For both seasons, the correlations between CPUE and DNA copy number for common carp were not significant ( $r_s = -.357$  spring,  $r_s = -.539$  autumn; Table 3).

## Discussion

Our results support the increasing evidence of the benefits of integrating eDNA methods into routine fish monitoring by management agencies. For all the target species, and in all cases except one, the eDNA method detected the target species in sites where it was captured by fyke nets. For less abundant species such as redbfin perch and Oriental weatherloach, the detection rate was higher for eDNA than for fyke nets. Hence, the application of the eDNA method, when paired with traditional detection, can increase the reliability of monitoring surveys particularly for rare and cryptic species.

The eDNA methodology failed to detect common carp DNA in one site during the autumn survey (Ngak Indau) although the species had been caught in the site with fyke nets. Non-detection using eDNA despite the presence of the animal in the environment could be a result of method errors (such as during the filtration, DNA isolation, extraction or amplification process) or insufficient detection sensitivity such as might arise from insufficient sampling (Furlan et al. 2015). In this study, we assume that the non-detection of carp DNA at one site was not due to method error



**Figure 2.** Boxplot comparing the seasonal variation in DNA copy numbers and CPUE among sites in common carp, Oriental weatherloach and redfin perch. Two sites, Splash Downstream of Log Crossing (Splash Down) and Upstream Splash (Up Splash), were not sampled using fyke nets in autumn. The dark line inside the boxes represent the median values, the bottom and top of the box indicates the 25th percentile and 75th percentile, and the T-bars extend to 1.5 times the height of the box or, if no case has a value in that range, to the minimum or maximum values. Values outside of the T-bars are tagged as outliers and are denoted by circles or asterisks (extreme outliers). Extreme outliers represent cases that have values more than three times the height of the boxes.

**Table 1.** Comparison of eDNA and fyke net detection of invasive fish species in eight sites in Katarapko, South Australia during two seasons, November 2014 (spring) and March 2015 (autumn). (+) indicates a positive detection in at least one of 36 PCR replicates per site, (-) indicates no positive detection, ND indicates no data. S = Spring, A = Autumn.

|  | Common Carp |   |           |    | Oriental Weatherloach |   |           |    | Redfin Perch |   |           |    |
|--|-------------|---|-----------|----|-----------------------|---|-----------|----|--------------|---|-----------|----|
|  | eDNA        |   | Fyke nets |    | eDNA                  |   | Fyke nets |    | eDNA         |   | Fyke nets |    |
|  | S           | A | S         | A  | S                     | A | S         | A  | S            | A | S         | A  |
| The Splash                             | +           | + | +         | +  | +                     | + | -         | -  | +            | + | -         | -  |
| The Splash Downstream of Log Crossing* | +           | + | +         | ND | +                     | + | +         | ND | +            | - | +         | ND |
| Sawmill Creek                          | +           | + | +         | +  | +                     | + | -         | -  | -            | - | -         | -  |
| Eckert Creek Wide Water                | +           | + | +         | +  | +                     | + | -         | -  | -            | - | -         | -  |
| Eckert Creek Southern Arm              | +           | + | +         | +  | -                     | + | -         | -  | -            | + | -         | -  |
| Eckert Creek Main                      | +           | + | +         | +  | +                     | + | -         | -  | -            | - | -         | -  |
| The Splash Upstream of Log Crossing*   | +           | + | +         | ND | +                     | + | -         | ND | -            | + | -         | ND |
| Ngak Indau                             | +           | - | +         | +  | +                     | + | +         | -  | -            | - | -         | -  |

\* Sites were not sampled with fyke nets during the Autumn survey

**Table 2.** Fixed effects from a negative binomial generalized linear mixed model predicting DNA copy number.

| Model Term                   | Coefficient | Std. Error | t      | Sig.  | 95% Confidence Interval |       | Estimated Means for Significant Effect<br>Target: DNA Copy No. |
|------------------------------|-------------|------------|--------|-------|-------------------------|-------|--|
|                              |             |            |        |       | Lower                   | Upper |  |
| <b>Common Carp</b>           |             |            |        |       |                         |       |  |
| Intercept                    | 2.545       | 0.466      | 5.463  | .000  | 1.620                   | 3.471 | Spring: 131  |
| Season                       | 2.350       | 0.341      | 6.891  | .000  | 1.673                   | 3.028 | Autumn: 12   |
| Location                     | -0.039      | 0.408      | -0.096 | .924  | -0.849                  | 0.771 |  |
| <b>Oriental weatherloach</b> |             |            |        |       |                         |       |  |
| Intercept                    | 0.889       | 0.273      | 3.258  | .002  | 0.347                   | 1.431 | Spring: 7  |
| Season                       | 1.151       | 0.395      | 2.917  | .004  | 0.367                   | 1.935 | Autumn: 2  |
| Location                     | -0.220      | 0.398      | -0.555 | .581  | -1.010                  | 0.569 |  |
| <b>Redfin perch</b>          |             |            |        |       |                         |       |  |
| Intercept                    | 0.160       | 0.827      | 0.194  | 0.847 | -1.486                  | 1.803 |  |
| Season                       | 1.280       | 0.835      | 0.153  | 0.129 | -0.378                  | 2.938 |  |
| Location                     | 0.464       | 0.826      | 0.562  | 0.576 | -1.176                  | 2.104 |  |

**Table 3.** Spearman's correlation coefficient showing the relationship between catch per unit effort and DNA copy number for three invasive fish species in Katarapko, South Australia over two seasons (summer - November 2014; autumn - March 2015),  $N = 8$ .

|                | Carp   |        | Weatherloach |        | Redfin |        |
|----------------|--------|--------|--------------|--------|--------|--------|
|                | Spring | Autumn | Spring       | Autumn | Spring | Autumn |
| Spearman's rho | -.357  | -.539  | .718*        | -      | .756*  | -      |
| Sig.           | .385   | .168   | .045         | -      | .030   | -      |

\*Correlation is significant at the 0.05 level (2-tailed)

because DNA was successfully amplified from another species (Oriental weatherloach) from the same samples and internal positive controls were also amplified. Six 2-L water samples per site were processed which recorded a 100% detection of common carp DNA during the 2014 spring survey when common carp CPUE was ten times higher compared to the autumn 2015 survey. It is possible that the sampling volume was sufficient in spring but insufficient during autumn when common carp CPUE was much lower. The seasonal variation in common carp eDNA seen in this study has also previously been reported by Turner et al. (2014a), indicating the importance of timing eDNA surveys when target species are known to be more abundant and active.

It is possible to obtain water samples without the target species' DNA particularly in large and complex systems. This has been shown previously where a patchy distribution and highly variable carp DNA concentrations ranging from zero to thousands of DNA copies in samples from the same site were found (Turner et al. 2014b). Similar variability was recorded in the common carp DNA concentrations in this study, including during the spring 2014 survey when common carp CPUE was higher. For example, five out of six samples from Sawmill Creek during the spring survey were negative for eDNA while the only positive sample had a concentration of 324 DNA copies/4 $\mu$ l. This result suggests that eDNA is heterogeneously distributed in the environment or occurs in clumps.

A higher eDNA detection rate for Oriental weatherloach was recorded compared to the fyke net results, perhaps because fyke netting is not the most appropriate sampling method for this mud burrowing benthic fish. Only fish of a certain diameter can be caught using fyke nets as larvae and small young of year pass through the mesh holes. The eDNA method is advantageous over fyke netting in these cases because the method can detect DNA from a species regardless of its size or age class. Using several fish sampling methods may also result in better estimates of species composition (Fago 1998) but often, the choice of sampling method depends upon the purpose of the activity and availability of resources. Electrofishing has been used to survey for Oriental weatherloach in Australia in addition to fyke nets (Keller and Lake 2007; Lintermans and Parks 1993). In the Katarapko/Eckert Anabranch system, other methodology (e.g., electrofishing) within some areas is not possible owing to access issues, snags, shallow areas and creek width etc. Future comparisons between the eDNA method and traditional method in detecting Oriental weatherloach should include other suitable methods in addition to fyke netting.

The eDNA approach has been reported to be a more sensitive method than netting (Jerde et al. 2011) or electrofishing (Wilcox et al. 2016) for fish detection, but it remains imperfect like other traditional survey techniques (Dejean et al. 2012; Schmidt et al. 2013; Yoccoz 2012). This suggests caution in interpreting negative eDNA results and vigilance in reducing the occurrence of false negatives. False negatives in eDNA surveys can be reduced by increasing sampling intensity (more samples/sites), but in most surveys this must be balanced with time and resource costs. The sensitivity of eDNA surveys can also be improved by the use of different filter paper types or extraction methods (Deiner et al. 2015; Liang and Keeley 2013). Recently, a few models have been developed using eDNA presence/absence data to estimate the sensitivity of eDNA surveys (Furlan et al. 2015; Schmidt et al. 2013). These models can be used to ascertain whether enough samples have been analysed to reach a desired probability of detection and is useful for planning subsequent eDNA surveys. The use of controls which can monitor method efficiency through all stages of the eDNA analysis is also important to distinguish method error from legitimate negative results (Furlan and Gleeson 2016a).

The eDNA method's sensitivity is an advantage but it can also be a pitfall as it may predispose to false positives (Wilcox et al. 2016). Minimizing contamination is paramount and this can be done through rigorous decontamination of bottles and

equipment, the use of designated trace DNA laboratories, and adequate quality checks (Furlan and Gleeson 2016a; Goldberg et al. 2016). All bottles and equipment in this study were decontaminated through immersion in a 10% bleach solution for a minimum of 10 minutes, similar to other eDNA protocols previously reported (Jerde et al. 2013; Turner et al. 2015; Wilson et al. 2014). However, low level contamination was still detected in two of the equipment blanks. Wilcox et al. (2016) also reported contamination in two equipment controls prompting them to use 50% bleach solution for later experiments. The current recommendation is decontamination of reused equipment/supplies using 50% commercial bleach solution (Goldberg et al. 2016).

A positive eDNA detection only signifies DNA presence in the environment but does not confirm whether the DNA is from a live organism. DNA can come from other sources such as dead or decaying organisms, excrement from predators, contaminated equipment or brought in from another area by flowing water. The sites sampled in this study are connected as a series of creeks within the Katarapko/ Eckert anabranch. Although physical barriers may prevent fish movement, water could still flow between sites especially when water levels are high. Transport and persistence of eDNA in flowing water is not well understood, although abiotic (flow rate, temperature, sunlight, DNA degradation rate, dilution, deposition and re-suspension) and biotic factors all play a part. A few studies have shown that eDNA can be detected anywhere within 5 m to 12 km from source in flowing water (Deiner and Altermatt 2014; Jane et al. 2015; Pilliod et al. 2014) although this would likely differ between species, sites and density. For this study, it cannot be discounted that the eDNA detected could have originated from upstream sites.

Both eDNA and fyke netting results indicated a decrease in the distribution of common carp and Oriental weatherloach in autumn compared to spring. Both species are known to spawn during spring, with carp's spawning season extending until autumn (Sivakumaran et al. 2003). The model used in this study suggested season as a significant predictor of eDNA copy number for carp and Oriental weatherloach. This result supports the recommendation of timing eDNA surveys during the breeding season or when the species are known to be more abundant. Carp are also known to use shallow wetlands during the warmer months (spring to autumn) and migrate to deeper river water during the colder months (Smith et al. 2009). Both the autumn and spring survey dates for this study occurred within the spawning season for carp in South Australia (October–April) (Smith and Walker 2004). Thus, we did not expect to see

such a marked difference in CPUE/eDNA copy numbers between the two monitoring seasons. Brown et al. (2005) however reported spawning peaks for carp occurring during spring. Thus, the increased spawning activity in spring could account for the higher eDNA and CPUE recorded during this time. It is also possible that carp have started to move into deeper water prior to our autumn survey. In addition, the Katarapko/Eckert Anabranch system is now the subject of substantial environmental rehabilitation effort wherein construction of a complex series of regulator structures and blocking banks to allow broad-scale engineered floodplain inundation in the absence of elevated discharge in the River Murray are planned (Bice et al. 2015). Some of these constructions had begun during our study period and it is probable that these affected the movement patterns of fish (and their DNA) in the area. The ability of the eDNA method to mirror the seasonal changes in CPUE shows its applicability as an indirect indicator of abundance.

There was no direct positive relationship between CPUE and DNA copy numbers for common carp in this study. This is similar to the findings of Turner et al. (2012) wherein no correlation was found between common carp DNA concentration and abundance using fyke nets in a coastal wetland site. In contrast, Pilliod et al. (2013) found that the amount of eDNA in streams was related to the density, biomass and occupancy of stream amphibians. It can be inferred that the differences in species, sites and sampling methods between studies could account for the varying results observed. Unlike lentic systems, correlating abundance with eDNA concentration in moving water is less straightforward because flow, discharge rate, channel morphology, downstream transport and other factors could complicate observations (Jane et al. 2015; Pilliod et al. 2013; Wilcox et al. 2016). Although this study found a positive relationship between CPUE and DNA copy number for redfin perch and Oriental weatherloach, this result should be interpreted with caution since the correlation is based on only a few data points and for one season only since no Oriental weatherloach or redfin perch were caught using fyke nets in the autumn survey.

The results showed no significant difference in eDNA concentration or number of positive PCR replicates from surface and subsurface water samples, even for a benthic fish like Oriental weatherloach. Water samples were collected near the banks of the creeks/streams where the water level at most of our survey sites was less than  $< 0.5$  m. It could be inferred that the water at this depth is relatively well-mixed thus accounting for our observation. Most eDNA studies obtain samples from the water surface

presumably out of ease of sampling. There are only a few studies including this study, that have investigated eDNA concentrations between surface and subsurface water samples. Moyer et al. (2014) found that surface water was the optimal location in the water column for the detection of African jewel fish (*Hemichromis letourneuxi*) in small ponds (average depth = 1.4m) whereas no significant difference in common carp eDNA detection and concentration was found between surface and subsurface samples in a small lake (average depth =  $< 2$ m) (Eichmiller et al. 2014). To the best knowledge of the authors, this study is the first study that investigated the difference in eDNA concentration between surface and subsurface water samples in flowing water. We wanted to determine if more DNA copies could be obtained from subsurface samples for a benthic fish such as Oriental weatherloach compared to the more pelagic redfin perch and common carp. Although more DNA and a higher PCR detection were recorded in subsurface samples for the benthic species (Oriental weatherloach), the difference from surface samples is not significant. Further studies are needed to investigate whether the same holds true for benthic fish in deeper flowing or static water.

Sampling surface water is easier and quicker than subsurface sampling and can be done without getting into the water wherein potential contamination can be introduced through clothing and footwear. These results give confidence that eDNA sampling from surface water in relatively shallow waterways for the three species studied will not compromise eDNA detection. Subsurface water samples also take a longer time to filter due to more sediment especially in waterways with a muddy substrate. Collecting sediments instead of water samples can also be an option as Turner et al. (2015) found that for bigheaded Asian carp, eDNA is more concentrated in sediments than surface water. However, using sediments for eDNA analysis have three main disadvantages: first, collection of sediments and decontamination of equipment is more laborious; second, PCR inhibition is very likely; and third, eDNA bound to sediments can persist for a long time, even months after a species is no longer present making spatiotemporal inferences complicated (Turner et al. 2015).

## Conclusion

Using the specific methodology outlined in this research (filtration, extraction, sample volume etc.), this study demonstrated that eDNA can be a sensitive detection tool but because the eDNA method also suffers from imperfect detection, it is more powerful

when used in conjunction with other survey methods as a way to enhance detection rates and to increase confidence in the monitoring results. Despite the advantages of eDNA detection, there is important ecological information such as fish recruitment and size / age classes that can only be gained through traditional monitoring. Correlating eDNA copy numbers to catch per unit effort in flowing systems needs further study. The patchy distribution of fish DNA and the large variation in copy numbers in samples from the same site currently precludes one from making inferences on fish abundance. However, eDNA can detect general trends or changes in abundance, which is still useful in monitoring. If used on its own, eDNA can alert managers to the presence or absence of a target species, allowing them to make more informed decisions especially for species of special concern such as threatened or invasive species.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Geo-referenced data points and description of the study sites in Katarapko/Eckert Creek Anabranh, South Australia.

**Table S2.** Summary statistics of DNA copy number in the eight study sites in Katarapko, South Australia.

**Figure S1.** Study sites in Katarapko/Eckert Creek Anabranh, South Australia.

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