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The value of quantitative environmental DNA analyses for the management of invasive and endangered native fish.

Running title

Value of quantitative eDNA in management.

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Abstract

1. Environmental DNA monitoring is a useful tool for species detection but its use to address management questions remains scarce. One factor limiting the use of eDNA as routine monitoring tool is uncertainty around the potential of eDNA data to estimate species abundance. While several confounding factors limit the ability of eDNA data to estimate absolute abundances at large spatial and temporal scales, eDNA data have the potential to estimate relative species abundances patterns at smaller scales, and this information can assist management.

2. Environmental DNA and conventional monitoring surveys were conducted in the Abercrombie River catchment (Australia) where an incursion of the invasive redfin perch (*Perca fluviatulis)* threatens the survival of a population of endangered Macquarie Perch (*Macquaria australasica)*. Species-specific assays were used to quantify eDNA concentrations from water samples and estimate the relative abundance of both species. Electrofishing and fyke netting surveys were used to validate key observations from the eDNA survey.

3. Environmental DNA of both species was detected at all sites except one, where redfin perch DNA was not detected. Between species comparisons of eDNA concentrations revealed a clear negative relationship between the eDNA concentrations of both species, consistent with other evidence of redfin perch having a negative impact on Macquarie perch populations. Between site comparisons of redfin perch eDNA concentrations showed evidence of a novel incursion of the species in the upper reaches of the Abercrombie River and conventional monitoring in the following year confirmed the pattern of increased redfin perch abundances from downstream to upstream sites.

4. Relative comparisons of eDNA concentrations of aquatic species can be used to assess species interactions and reveal unexpected species abundance patterns (e.g. allowing inferences of novel incursions of invasive species). This information is critical to evaluate current, and design future, management strategies. Consequently, while deriving absolute species abundances from quantitative

eDNA data may remain challenging, the use of quantitative eDNA surveys can provide relative abundance patterns valuable to the conservation and management of invasive and endangered species.

5. The quantitative nature of eDNA survey data has been debated extensively in the current literature because of potential confounding influences. Current study results show that these confounding influences may be less problematic at small spatial scales and quantitative eDNA data can be effective to monitor relative species abundances patterns.

Introduction

Ever since it was recognised that trace DNA fragments left behind by organisms in the environment (environmental DNA or eDNA) can be used to monitor rare, cryptic and invasive biodiversity (Ficetola, Miaud, Pompanon, & Taberlet, 2008), interest in the research field has increased along with the number of scientific publications (Jarman, Berry, & Bunce, 2018). While methods for eDNA-based biodiversity monitoring continue to be improved (Geerts, Boets, Van den Heede, Goethals, & Van der heyden, 2018; Hinlo, Gleeson, Lintermans, & Furlan, 2017; Sepulveda et al., 2019; Thomas, Nguyen, Howard, & Goldberg, 2019; Tsuji, Takahara, Doi, Shibata, & Yamanaka, 2019), the use of eDNA-based methods to address questions relevant to environmental managers remains under-utilised.

Environmental DNA has proven valuable for monitoring single species at low densities (Ficetola et al., 2008; Ikeda, Doi, Tanaka, Kawai, & Negishi, 2016; Sigsgaard, Carl, Møller, & Thomsen, 2015); with applications that include improving species distribution estimates of invasive and endangered species (Bylemans, Furlan, Pearce, Daly, & Gleeson, 2016; Doi, Katano, et al., 2017; Gold et al., 2020; Mauvisseau et al., 2020; Smart, Tingley, Weeks, Van Rooyen, & McCarthy, 2015), evaluating eradication efforts (Davison, Copp, Créach, Vilizzi, & Britton, 2017; Furlan, Gleeson, Wisniewski, Yick, & Duncan, 2019; Robinson, Garcia de Leaniz, Rolla, & Consuegra, 2019),

74 monitoring reintroductions and post-release survival of species (Hempel et al., 2020; Rojahn,
75 Gleeson, & Furlan, 2018) and determining the timing and location of reproductive activity (Bylemans
76 et al., 2017; Erickson et al., 2016). Environmental DNA can also be used to obtain information on
77 community composition through eDNA metabarcoding. Metabarcoding has been used successfully
78 to monitor temporal shifts in community composition (Bista et al., 2017), assess the ecological health
79 of water bodies (Li et al., 2018) and to determine population genetic diversity (Sigsgaard et al., 2016).
80 For both single and multiple species, several studies have indicated that data on eDNA concentration
81 can be used to estimate species abundances (Evans et al., 2015; Knudsen et al., 2019; Lacoursière-
82 Roussel, Côté, Leclerc, & Bernatchez, 2015; Ushio et al., 2018). However, correlations between
83 eDNA concentrations and species abundances in natural systems are generally weak compared to
84 laboratory studies (Yates, Fraser, & Derry, 2019), particularly in riverine systems (Hinlo, Lintermans,
85 Gleeson, Broadhurst, & Furlan, 2018; Spear, Groves, Williams, & Waits, 2015). In addition to the
86 different processes that influence eDNA shedding and degradation rates (e.g. seasonality,
87 temperature, pH, etc.) (Sassoubre, Yamahara, Gardner, Block, & Boehm, 2016; Strickler, Fremier, &
88 Goldberg, 2014; Tsuji, Ushio, Sakurai, Minamoto, & Yamanaka, 2017), a quantitative interpretation
89 of eDNA data in riverine systems is further complicated by water flow, eDNA transport and eDNA
90 retention in the substrate (Fremier, Strickler, Parzych, Powers, & Goldberg, 2019; Jane et al., 2015;
91 Shogren et al., 2018; Shogren, Tank, Egan, Bolster, & Riis, 2019). Nevertheless, it may be possible
92 to compare relative eDNA concentrations among species at the same or similar sites where the
93 influence of confounding factors (i.e. turbidity, pH, temperature, etc.) are likely reduced.
94 A system of particularly high conservation value where quantitative eDNA data can provide
95 useful information for future management is the Abercrombie River catchment in central-west New
96 South Wales (NSW) (Australia). The Abercrombie River is part of the Lachlan River catchment
97 which historically supported a large population of the national endangered Macquarie perch

98 (*Macquaria australasica*, Percichthyidae) (Gilligan, McGarry, & Carter, 2010). Currently, the
99 catchment remains one of the last strongholds for Macquarie perch, as habitat degradation and the
100 spread of the invasive redfin perch (*Perca fluviatulis*, Percidae) have caused dramatic declines and
101 the likely extirpation of the adjacent Lachlan River Macquarie perch population (Gilligan et al., 2010;
102 Lintermans, 2007). However, recent surveys have reported the presence of redfin perch in the lower
103 reaches of the Abercrombie River (Figure 1) and the further spread of redfin perch may threaten the
104 long-term survival of the remnant Macquarie perch population. A captive breeding program was
105 initiated by NSW Department of Primary Industries to establish a refuge population in the Retreat
106 River, a side tributary of the Abercrombie River. The Retreat River contains sufficient Macquarie
107 perch habitat to support a population and a series of waterfalls in its lower reaches (2-3 km upstream
108 from the confluence of the Retreat and Abercrombie River) form a natural barrier reducing the
109 chances of redfin perch invasions (Figure 1) (Gilligan et al., 2010; Pearce, 2013). From 2010 to 2014,
110 more than 19,000 Macquarie perch fingerlings were released into the Retreat River (Pearce, 2013).
111 Subsequent monitoring of the Retreat River population has shown that Macquarie perch have
112 persisted (recaptures of released fish) and have bred successfully (capture of several wild born
113 fingerlings in 2017 and 2018).

114 This study aimed to assess the value of quantitative eDNA data in guiding future management
115 of redfin and Macquarie perch in the Abercrombie and Retreat Rivers. If quantitative eDNA data
116 accurately reflects relative species abundance patterns it can be predicted that: 1) the current upstream
117 expansion of redfin perch will create a gradient of decreasing eDNA concentration from the lower to
118 upper sites in the Abercrombie River; and 2) the negative interactions between redfin and Macquarie
119 perch would result in a negative correlation between the observed eDNA concentrations of the two
120 species. Finally, the general benefits of quantitative eDNA surveys are discussed as well as the
121 specific management implications.

Materials and methods

Environmental DNA monitoring

Environmental DNA samples were collected from seven sites in the Abercrombie River and two sites in the Retreat River over a four-day period in May 2018 (Figure 1). Both rivers are located within the Abercrombie River National Park which is characterised by steep terrain and limited access along river corridors, partly due to extensive vegetation and remnant bushland. During the sample period, river systems varied in size and flow with some smaller river segments dry. Sample sites were therefore selected based on current knowledge of the target species' distributions, accessibility, and suitability (i.e. presence of large pools). Samples were collected beyond the currently known distribution limits of redfin perch as previous studies have indicated that conventional monitoring surveys generally underestimate the distribution of species compared to eDNA surveys (Figure 1) (Bylemans et al., 2016; Jerde, Mahon, Chadderton, & Lodge, 2011). Sites in the Retreat River were situated below and above the waterfalls to assess the potential effectiveness of this natural migration barrier.

Eight water samples (1 L) were collected per site using clean DNA-free Nalgene bottles (i.e. treated with a 20% bleach solution and rinsed with UV-treated tap water). At most sites a single pool was sampled from either the main river channel or isolated pool, with sampling focussed on areas containing vegetation (i.e. redfin perch habitat) (Lintermans, 2007; Westrelin, Roy, Tissot-Rey, Bergès, & Argillier, 2018). If vegetation was lacking or difficult to access, samples were collected at approximate evenly spaced intervals around the edge of the pool. For two sites where water levels were low and a single large sampling pool could not be identified, samples were collected from two smaller pools. One blank field control (BFC) was included per site consisting of a 1 L bottle filled with UV-treated water which was opened on site, exposed to the air for *ca*. 1 min, closed and submerged. Samples were stored on ice and eDNA was captured within 12 hours using 1.2 μm, 47

146 mm cellulose-nitrate filter papers (Sarstedt, Nümbrecht, Germany). Prior to filtering, all equipment
147 was cleaned using the protocol described earlier and negative equipment controls (NEC) were
148 obtained by filtering 0.5 L of UV-treated tap water before processing eDNA samples. A maximum
149 of three individual filter papers was used per sample to maximize the total volume of filtered water.
150 Filter papers were stored in 5 mL tubes, placed on ice during the sampling campaign (≤ 4 days) and
151 stored at -20 ºC after returning to the University of Canberra (ACT, Australia). Environmental DNA
152 was extracted from filter papers in a dedicated trace DNA laboratory at the University of Canberra.
153 During each batch extraction, BFCs and NECs were included to monitor potential cross-
154 contamination. The Qiagen DNeasy® kit (Qiagen, Hilden, Germany) was used to extract eDNA with
155 slight modification to the protocol (Hinlo et al., 2017; Renshaw, Olds, Jerde, McVeigh, & Lodge,
156 2015). Environmental DNA extracts were eluted in 100 µL of Buffer AE and stored at -20 ºC until
157 further analyses.

158 Quantitative Real-Time PCR (qPCR) was used to determine the presence/absence of target
159 DNA and simultaneously quantify target eDNA concentrations. A redfin perch specific Taqman™
160 assay was previously designed and validated (Furlan & Gleeson, 2016a) while the Macquarie perch
161 assay consisted of previously designed and validated primers and a newly designed minor groove
162 binding hydrolysis probe (5'- ACAGCCCAAAACGTCAGGTCGAGG-3') (Bylemans et al., 2017).
163 For each target, six qPCR replicates were performed per sample and a generic fish assay was included
164 to evaluate the sample processing workflow and assess the occurrence of PCR inhibition (Furlan &
165 Gleeson, 2016b). For three sites by species combinations the initial PCR replicates showed low levels
166 of amplification for either assays and thus an additional six PCR replicates were performed (Table
167 1). PCR setups were performed in a physically separated room with positive air pressure within the
168 Trace DNA laboratory to minimise contamination risk. An epMotion® 5075 Liquid Handling
169 Workstation (Eppendorf, Hamburg, Germany) was used to setup qPCR reactions in a 384 well plate

format. Individual reactions contained 7.5 µL of Taqman™ Environmental Master Mix 2.0, the target-specific assay (1x), the generic fish assay (0.75x), 2 µL of template eDNA and DEPC water to a final volume of 15 µL. Each setup included a 6-point standard curve consisting of target specific PCR amplicons with concentrations ranging from 3,000,000 to 30 copies per reaction. Furthermore, non-template controls (NTC) were included during each setup to assess potential cross contamination. The ViiA™ 7 Real-Time PCR machine (Applied Biosystems, Foster City, USA) was used to run qPCR analyses with cycle conditions set at 95 ºC for 10 mins followed by 55 cycles of 95 ºC for 15 sec and 60 ºC for 30 sec. Results were visually inspected, and reactions were only considered valid if a clear exponential amplification curve could be observed for at least one of the assays (i.e. a failed amplification for both assays indicates improper sample processing or the presence of PCR inhibitors).

Conventional monitoring

Conventional fish monitoring surveys were conducted at 12 sites during April 2018 (8 sites) and May 2019 (8 sites) (Table 2). During both years, the primary purpose of the survey was to assess the status of the Macquarie perch population in the Retreat River and consequently most sites were located in this tributary (all 8 sites surveyed in 2018 and 5 out of 8 sites surveyed in 2019). However, in the 2019 survey 3 sites were sampled in the upstream section of the Abercrombie River to help validate some the results of the eDNA survey.

Fish sampling protocols used were those developed for the Murray-Darling Basin Authority's Sustainable River Audit – Fish theme (Davies, Stewardson, Hillman, Roberts, & Thoms, 2012). Electrofishing consisted of eight 150 second (power-on time) single-pass operations with a Smith-Root model LR24 backpack electrofishing unit (Smith-Root Inc, Vancouver, USA). In addition to electrofishing, 5 fyke nets (single wing, 5 meters, 6 hoops with a front 'D' 60cm drop and 19mm mesh) were set overnight for a minimum period of 12 hours at 4 sites (1 site in 2018 and 4 sites in

194 2019). All captured fish were identified to species level, length measurements were taken to the
195 nearest millimetre and the weight of individuals > 100 mm in length was measured to the nearest
196 gram.

197 ## Data analyses

198 The eDNA survey results were summarized to show the total number of PCR replicates, and the
199 number of valid and positive PCR replicates for each site and species. The conventional monitoring
200 results were summarized to show the total number of Macquarie perch and redfin perch caught at
201 each site for each method.

202 Detailed analysis of the quantitative eDNA data was performed using an occupancy-detection
203 model modified from a previously published hierarchical Bayesian model used to estimate eDNA
204 concentration from PCR replicates (see Supporting Information) (Furlan, Gleeson, Hardy, & Duncan,
205 2016). We used this approach to estimate the probability of species presence and the concentration
206 of eDNA at each site conditional on presence, accounting for the patchy distribution of eDNA in the
207 water samples. Briefly, the eDNA copy numbers per PCR replicate were modelled as drawn from a
208 Poisson distribution with mean proportional to the total number of eDNA molecules in each water
209 sample. The number of molecules per water sample were modelled as drawn from a negative binomial
210 distribution with mean proportional to the concentration of eDNA at a site conditional on species
211 presence and the volume of water filtered. If a species eDNA was detected in a sample, the probability
212 of species presence at a site was one and the proportion of samples with positive detections could be
213 used to estimate the detection probability. Given the detection probability, the probability of false
214 negative detections (i.e. the species was present but remained undetected) could then be estimated.
215 The distribution of positive detections among samples and PCR replicates allowed an estimate of the
216 concentration and dispersion of eDNA at each sample site (see Furlan *et al*., 2016). The model was
217 fitted to the data in R using the package jagsUI (Kellner, 2015; R Development Core Team, 2010).

Relatively uninformative priors (parameters were given flat normal priors with mean = 0 and variance = 100) were specified to allow the data to drive parameter estimation.

The Bayesian model produced posterior distributions describing the estimated concentration of eDNA for each species at each site. Posterior distributions were summarized using the mean and 95% quantiles to generate 95% credible intervals. For all sites within the main Abercrombie River channel, the estimated concentrations were used to test for a negative correlation between redfin and Macquarie perch abundances. The estimated Macquarie perch eDNA concentrations were plotted against the redfin perch eDNA concentrations, and a generalized linear model was fitted to the log-transformed mean eDNA concentrations.

Results

Environmental DNA monitoring

All negative controls performed as expected except for one NTC replicate which amplified for redfin perch. The PCR well that produced this positive amplification was located directly adjacent to a well containing the standard curve reactions. Consequently, this positive amplification is likely the result of cross contamination when loading the standard curve samples on the plate. None of the other negative controls (BFC and NEC) in the same run produced a positive amplification, indicating the contamination was localized.

The percentage of valid PCR replicates for each site and target species ranged from 25-100% (Table 1). Positive amplification of the Macquarie perch assay was observed at all sites, with the percentage of positive PCR replicates ranging from 35.71-100%. Amplification of redfin perch DNA occurred at all but one site, with the percentage of positive PCR replicates ranging from 36.36-100%. The only site at which redfin perch DNA was not detected was also the only site located above the

240 waterfalls in the Retreat River. The model indicated a 0.08 probability of redfin perch being present
241 at this site even though no eDNA was detected.

242 Macquarie perch standard curves indicated an average PCR efficiency of 84.48% (±19.94)
243 and an R^2 value of 0.993 (±0.003) while redfin perch standard curve samples showed an average PCR
244 efficiency of 81.14% (±3.74) and an R^2 value of 0.997 (±0.003). The posterior estimates of eDNA
245 concentrations showed overall higher redfin perch eDNA concentrations in the Abercrombie River
246 relative to Macquarie perch eDNA concentrations (Figure 2), with a general increase in redfin perch
247 eDNA concentrations from downstream to upstream sites (Figure 2). In contrast, Macquarie perch
248 eDNA concentrations in the Abercrombie River tended to decrease from downstream to upstream
249 sites (Figure 2). These contrasting trends in eDNA concentrations were also evident in the regression
250 analysis, which revealed a clear negative relationship between redfin and Macquarie perch eDNA
251 concentrations (Figure 3).

252 ## Conventional monitoring

253 Conventional monitoring results from the 12 sites sampled in 2018 and 2019 are summarized in Table
254 2. Surveys in the Retreat River showed no evidence of the invasive redfin perch while Macquarie
255 perch were caught at six out of the nine sites (Table 2). In two out the three sites surveyed in the upper
256 Abercrombie River in 2019 (i.e. Binacrombie and Jerrong), high numbers of redfin perch were
257 caught. Macquarie perch were only caught in the most upstream site (i.e. Binacrombie) within the
258 Abercrombie River, this is however further upstream than they have previously been detected (Figure
259 1). When evaluating the capture success of both methods used, the most notable pattern is the higher
260 success rate of capturing Macquarie perch using fyke nets compared to electrofishing (Table 2).

Discussion

We aimed to assess the value of a quantitative eDNA survey to guide management of an invasive fish that is impacting on a native endangered fish species. Data revealed a clear negative relationship between eDNA concentrations of each species, consistent with other evidence highlighting a negative impact of invasive redfin perch on the remaining Macquarie perch populations. Furthermore, while conventional surveys conducted prior to this study indicated that invasive redfin perch are actively moving upstream in the Abercrombie River, the quantitative eDNA data provides evidence of a new incursion as redfin perch eDNA concentrations generally increased in more upstream sites. These findings give valuable insights into the ecological interpretation of quantitative eDNA data while also providing detailed background information to guide future management actions in the study system.

Evaluating the relationship between eDNA concentrations and species abundance has received considerable attention in current literature (Doi, Inui, et al., 2017; Knudsen et al., 2019; Lacoursière-Roussel et al., 2015). However, the value of quantitative eDNA data to provide estimates of absolute species abundance (i.e. biomass or number of individuals) remains questionable due to multiple confounding influences (Hinlo et al., 2018; Shogren et al., 2019). Nonetheless, the results of this study show that quantitative eDNA data can reveal relative species abundance patterns within a river system. Firstly, comparisons of between species eDNA concentrations show evidence of a negative impact of redfin perch on the abundance of Macquarie perch. While this negative interaction between the two target species is not novel *per se* (Arthington & McKenzie, 1997; Lintermans, 2007), it highlights the value of quantitative eDNA data to infer species interactions. However, caution is needed when interpreting regression analyses as the existence of a correlation does not necessary indicate a causal relationship. For example, sites able to support high numbers of one species may be ecologically unsuitable for the other species and *vice versa* and thus the observed relationship may only reflect differences in habitat requirements. Secondly, between site comparisons of redfin perch

285 eDNA concentrations indicate, in contrast to prior expectations, a higher abundance or redfin perch
286 in the upstream sites in the Abercrombie river. A possible explanation for this observed pattern is the
287 occurrence of a relatively recent and novel redfin perch incursion in the upper section of the
288 Abercrombie River. Conventional monitoring efforts in the subsequent year provided further support
289 for this explanation as a high number of redfin perch were captured in two out of three upstream sites
290 while surveys from 2014 to 2018 only indicated a low abundance of redfin perch in low sections of
291 the Abercrombie river (data not shown). These results show the ability of quantitative eDNA data to
292 reliably estimate relative species abundance patterns within a riverine system.

293 The results also give valuable insights from a conservation management perspective. The
294 absence of redfin perch detections in the Retreat River above the waterfalls, strongly indicates they
295 are currently absent in this river section. However, future redfin perch incursions are a realistic threat
296 and should be considered in management through, for example, increased community awareness to
297 avoid future deliberate/accidental releases of redfin perch. Current threats to the Abercrombie River
298 Macquarie perch populations also significantly increases the conservation value of the Retreat River
299 refuge populations (Pearce, 2013). In particular, the Lachlan River Catchment Macquarie perch
300 populations have been found to be genetically divergent from the rest of the MDB and the
301 Abercrombie River population is likely to serve as source for the any re-colonisation of the Lachlan
302 River (Faulks, Gilligan, & Beheregaray, 2011; Pavlova et al., 2017). The further decline of the
303 Abercrombie River population could thus lead to the overall disappearance of the Macquarie perch
304 from the Lachlan River catchment and the loss of unique genetic diversity. While some of this
305 diversity would be preserved in the refuge population it is well known that captive breeding programs
306 typically reduce genetic diversity through founder effects and genetic drift (Rourke, McPartlan,
307 Ingram, & Taylor, 2009; Ryman & Laikre, 1991). It is highly recommended that future management
308 strategies are designed to maximize the preservation of the genetic diversity of the Macquarie perch

populations in the broader Lachlan River catchment. Re-establishing a captive breeding program and establishing additional refuge populations may be possible avenues but detailed genetic/genomic monitoring would be needed.

Conclusion

Environmental DNA monitoring requires reliable methods and interpretation of data to provide outcomes useful to management. In particular, the interpretation of quantitative eDNA may be challenging as direct correlations between eDNA concentrations and a species abundance can be questionable. Here we showed that relative comparisons of quantitative eDNA data (i.e. between site and species comparisons) can indeed reveal relative species abundance patterns. The information gained from this data provided insights into species dynamics which in turn can guide future management decisions for both invasive and endangered native species.

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Data accessibility

The Rscript to produce the different tables and figures is given in the supporting information. The full data associated with this study have been uploaded to the Figshare data repository and is available at https://doi.org/10.6084/m9.figshare.14569425.

Author contribution

JR, LP, DMG, DG and JB designed the study; JR and LP led the field work, JR and JB performed the laboratory work; JR, JB and RPD performed the data analyses. JR and JB led the writting of the manuscript with significant contributions from all co-authors.

Tables

Table 1. Details of the sampling sites, sampling effort and detection results for the environmental DNA based monitoring survey. Detection results are given as the total number of PCR replicates performed, the number of valid and positive PCR replicates for each site and both target species (i.e. Macquarie perch (*Macquaria australasica*) and redfin perch (*Perca fluviatilis*)).

Waterway	Location	ID	Latitude	Longitude	Month	Year	Target	No samples	No. PCRs		
									Total	Valid	Positive
Abercrombie River	The Junction	JU	-34.011370	149.466916	May	2018	*M. australasica*	8	48	41	38
							P. fluviatilis	8	96	55	20
	Millvale	MV	-34.093805	149.550849	May	2018	*M. australasica*	8	48	41	41
							P. fluviatilis	8	96	55	35
	Smiths Crossing	SC	-34.105458	149.585993	May	2018	*M. australasica*	8	48	41	38
							P. fluviatilis	8	48	40	39
	The Beach	TB	-34.128663	149.634285	May	2018	*M. australasica*	8	48	38	38
							P. fluviatilis	8	48	42	42
	Tween Cabin	TC	-34.174962	149.671264	May	2018	*M. australasica*	8	48	41	34
							P. fluviatilis	8	48	37	37
	Bummaroo Ford	BuF	-34.194049	149.738498	May	2018	*M. australasica*	8	48	30	16
							P. fluviatilis	8	48	22	21
	Jerrong	JE	-34.184885	149.888472	May	2018	*M. australasica*	8	96	24	16
							P. fluviatilis	8	48	28	28
Retreat River	Retreat crossing	RC	-34.119052	149.639486	May	2018	*M. australasica*	8	48	48	36
							P. fluviatilis	8	48	32	19
	The Sink	TS	-34.097073	149.659142	May	2018	*M. australasica*	8	48	28	10
							P. fluviatilis	8	48	17	0

528

529 **Table 2.** Details of the sampling sites, sampling effort and the catch results of the conventional fisheries surveys. Catch data are presented as
530 the total individuals caught for both Macquarie perch (*Macquaria australasica*) and redfin perch (*Perca fluviatilis*).

Waterway	Location	ID	Latitude	Longitude	Month	Year	Method	*M. australasica*	*P. fluviatilis*
Abercrombie River	Binacrombie	Bi	-34.191249	149.771151	May	2019	Fyke netting (n=5)	2	35
	Jerrong	JE	-34.184885	149.888472	May	2019	Electrofishing (n=8)	0	24
					May	2019	Fyke netting (n=5)	0	1
	Parliament Hill	PH	-34.182981	149.918924	May	2019	Electrofishing (n=8)	0	0
Retreat River	The Falls	ThF	-34.118000	149.644940	May	2019	Electrofishing (n=8)	0	0
	Lower Retreat	LR	-34.111000	149.657000	April	2018	Electrofishing (n=8)	0	0
	Mid Retreat	MR	-34.104810	149.662860	April	2018	Electrofishing (n=8)	2	0
					May	2019	Electrofishing (n=8)	0	0
	The Sink	TS	-34.097073	149.659142	April	2018	Electrofishing (n=8)	1	0
					May	2019	Electrofishing (n=8)	0	0
					May	2019	Fyke netting (n=5)	7	0
	Gates Tunnel	GT	-34.052000	149.649000	April	2018	Electrofishing (n=8)	3	0
	Ledinghams Hut	LH	-34.046700	149.663207	April	2018	Electrofishing (n=3)	1	0
					April	2018	Fyke netting (n=5)	17	0
					May	2019	Electrofishing (n=8)	0	0
					May	2019	Fyke netting (n=5)	2	0
	Creek Walk	CW	-34.044000	149.676000	April	2018	Electrofishing (n=8)	0	0
					May	2019	Electrofishing (n=8)	0	0
	Claytons Release	CR	-34.031580	149.697220	April	2018	Electrofishing (n=8)	1	0
	U/S Claytons	Cus	-34.024660	149.701800	April	2018	Electrofishing (n=8)	2	0

531

Figures

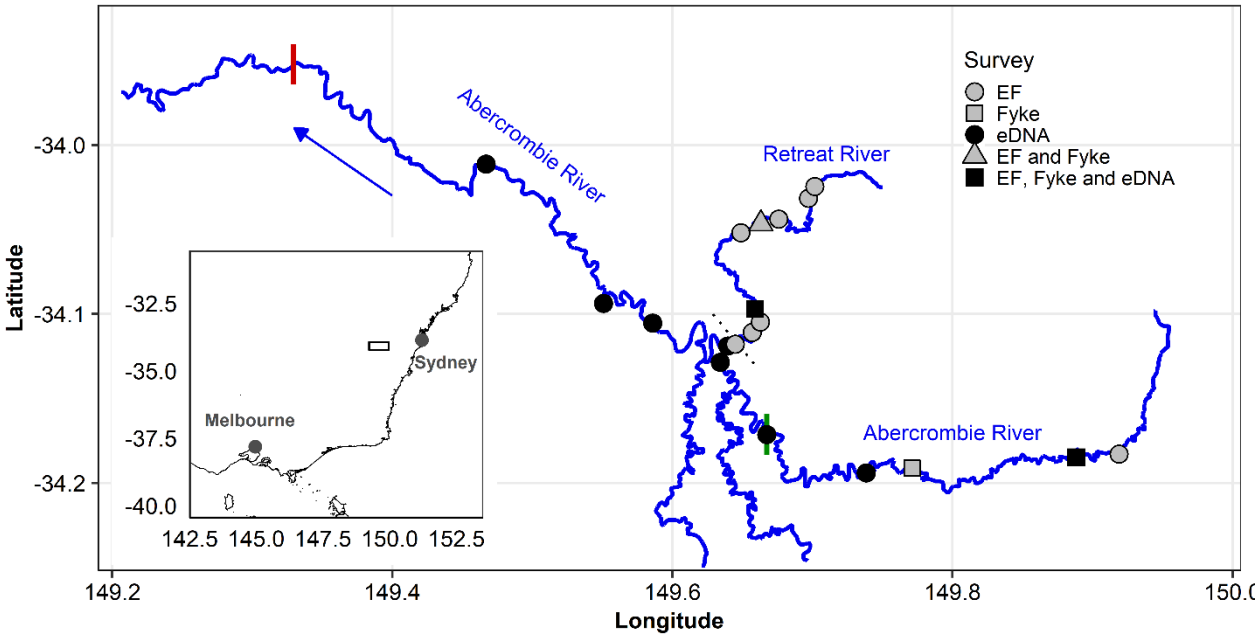

Figure 1. Map of the general study area with all sampling sites and the direction of water flow indicated by the blue arrow. The most upstream distribution limits of both Macquarie and redfin perch, as determined by standard monitoring surveys conducted prior to 2018, are indicated by the green and red vertical bars, respectively. The location of the waterfalls in the lower reaches of the Retreat River, which are believed to be an effective barrier for the future spread of redfin perch, are shown by the dotted black line.

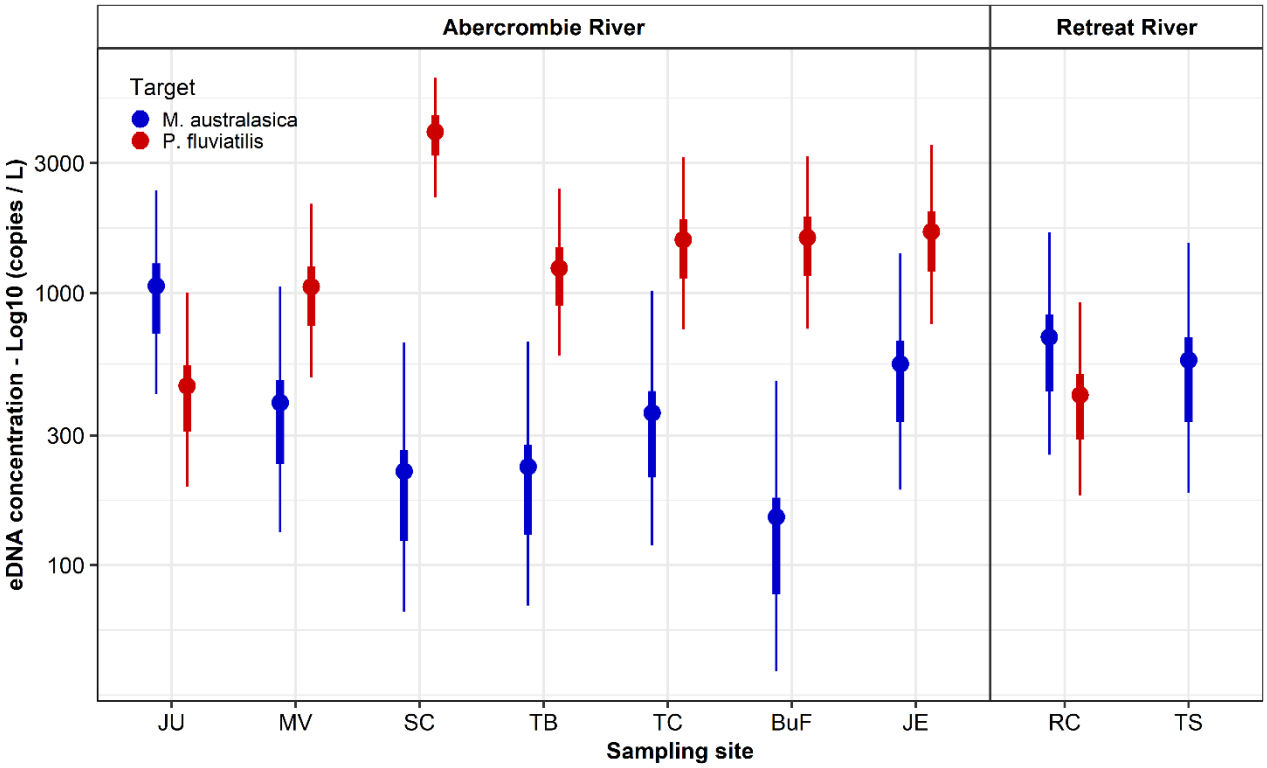

Figure 2. The environmental DNA (eDNA) concentrations for both Macquarie (blue) and redfin (red) perch for all the different eDNA sampling sites in the Abercrombie and Retreat Rivers. Mean eDNA concentrations are shown by the solid points while the wide and narrow lines represent the 50% and 95% credibility intervals, respectively. For each river, sites are ordered along the x-axis going from most downstream (left) to most upstream (right).

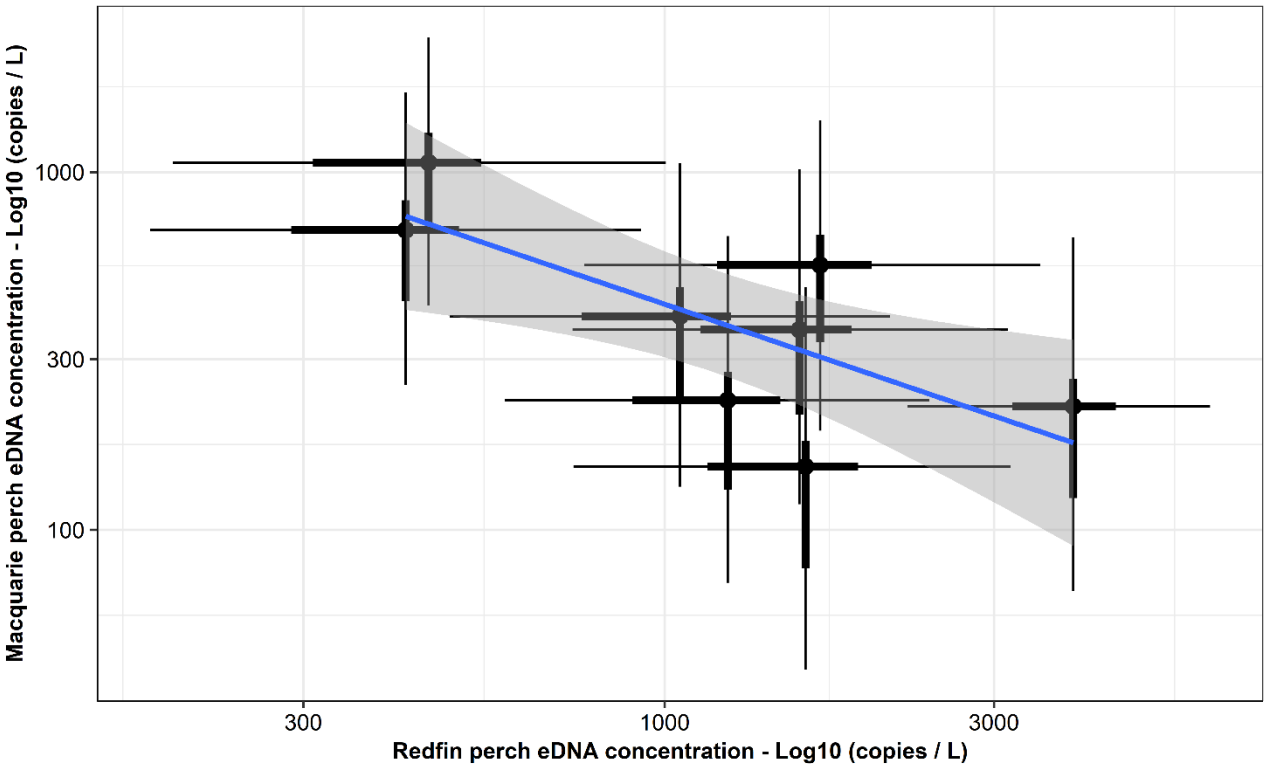

Figure 3. Linear regression showing the relationship between the mean environmental DNA (eDNA) concentrations for Macquarie perch and redfin perch considering all sites within the Abercrombie River where eDNA of both species was detected. Solid black points show the mean eDNA concentrations while the wide and narrow black lines represent the 50% and 95% credibility intervals, respectively.