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RESEARCH PAPER

A rapid assessment of non-native fish distributions in two English river basins using environmental DNA

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Abstract. Environmental DNA (eDNA) surveys are increasingly used to inform management decisions for non-native species, for example, by detecting the presence and plotting distributions of species that may be in too low abundance for easy detection by conventional means. A recently-developed nested PCR protocol was used to assess the distributions of three non-native fish species in two river basins of southern England (River Test, Hampshire; River Ouse, Sussex). These river basins were known to contain three non-native fishes, either in the recent past or currently: two invasive small-bodied fish species (topmouth gudgeon *Pseudorasbora parva*, sunbleak *Leucaspius delineatus*), as well as a currently non-invasive species predicted to become invasive under future climate conditions, pumpkinseed *Lepomis gibbosus*. Water samples were collected at locations from headwater streams to estuary. Pumpkinseed and sunbleak were both detected downstream of an angling venue in the Sussex Ouse catchment known to contain those species, with an upstream expansion of sunbleak suggested by the detection of eDNA at a few upstream locations. Neither sunbleak nor topmouth gudgeon was detected in water samples from the River Test catchment, suggesting that neither species has persistent populations in that river catchment.

Key words: invasive species, alien species, qPCR, lotic fish monitoring

Introduction

Accurate mapping of biological invasions is essential to inform management decisions on eradication, control or containment strategies to avoid or minimise impacts (Simberloff et al. 2005, Davison et al. 2017). Molecular techniques based on environmental DNA (eDNA) shed by organisms into the water have successfully detected a range of taxa (e.g. Darling & Mahon 2011, Rees et al. 2014, Ruppert et al. 2019), and in particular, fish species at low densities in still waters (e.g. Takahara et al. 2013, Lacoursière-Roussel et al. 2016, Davison et al. 2019) and in lotic systems (e.g. Minamoto et al. 2012, Keskin 2014, Bylemans et al. 2019). Species-targeted eDNA assays have sometimes outperformed traditional survey methods in mapping the distribution of small-bodied cryptic fishes in river catchments, detecting them outside of their previously known ranges (Paine et al. 2021, Westhoff et al. 2022). These assays can use either: 1) conventional PCR (cPCR), in which final amplification products are run on an agarose gel to determine whether the species is present; or 2) quantitative real-time PCR (qPCR), during which the

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machine measures fluorescence at each amplification cycle. These two approaches can be combined in a nested PCR protocol whereby the product of the cPCR amplifications is subjected, in the event of non-detection of the target species, by a round of qPCR amplification. In a previous laboratory study, a nested PCR protocol was 100´ more sensitive than cPCR or qPCR in sensitivity tests on DNA derived from topmouth gudgeon tissue (Davison et al. 2019).

Non-native fishes introduced to floodplain water bodies can be particularly problematic if they gain access to the main river, which can serve as a dispersal pathway to invade other floodplain water bodies (Fobert et al. 2013). Monitoring methods for freshwater fishes in water courses have generally successfully captured larger-bodied, more abundant species, with small-bodied fishes in low density potentially avoiding detection (e.g. Bohlin et al. 1989, Rogers et al. 2003). However, even if a target species occurs at very low densities, its eDNA is potentially detectable, providing information on distributions essential for developing management strategies, including control and containment measures (Davison et al. 2017).

Our study aimed to assess the distribution of three non-native fish species in two river catchments in southern England in a synchronic manner (*sensu* Amoros et al. 1987), i.e. the simultaneous sampling of an entire system, which resembles the approaches developed by government agencies to monitor fish stocks annually (e.g. Adjers et al. 2006) or at longer intervals (e.g. Coles et al. 1985, Persat & Chessel 1989). The three species are the: 1) North American freshwater sunfish, pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), which was introduced to England as an ornamental fish during the "acclimation society" era of the late 1800s or early 1900s (Copp et al. 2002); 2) sunbleak *Leucaspius delineatus* (Heckel & Kner, 1858), a small-bodied cyprinid native to continental Europe that was introduced around 1990 to southwest England, where it is considered invasive despite being in decline in its native range (e.g. Carpentier et al. 2007); and 3) topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846), a small-bodied invasive cyprinid native to parts of Asia that was first reported in England in 1996 (Domaniewski & Wheeler 1996) and is of particular concern due to its threat as a healthy host of the pathogen *Sphaerothecum destruens* and other diseases, and rapid spread within Europe (Gozlan et al. 2010).

Pumpkinseed has existed in (or in the vicinity of) the River Ouse in East Sussex (henceforth, the Sussex Ouse) since at least as early as the 1910s (Wheeler & Maitland 1973). During an electrofishing survey of two tributaries in 2001, pumpkinseed was relatively widespread but low-to-moderate abundance (Klaar et al. 2004), persisting at these densities for about a decade after that (Copp et al. 2010a, Jackson et al. 2016). Whereas, the introductions of both topmouth gudgeon and sunbleak to Great Britain (GB), which were more recent (the 1990s), have been linked to imports of the golden orfe, a variety of the ide *Leuciscus idus* (Linnaeus, 1758), to an ornamental fish farm (now defunct) at Crampmoor on Tadburn Lake Stream, River Test catchment, Hampshire (Farr-Cox et al. 1996, Pinder & Gozlan 2003). The known GB distribution of sunbleak remained limited to the southern English counties of Hampshire and Somerset (Pinder & Gozlan 2003) until discoveries of populations in 2003 and 2006 further east at locations in the catchment of the Sussex Ouse (Zięba et al. 2010). Whereas, topmouth gudgeon had dispersed much more widely in GB (Pinder & Gozlan 2003) as a contaminant of fish movements (Copp et al. 2010b), though this wider distribution has since been reduced thanks to a national eradication programme (Environment Agency 2019).

The specific objectives of our study were to: 1) design qPCR primers for the detection of pumpkinseed and sunbleak for use in a nested PCR protocol recently developed for high-precision detection of topmouth gudgeon (Davison et al. 2019); 2) carry out eDNA surveys along two river catchments in southern England where recent surveys had confirmed the presences of the target species (pumpkinseed in the Sussex Ouse catchment, topmouth gudgeon in the River Test catchment, and sunbleak in both catchments); and 3) assess whether the use of a nested PCR protocol can result in increased field detections when compared to cPCR alone.

Material and Methods

Study site

Sampling along the Sussex Ouse catchment (Fig. 1) focused on two second-order tributaries, Sheffield Stream and Batts Stream, the latter also known as Batts Bridge Stream. Sheffield Stream is known to contain pumpkinseed (Klaar et al. 2004, Villeneuve et al. 2005, Stakėnas et al. 2013) and possibly sunbleak, which was discovered in an upstream commercial angling venue (latitude, longitude: 51.023535, 0.009485) near Danehill, East Sussex (Zięba et al. 2010). Overflow from this venue discharges, via a vertical drain-pipe system, into the adjacent Sheffield Stream (Fobert

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et al. 2013). Both pumpkinseed and sunbleak have established self-sustaining populations in the angling ponds (Zięba et al. 2010, Copp et al. 2017, Bašić et al. 2018).

In the Batts Stream catchment, pumpkinseed is known to have been established in Boringwheel Lake (Villeneuve et al. 2005, Fobert et al. 2013), a 400-year-old, 2.6 ha former mill pond now used as a commercial trout fishery. After overtopping a gated weir, Batts Stream passes through this pond, continuing along the stream bed. Both tributaries, which are of variable width (1.0-4.3 m) and depth (0.05-1.5 m), are described in Fobert et al. (2013).

Water sampling sites in the River Test (Fig. 1), which is a chalk river of high conservation interest (Natural England 1997), focused on the receiving water course of a former fish farm at Crampmoor (Fig. 2). Fish escape from the fish farm was possible, via an unscreened discharge pipe, into Crampmoor Stream, which joins Tadburn Lake Stream (width = 0.7-2.0 m) and eventually joining the River Test at Romsey (50.984171, –1.503319). An established population of topmouth gudgeon in the fish ponds and possibly in the stream (Beyer et al. 2007) was eradicated in 2014-2015 as part of a national programme (Environment Agency 2014, Great Britain Non-native Species Secretariat 2015). Following the eradication of topmouth gudgeon, a pathogen known to be associated with the fish species, *S. destruens* (Gozlan et al. 2005), was detected in water samples collected downstream of the fish farm (Sana et al. 2018). The persistence of sunbleak in the Test system following its initial introduction in the 1990s remained unknown, hence our interest in testing for that species.

Water sample collection and preparation

Water samples were collected on 14-15 June 2016 (Sussex Ouse) and 16 June 2016 (Hampshire Test) at about 1.5 m distance from one bank, or from the mid-point of the stream at sites where the stream was < 3 m wide. A 500 mL polypropylene sampling cup, attached to a polypropylene sampling pole of 183 cm length (Camlab Ltd, Cambridge, UK), was used to collect the water, avoiding contact with the bottom sediment. At each sampling location, three replicates of 300 mL water were injected through a Sterivex-GP 0.22 µm sterile filter cartridge (EMD Millipore, Billerica, MA, USA) using a 50 mL sterile syringe (Thermo Scientific) attached to the cartridge's input opening. Cartridges from each location were sealed in individual plastic bags and immediately frozen (–20 °C) for transportation back to the laboratory. Contamination between sites was avoided by thoroughly disinfecting the pole and cup using Microsol 3+ sterilising solution (Anachem Ltd, Luton, UK) and then rinsing with de-ionised water. On each sampling day, water from a sterilised bottle of de-ionised water from the laboratory was filtered,

Table 1. Primers used for conventional PCR (cPCR) and quantitative PCR (qPCR) of topmouth gudgeon *Pseudorasbora parva*, pumpkinseed *Lepomis gibbosus* and sunbleak *Leucaspius delineatus*. For the present study, the cPCR primers for all species were designed by Davison et al. (2017). The qPCR primers for topmouth gudgeon were designed by Davison et al. (2019) and for pumpkinseed and sunbleak by P.I. Davison for the present study.

	Forward primer (5′-3′)	Reverse primer (5′-3′)	FAM probe (5′-3′)	Amplicon size (bp)
P. parva cPCR	CCTCTTCCGGA GTAGAGGCT	TAGGATTGGG TCTCCTCCCC	Not applicable	350
P. parva qPCR	GTGTTTCATCAAT TCTAGGCGCAAT	AGCTCATACAAAT AAGGGCGTTTGA	ATATAAAACCTCC AGCTATTTCC	101
L. gibbosus cPCR	CTAATAATTGGCG CCCCCGA	CGGACCAGACA AACAGTGGT	Not applicable	310
L. gibbosus qPCR	GCTGGCACGGGCTGAA	GAGAAAATAGTGA GATCAACGGATGCT	CCGGCAACCTAGC CCACGCC	83
L. delineatus cPCR	TTCGAGCCGAAC TAAGCCAR	GGCCTCAACCC CAGAAGAAG	Not applicable	251
L. delineatus qPCR	CCCACGCCTTCGT AATAATTTTCTT	CGGGCGCACCAA TCATTAG	CGGGTTTGGAAAC TGACTCGT	93

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handled and transported in the same manner as the stream samples and analysed in the laboratory to test for contamination.

In the laboratory, DNA was extracted from the cartridges using a PowerWater Sterivex™ DNA Isolation Kit (MoBio, Carlsbad, CA, USA), producing a final elution volume of 100 mL. The extracted sample was then diluted 1:5 in deionised water to dilute potential inhibitors (McKee et al. 2015).

Primer design and nested PCR protocol

To complement the existing qPCR primer for topmouth gudgeon (Davison et al. 2019), qPCR primers were designed for pumpkinseed and sunbleak (Table 1) to amplify a section of the mitochondrial cytochrome *c* oxidase I (COI) gene. Conventional PCR primers for all three species were designed and tested for sensitivity and specificity in a previous study (Davison et al. 2016).

DNA extracted from dorsal muscle tissue of individuals of all three target species collected from populations in southern England was used to test the primers' sensitivity as a positive control in cPCRs and to calibrate qPCRs. Concentrations of tissue-extracted DNA were measured using a Nanodrop® ND1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and calculated with the software ND-1000 v3.8.1 (Thermo Scientific).

The specificity of all primers was tested *in silico* against sequences in the NCBI Genbank database using the NCBI Primer Blast software (www.ncbi.nlm.nih.gov/tools/primer-blast/; Ye et al. 2012), and tested experimentally against 0.1 ng genomic DNA extracts from fish species likely to co-occur at the study sites: common carp *Cyprinus carpio* (Linnaeus, 1758), common bream *Abramis brama* (Cuvier, 1816), roach *Rutilus rutilus* (Linnaeus, 1758) and rudd *Scardinius erythrophthalmus* (Linnaeus, 1758). No amplification for any of these species was shown by either the cPCR or qPCR assays for pumpkinseed, sunbleak or topmouth gudgeon.

The nested PCR (nPCR) protocol described by Davison et al. (2019) involves two steps: 1) a cPCR, which in the event of a non-detection is followed by 2) a qPCR, using primers that are internal with respect to the cPCR amplified product, on the product of Step 1. On each sample, cPCRs were performed on 6 µL of eDNA sample, with the reaction mixture containing 0.5 µM of each specific primer, 10 µL of HotStar Taq® Plus DNA polymerase 2× (Qiagen Fast Cycling PCR Kit) and 2 µL of Coral Load Fast Cycling Dye 10× (Qiagen), made up to 20 µL with the addition of de-ionised water. The PCR cycling conditions consisted of an initial denaturation step of 95 °C for 5 min, followed by 32 cycles of 96 °C for 5 s, 62 °C for 5 s and 68 °C for 12 s, with a final extension step at 72 °C for 1 min. PCR products were visualised after 60 min of electrophoresis migration on 2% agarose gel, stained with SYBR™ Gold Nucleic Acid Gel Stain (Invitrogen). Five cPCR replicates were analysed for each sample location.

Samples of eDNA that provided a negative result at the cPCR (Step 1) stage were subjected to further (Step 2) analysis, with qPCR performed on the products from three of the five cPCR replicates. Real-time qPCRs were performed on an Applied Biosystems Step One™ system (Applied Biosystems, Foster City, CA, USA) in a 20 µL reaction mixture containing 2 µL of DNA sample, 1 µL of assay mix (18 µM forward and reverse primers and 5 µM probe) for the targeted species (Applied Biosystems), 10 µL of TaqMan® Genotyping Master Mix (Applied Biosystems) and 7 µL of de-ionised water. The thermocycling profile used was 2 min at 50 °C, 10 min at 95 °C, followed by 35 cycles of 15 s denaturation at 95 °C and 60 s annealing-extension at 60 °C. The standard curve, applied on each qPCR run, comprised a range of five 10-fold dilutions of tissue-derived DNA (10^{-2} to 10^{-6} ng µL^{-1}).

Sample extraction, PCR preparation and post-PCR analysis were performed in separate rooms of a dedicated molecular biology laboratory, observing strict anti-contamination procedures (no transfer of equipment between rooms; changing of lab coats when moving between rooms; thorough cleaning of all equipment and surfaces; treating of equipment under UV light; use of sterile filter tips for pipettes). An important consideration when using nested PCR protocols is the increased risk of sample contamination due to increased handling of amplified DNA. This risk was minimised by preparing reagents in a separate room from that in which the completed cPCR product was added.

Results

Positive eDNA detections of both sunbleak and pumpkinseed were obtained for the Sussex Ouse catchment at locations in the Sheffield Stream (Fig. 1), but there were no detections of either species in samples from Batts Stream or from the furthest downstream location in the Ouse's upper estuary

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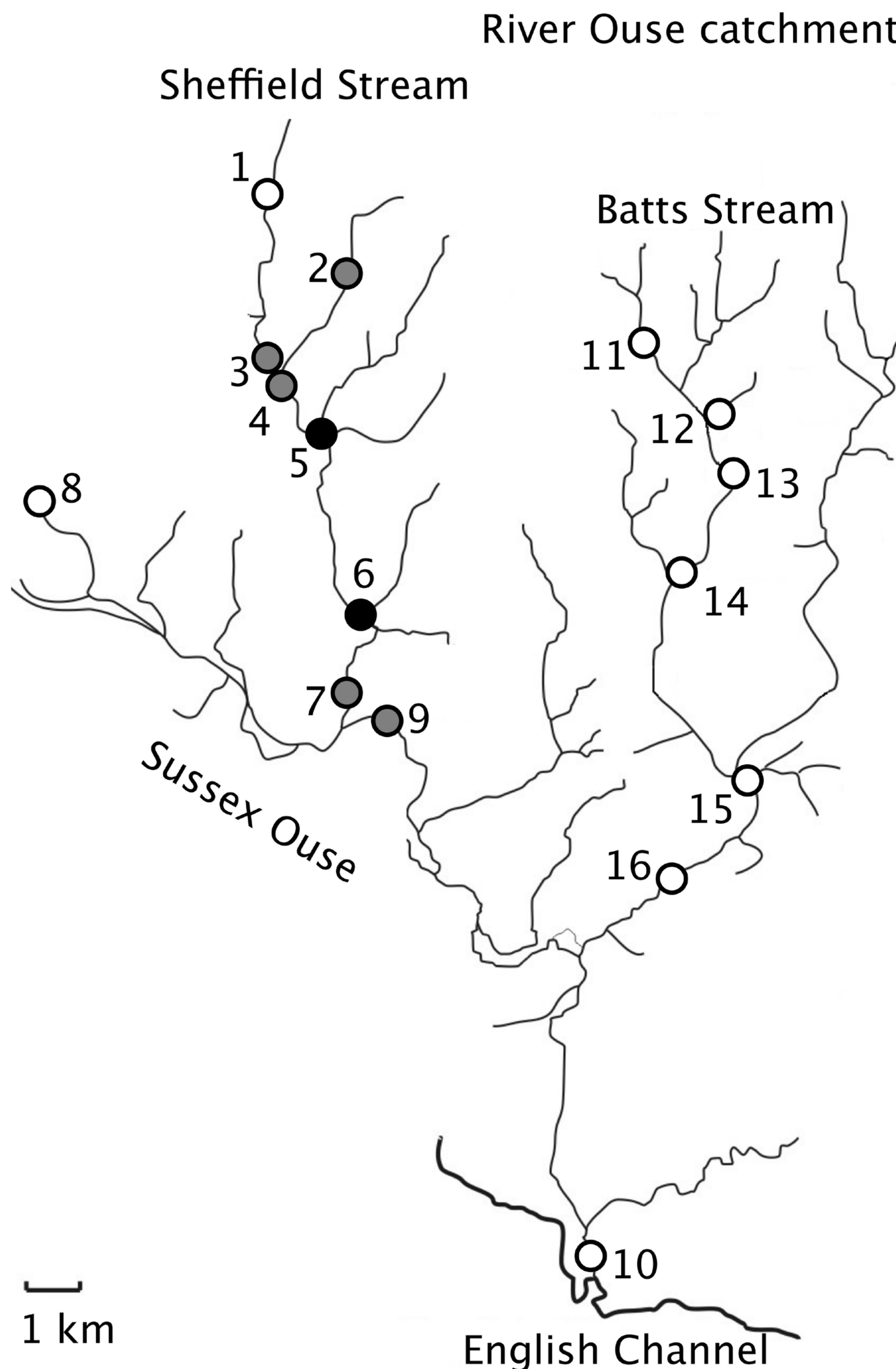

Fig. 1. Map of water collection sites (WGS84 coordinates) in the River Ouse catchment (Sussex) for eDNA analysis of pumpkinseed *Lepomis gibbosus* (all sites) and sunbleak *Leucaspius delineatus* (sites 1-10). Black-filled circles indicate sites where both species were detected in all five replicates at the cPCR step. Grey-filled circles indicate sites where only *L. delineatus* was detected (in less than five replicates at the cPCR step, but all replicates in the nPCR). Open circles indicate sites with no detections. 1 = Sheffield Stream at Collingford (51.028885, 0.007233); 2 = Tanyards Farm Stream (51.027380, 0.013906); 3 = Sheffield Stream above an angling venue previously known as Tanyards Fishery (51.023912, 0.009229); 4 = Sheffield Stream at upper end of Tanyards Fishery (51.023486, 0.009990); 5 = Sheffield Stream below Tanyards Fishery (51.018459, 0.014657); 6 = Sheffield Stream at Eastbridge (51.007024, 0.017481); 7 = Sheffield Stream at lower end of Sheffield Park (51.993436, 0.019276); 8 = River Ouse at Ardingly (51.028445, –0.0894816); 9 = River Ouse at Fleching Mill (50.987522, 0.027771); 10 = River Ouse estuary at Piddinghoe (50.810306, 0.033544); 11 = Batts Stream at Batts Bridge (50.991580, 0.068360); 12 = Batts Stream at Cackle Street (51.016889, 0.075606); 13 = Batts Stream at Old Forge Lane (51.013028, 0.078568); 14 = Batts Stream at Hole Farm (51.020853, 0.072426); 15 = Confluence of Batts Stream and Shortbridge Stream at Powder Mill (50.991621, 0.068564); 16 = Shortbridge Stream (50.992533, 0.094205).

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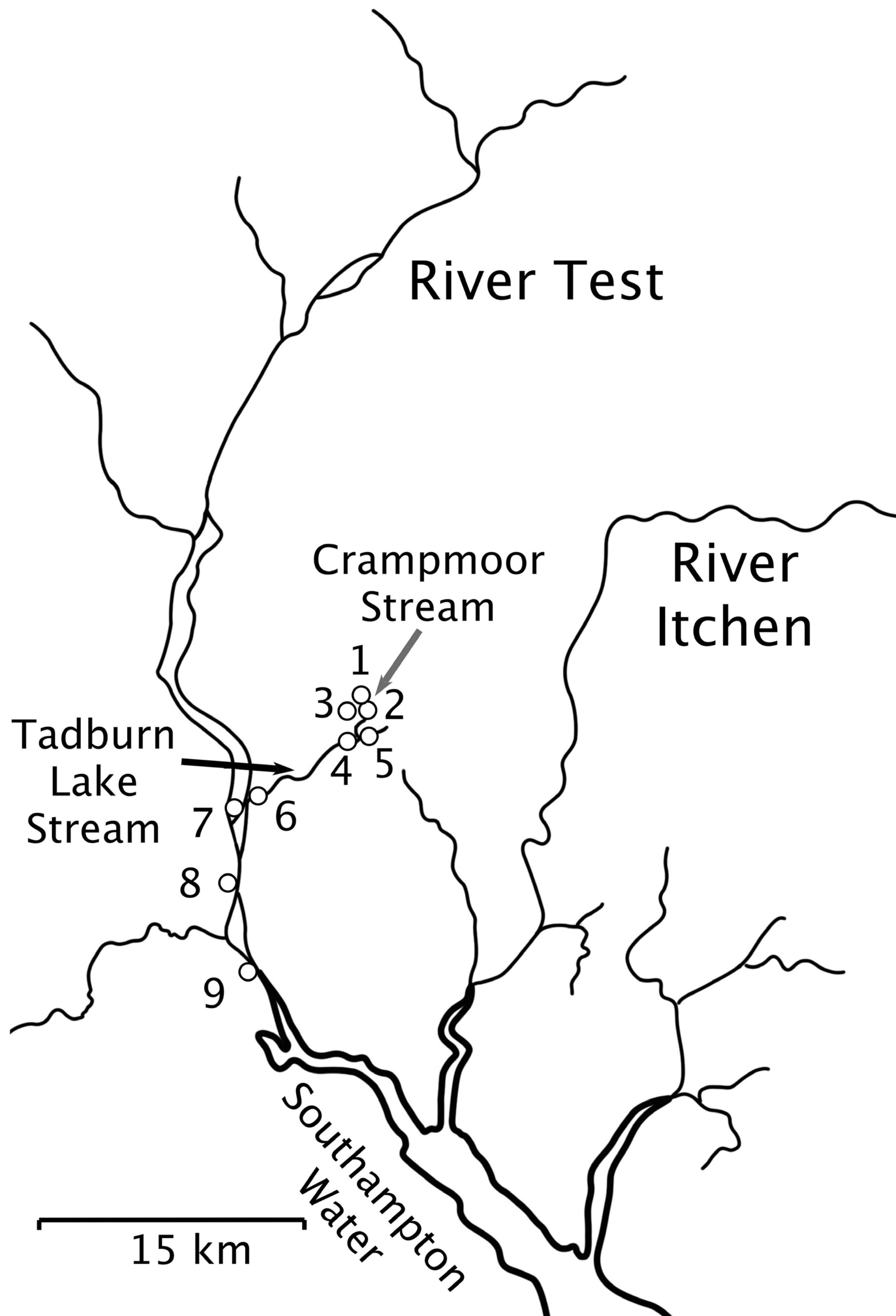

Fig. 2. Map of water sampling sites (WGS84 coordinates) on the River Test catchment, Hampshire. Open circles indicate sites with no detection of topmouth gudgeon *Pseudorasbora parva* or sunbleak *Leucaspius delineatus*. 1 = Crampmoor Fishery stream, above ponds (51.001136, –1.44476); 2 = Crampmoor Fishery stream, adjacent to ponds (50.999826, –1.447869); 3 = Crampmoor Fishery stream, below ponds (50.999556, –1.451109); 4 = Crampmoor Stream, at the confluence with Tadburn (50.996450, –1.454585); 5 = Tadburn Lake (50.996234, –1.454135); 6 = Tadburn, in Romsey (50.986037, –1.498767); 7 = River Test, in Romsey, above the confluence with Tadburn (50.984605, –1.504131); 8 = River Test, at Lee Farm, Longbridge (50.959004, –1.496252); 9 = River Test, at Salmon's Leap (50.926528, –1.486266).

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(Site 10). Along Sheffield Stream, both species were recorded at two sites, one immediately downstream of the commercial angling venue (Site 5), which has lakes known to contain both species (Zięba et al. 2010, Fobert et al. 2013), and the subsequent downstream site (Fig. 1, Site 6). Additionally, sunbleak DNA was detected at three sites upstream of the angling venue and at two sites further downstream: one just before (Site 7) and one just below (Site 9) the confluence of Sheffield Stream with the Sussex Ouse (Fig. 1). At both locations with positive detections for eDNA of pumpkinseed (sites 5 and 6), all five replicates of the cPCR provided a positive result. The eDNA signal for sunbleak was strongest at the location (Site 5) immediately downstream of the angling venue (Fig. 1), with all five cPCR replicates proving positive. At five locations with sunbleak detections (sites 2, 3, 4, 7, 9), only 1-2 cPCR replicates proved positive, with comparatively faint bands, but in each case, proceeding with the nPCR on three of the negative replicates produced a positive result.

In the River Test catchment (Fig. 2), no positive eDNA detections were obtained for either sunbleak or topmouth gudgeon, including the stretch of Crampmoor Stream that receives outflow discharges from the fish ponds of the former Crampmoor Fish Farm and discharges into Tadburn Lake Stream at Site 4.

The negative field controls (de-ionised water processed in the field on each sampling day) did not produce detections at either the cPCR or nPCR stage for any of the three species. Likewise, no positive results were obtained from the negative PCR controls, which utilised deionised water in place of the sample on every cPCR and qPCR run.

Discussion

The distributions of the target species within the two river catchments at the time of the surveys were obtained relatively rapidly from the eDNA survey (Fig. 1), i.e. one day of water sampling in each catchment and 24-39 h of laboratory analysis per species per catchment. Detections of pumpkinseed eDNA in Sheffield Stream (Sussex Ouse catchment) were consistent with previous surveys (e.g. Klaar et al. 2004, Copp et al. 2010a, Jackson et al. 2016) and further substantiated by tagging and drift-net studies (Fobert et al. 2013).

Despite the demonstrated capacity of pumpkinseed to make seasonal up- and down-stream movements in Sussex Ouse tributaries (Stakėnas et al. 2013), no pumpkinseed eDNA was detected upstream of the angling venue in which the species has been established in several floodplain ponds for at least three decades (Klaar et al. 2004, Villeneuve et al. 2005, Fobert et al. 2013, Copp et al. 2017). Sunbleak was also inadvertently introduced into these angling ponds and established sometime before 2006 (Zięba et al. 2010, Bašić et al. 2018). Detections of sunbleak were more widely dispersed along Sheffield Stream, including a few locations upstream of the established populations at the angling venue. This finding suggests an expansion of the species up the catchment, including the possibility of an established population at the in-stream pond just above Site 2 (Fig. 1).

An inherent limitation when using eDNA detections to map fish distributions in rivers or streams is the possibility of downstream movement of DNA to sections where the fish itself is not present. The DNA signal from caged fish has been shown to peak at a downstream distance of between 300 m and 2 km (Van Driessche et al. 2022), although this will be influenced by site-specific differences in the water body, such as discharge rates. Consequently, eDNA surveys might be most effectively employed to delimit the upstream range extent of fish species (Penaluna et al. 2021), with other detection methods providing potentially more-accurate data on downstream limits. For example, detections of sunbleak and pumpkinseed at sites 7 and 9 on the Sussex Ouse could relate to the presence of live fish or their DNA emanating from floodplain ponds that discharge water into the water course. Further attempts to map species' presence in river catchments would benefit from greater knowledge of the dynamics of eDNA in lotic systems, given that little is known of the distance that DNA can travel downstream from a given source. Also, there are several small reservoirs along small-stream tributaries of Batts Stream where pumpkinseed populations may persist (Villeneuve et al. 2005). However, resource limitations did not permit the collection of water samples from those water bodies, which may discharge into Batts Stream during periods of intense precipitation.

The lack of topmouth gudgeon and sunbleak detections from Crampmoor and Tadburn Lake streams suggests that these species are no longer present in that part of the catchment or occur at a density below the detection limit of our survey. Because sunbleak is known to be present in some stillwater fisheries that discharge into tributaries of

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the rivers Test and Itchen (Gozlan et al. 2002, 2003, Beyer 2008), such cases of negative detection suggest a need for further, more intensive surveying. Imperfect detection rates by eDNA or conventional methods (e.g. Britton et al. 2011c) are particularly important when the results form the basis of management decisions. Stochastic variability in detection should be expected when detecting low DNA concentrations in streams (Wilcox et al. 2016). A higher level of field sampling (larger water volume filtered or increased spatial or temporal repetition) might have produced positive results at more locations in this study.

Implications for management

The lack of pumpkinseed detections along Batts Stream (Fig. 1), which receives outflow directly from Boringwheel Lake, is of interest regarding non-native species management. Pumpkinseed were present in Boringwheel Lake up until at least 2007 (Copp et al. 2010a, Fobert et al. 2013), but none were captured in subsequent surveys of Batts Stream in 2009 or 2010 (Jackson et al. 2016). This situation suggests that pumpkinseed may have disappeared in (or been extirpated from) Boringwheel Lake at about the same time as its apparent extirpation in Batts Stream due to repeated removals of specimens for scientific study (Jackson et al. 2016). However, pumpkinseed remain in the Batts Stream catchment, such as in the garden pond at Watersmeet (adjacent to Site 12, Fig. 1), into which the species gained entry during a flood and established a population in 2007 (Fobert et al. 2013). Furthermore, pumpkinseed were still present in Watersmeet pond in July 2016 when specimens were collected for a life-history study (Copp & Fox 2020), which indicates that this stream remains at risk of being re-invaded by pumpkinseed during the next extreme discharge event. This scenario is feasible, given that elevated water temperatures and more extreme discharge events are predicted for watercourses in southern England (Environment Agency 2022).

Analytically, nPCR analysis produced no detections of the target species from any locations where they had not already been detected using cPCR. However, in the limited number of cPCR replicates that produced negative detections for sunbleak in Step 1 (at sites where other replicates had proved positive), the species was subsequently detected in Step 2 using nPCR. This finding suggests that the increased sensitivity of the nested PCR technique compared with cPCR or qPCR alone, as demonstrated in the laboratory (Davison et al. 2019), could, in some cases, improve field detectability. In addition, the enhanced sensitivity of the nested PCR protocol, which could be the result of both an increased number of cycles and the refreshed reagents provided for the second qPCR step, provides increased confidence that species present in water samples are not being missed as “false negatives”; but, this comes at an increased financial cost. However, nPCR protocols should be used cautiously because of the increased risk of “false positives” from contamination when re-analysing amplified DNA. Therefore, results need to be interpreted with care.

Advantages of eDNA surveying include the ability to undertake surveys where factors such as weather or water depth preclude electrofishing and other capture methods or limit their effectiveness. Regarding financial cost, eDNA surveys are often considered less expensive than conventional methods (Biggs et al. 2015, Sigsgaard et al. 2015, Evans et al. 2017). In our study, eDNA surveying provided a wider geographic coverage more rapidly (two days in the field) than would be possible if employing conventional methods at each sampling site, enabling an initial “screening” of the catchment for the target species. This approach to water sampling could be combined with metabarcoding to detect a broader suite of potential non-native species within the fish community of that catchment (Antognazza et al. 2021). Further research is needed to identify the sampling regime required to achieve an acceptable probability of detection for fish species of restricted distribution within a river. The potential power of eDNA-based approaches has been demonstrated in a modelling study (Wood et al. 2021), which suggested that 110 samples collected at 400 m intervals would be sufficient to detect a single juvenile salmonid in a 44 km stream. Analysis of water samples for eDNA has been suggested as a tool for regulatory monitoring of fish communities in river systems (Pont et al. 2021), and eDNA analysis of water samples has already proved useful for monitoring invasive fishes before and following eradication procedures (Davison et al. 2019). As such, eDNA offers the potential to play a key role in the early detection of newly-arrived non-native species at the catchment level and thereby inform decision-makers of a species’ persistence, or lack thereof, so that they identify an appropriate and effective management strategy (Britton et al. 2011a, b).

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Author Contributions

P.I. Davison and G.H. Copp designed the study, conducted field sampling, analysed the data, and contributed to all manuscript drafts. P.I. Davison conducted the laboratory analysis and wrote the initial draft.

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