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DOI
10.1007/s00114-019-1605-1

Publication date
2019

Document Version
Final published version

Published in
Naturwissenschaften

The Science of Nature (2019) 106: 9
https://doi.org/10.1007/s00114-019-1605-1

ORIGINAL PAPER

# Evaluating monitoring options for conservation: comparing traditional and environmental DNA tools for a critically endangered mammal

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Received: 3 August 2018 / Revised: 6 December 2018 / Accepted: 30 January 2019 / Published online: 18 February 2019


## Abstract

While conservation management has made tremendous strides to date, deciding where, when and how to invest limited monitoring budgets is a central concern for impactful decision-making. New analytical tools, such as environmental DNA (eDNA), are now facilitating broader biodiversity monitoring at unprecedented scales, in part, due to time, and presumably cost, of methodological efficiency. Genetic approaches vary from conventional PCR (cPCR; species presence), to metabarcoding (community structure), and qPCR (relative DNA abundance, detection sensitivity). Knowing when to employ these techniques over traditional protocols could enable practitioners to make more informed choices concerning data collection. Using 12 species-specific primers designed for cPCR, eDNA analysis of the Yangtze finless porpoise (YFP; *Neophocaena asiaeorientalis asiaeorientalis*), a critically endangered aquatic mammal within the Yangtze River, we validated and optimized these primers for use in qPCR. We tested repeatability and sensitivity to detect YFP eDNA and subsequently compared the cost of traditional (visual and capture) sampling to eDNA tools. Our results suggest cPCR as the least expensive sampling option but the lack of PCR sensitivity suggests it may not be the most robust method for this taxon, predominately useful as a supplementary tool or with large expected populations. Alternatively, qPCR remained less expensive than traditional surveys, representing a highly repeatable and sensitive method for this behaviorally elusive species. Cost comparisons of surveying practices have scarcely been discussed; however, given budgetary constraints particularly for developing countries with limited local oversight but high endemism, we encourage managers to carefully consider the trade-offs among accuracy, cost, coverage, and speed for biodiversity monitoring.

**Keywords** Yangtze finless porpoise · Environmental DNA · Systematic conservation planning

## Introduction

As a discipline, systematic conservation planning (e.g., Margules and Pressey 2000) has now surpassed three decades, and its achievements around the world have been remarkable. Still, the social and political environment in which conservation issues are addressed is highly complex, often forming a nexus between the cost, speed, and accuracy of collecting necessary biodiversity data. Indeed, so much of conservation fundamentally involves choices about *where, when* and *how* to make investments, thus requiring organismal information at scales commensurate with both goals and monetary availability. Therefore, there remains a vital need to continually highlight new analytical frameworks, innovations, and advances in our collective understanding and approaches to conservation's core objectives.

Despite the prerequisite for comprehensive monitoring initiatives in conservation programs, a thorough knowledge of organismal distribution and abundance is often prohibitive due largely to difficulties in data collection for hard-to-study taxa (e.g., cryptic, behaviourally elusive, low site fidelity, or rare), difficult to sample locales (e.g., aquatic environments), and affiliated costs, particularly in developing countries (Danielsen et al. 2003). Advancements in biodiversity data monitoring via DNA sampled straight from the environment

Communicated by: Matthias Waltert

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s00114-019-1605-1) contains supplementary material, which is available to authorized users.

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(without invasively targeting taxa) known as eDNA has recently revolutionized conservation biology.

Advocated as a time and cost-effective alternative to traditional methods of biodiversity data monitoring, eDNA is a highly sensitive technology that has been successfully employed for myriad species and goals (e.g., Ficetola et al. 2008; Lodge et al. 2012; Fukumoto et al. 2015; Ma et al. 2015; Dougherty et al. 2016). However, just as surveying methods are varied, so too are eDNA applications, and few studies to date have assessed and compared the cost of these protocols. Applications of eDNA methodology can range from detecting the presence/absence of species with conventional PCR (cPCR) (e.g., Jerde et al. 2011; Dejean et al. 2012; Thomsen et al. 2012; Mahon et al. 2013; Piaggio et al. 2014; Fukumoto et al. 2015) or community constituents via metabarcoding (e.g., Evans et al. 2015; Valentini et al. 2016; Hänfling et al. 2016; Shaw et al. 2016), to quantifying the relative abundance of DNA sequences (proxies for relative species abundance) or increasing detection sensitivity via quantitative PCR (qPCR) (e.g., Takahara et al. 2012; Goldberg et al. 2013; Pilliod et al. 2013; Klymus et al. 2015; Laramie et al. 2015; Balasingham et al. 2017). Still, it is difficult to discern when and how to match appropriate sampling protocols with conservation goals in the most cost-effective manner; this understanding could propel the entire field of conservation biology forward and galvanize eDNA as a tractable tool for managers.

At present, the Yangtze finless porpoise, *Neophocaena asiaeorientalis asiaeorientalis* (henceforthYFP), is the only cetacean species to be found within the Yangtze River and its tributaries (China), the world's only freshwater porpoise, and is currently classified as a critically endangered species by the IUCN (estimated to encompass less than 1050 individuals in the wild; Wan et al. 2016; Mei et al. 2012). The YFP is a far-ranging aquatic species distributed across the middle-to-lower Yangtze River, many lake systems, and an oxbow lake reserve hydrologically linked to the Yangtze River, totally approximately 1890 km in length (Li 2011). Although a conservation priority for China, YFP natural history make it difficult to survey: i) they remain behaviorally elusive, ii) are benthic feeders infrequently surfacing to breathe, and iii) their dark coloration and lack of dorsal fin to break the water surface makes visual detection difficult (Stewart et al. 2017). Thus, current traditional population monitoring for this taxon is primarily restricted to presence/absence information via visual or acoustic surveying unless individuals are physically captured for demographic information (although the invasiveness of the capture method remains a concern). YFP's extensive distribution across the Yangtze River and its tributaries also makes approaches to population management and data collection costly, an important consideration for this developing country.

Previous research has successfully developed 12 species-specific YFP primer pairs used in cPCR for eDNA sampling (Ma et al. 2016). However, this eDNA approach only allows for the identification of presence/absence data. While more informative (e.g., abundance information for target taxa), qPCR is further suggested to be more sensitive for eDNA detection (Balasingham et al. 2017). Thus, for this study we optimize and validate species-specific primer pairs for qPCR amplification (quantifying relative DNA abundance), simultaneously testing and comparing the sensitivity (theoretical limits of detection) of each primer pair for eDNA applications. We additionally conduct a cost-assessment of YFP traditional visual monitoring to conventional and quantitative PCR data collections. We ultimately aim to generate management recommendations for conservation practitioners commensurate with the scale, goal, and financial feasibility of each sampling application currently available (i.e., compare different eDNA detection methods to that of traditional surveying).

## Methods

### Primer optimization and validation

Using 12 published YFP primers pairs validated for use in cPCR (Ma et al. 2016), we optimized qPCR protocols for use in eDNA surveying. Species-specificity was previously determined for cPCR *in silico* (bioinformatically) via the comparison of 20 complete YFP cytochrome *b* (cyt *b*) sequences (range of haplotypes) to other geographically overlapping species; *in vitro* (blood and tissue samples) from YFP and sister species, and *in situ* (within environments where YFP are known to be present, positive control, and where they are absent, negative control) (for details see Ma et al. 2016). Briefly for cPCR, DNA was extracted from both blood and environmental samples using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) in a final elution volume of 200 μL. DNA concentrations were measured with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and proceeding reaction volumes were 25 μL, consisting of 1 μL of 1–20 ng/μL template DNA, 12.5 μL of 1 × Taq PCR Master Mix (BIO BASIC CANADA INC.), 1 μL each of 0.2 μM each of forward and reverse primer, and 9.5 μL of $ddH_20$. cPCR amplification included the following thermal cycling profile: 94 °C initial denaturation for 4 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 20 s, and one final extension step at 72 °C for 5 min. cPCR products were visualized using electrophoresis on 2% agarose gel, stained with 7 μL of 4S Red Plus Nucleic Acid Stain (BBI) per gel, and all amplifications were repeated in triplicate (following Ficetola et al. 2015). Finally, cPCR products of the target sequences were sent to Sangon Biotech (Shanghai, China) utilizing an ABI 3730XL sequencer to validate PCR products as indeed being from YFP. Sequences were visualized using MEGA6 (Tamura

et al. 2013) and then compared to all available sequences in GenBank using Primer-BLAST (Ye et al. 2012). For sensitivity analyses, concentration assays involved diluting the DNA extracted from blood samples with sterilized dd$H_2O$ to 4 ratios: 1:2, 1:10, 1:100, 1:1000, replicating PCR amplification in triplicate, and visualizing the electrophoresis results to note maximum detection sensitivity of each primer pair. Detection using cPCR was denoted as positive if at least 2 of the 3 replicates amplified YFP DNA. If only 1 out of 3 demonstrated positive amplification, we re-ran another triplicate test to eliminate possible contamination.

For optimization of qPCR, we first acquired a synthetic gene solution as template DNA. This synthetic gene was then used to optimize amplification and explore the sensitivity/limit of detection (LOD; the minimum level of target DNA detected in a sample) of these primer pairs. The synthetic cyt *b* gene sequence (1140 bp) was retrieved from GenBank (accession number KJ472902), and encompassed the entire region in which all 12 primer pairs are located for YFP (Ma et al. 2016). Each qPCR reaction contained 10 μL of SYBR Premix Ex Taq II (Tli RNaseH Plus) (2×) (TaKaRa.), 0.4 μL of ROX Reference Dye II (50×) (TaKaRa.), 6.4 μL of RNase-free water, 0.6 μL of forward primer (10 μM), 0.6 μL of reverse primer (10 μM), and 2 μL of template DNA, for a total volume of 20 μL. All amplifications were performed on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies) with qPCR thermal profile as follows: holding stage (95 °C for 30s), followed by cycling stage (40 cycles of 95 °C for 5 s and 64 °C for 34 s), then followed to a melt-curve stage (95 °C for 15 s, 60 °C for 1 min, 95 °C for 30 s, and 60 °C for 15 s).

We judged qPCR amplification to be successful if the results met the following requirements: 1) qPCR standards (or samples) displayed the expected amplification curve; 2) amplifications displayed an expected melting curve peak; and 3) negative controls showed no amplification prior to critical threshold. qPCR reactions were performed in triplicate at concentrations of 500 and 5000 copies/μL due to high repeatability (near 100% positive amplification), but to increase our confidence in the amplification results at lower concentrations (5–50 copies/μL), qPCR reactions were tested in 13 technical replicates including negative controls (RNase-free water). Amplifications were recorded as zero detection when reactions did not cross the fluorescent threshold prior to the 40th cycle (Ct; 40), and quantified as a positive detection when samples demonstrated amplification prior to Ct.

Amplification efficiency (%) for qPCR reactions was additionally calculated for each primer pair based on the slope of the standard curve, assuming a slope of −3.322 with an amplification factor of 2, which equates to 100% efficiency.

To test the sensitivity of each primer pair for use in eDNA analysis, we amplified the synthetic gene at concentrations of 5, 25, 50, 500, and 5000 copies/μL. The synthetic gene solution concentrations in ng/μL (as measured via NanoDrop Lite spectrophotometer; Thermo Fisher Scientific, Wilmington, DE, USA), were converted from *x* ng/μL to *y* copies/μL using the standard formula:

$$y = \frac{x \times 10^{-9}}{650 \times (length)} \times 6.02 \times 10^{23}$$

For this calculation, we assumed 650 Da the weight of each base pair and $6.022 \times 10^{23}$ for the conversion to molecules/mol (Avogadro's number). For use in qPCR calculations, we used the combined synthetic gene length of 1140 bp and 2710 bp for the synthetic gene clone vector length (pUC57), and for PCR calculations we used the length of each primer amplicon (bp). Maximum cPCR sensitivity data was extracted from Ma et al. (2016) in which extracted DNA from YFP blood samples were diluted to a minimum level of detection (ng/μL converted to copies/μL).

## Validation via eDNA collection

To verify primer efficiency, we tested whether these primer pairs could amplify YFP eDNA from a location where we knew this species to be present. Thus for a positive control, we collected water from the "netted cage" (approximately a 15 m × 15 m enclosure) within Tian-e-Zhou Baiji National Nature Reserve (29° 51′ 11 N, 112° 35′ 15E), a natural protected area for the YFP which runs parallel, and is hydrologically linked to, the Yangtze River (similar in protocol to Foote et al. 2012), and acts as a lake due to limited water flow (Stewart et al. 2017). This reserve is hydrologically similar to other natural lake systems within which over half the YFP species populations are found in China (e.g., Dongting and Poyang lake). Within the netted cage area, 2 YFP individuals were temporarily housed for breeding research purposes before being released into the reserve. The water within the netted cage was connected, and freely flowed to the rest of the reserve which further housed approximately 58 other YFP individuals at the time of sampling (April 2015). Water was sampled 0.4 m below water surface and acquired with a VanDorn water sampler (GRASP CG-00, GRASP Science &Technology Co., Ltd.) which had been sterilized with 20% diluted bleach, rinsed with sterilized water, and then dried. The water sample was then filtered using a portable field peristaltic pump (Spectra Scientific Inc. Spectra Filed-Pro Professional Grade) with 47 mm diameter mixed cellulose esters (MCE) filter paper, 0.45 μm pore size, utilizing a sterilized, reusable filter holder (Sterifil 47 mm Filter Holder, MILLIPORE). To prevent clogging the filter paper with large suspended particles (e.g., algae), we wrapped the inlet of the filter holder with sterilized, disposable, medical-grade gauze. Based on collection procedures and sensitivity measures from cPCR protocols (Ma et al. 2016), we collected and filtered approximately 3-1 L samples of water, calculating

final extracted DNA concentration from each sample as a proportion of their original sample volume. After filtration, we folded the filter paper using sterilized tweezers and placed the filter paper into an Eppendorf tube with 95% ethanol. Samples were immediately stored in a −20 °C freezer until DNA extraction. For our negative control, we chose Gonghuwan (31° 27′ 21″ N, 120° 20′ E), an artificial lake in Wuxi, China, where porpoises are absent and water supply is not linked with the Yangtze River or any other waterway containing YFP.

DNA was subsequently extracted from stored, dried filter paper with the PowerWater® DNA Isolation Kit (Mo Bio Laboratories, Inc) via placing filter paper into a 5-mL PowerWater® Bead Tube and following manufacturer's protocol. After DNA extraction, the mean total eDNA concentration was measured with a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and tested with each primer pair using both cPCR and qPCR protocols. Collected eDNA was also diluted 1:10, 1:20, and 1:30 to check for PCR inhibition (Davy et al. 2015) such as those caused by accidental humic acid collection in samples (Thomsen and Willerslev 2015).

To minimize contamination among samples, we utilized filtered pipette tips, separate clean rooms for DNA extraction and PCR amplification, and all equipment was thoroughly sterilized with 20% diluted bleach, rinsed with sterilized water, and then dried under UV light for 30 min. Latex gloves were also used for each sample collected. After each filtering session, medical-grade sterilized gauze covering the inlet of the filter holder was replaced, and the filter holder and tweezers were washed in a 20% bleach dilution, rinsed with sterilized water, and allowed to be fully dry (Davy et al. 2015).

All collections were ethically conducted with permission from conservation managers and WWF officers affiliated with the Tian-e-Zhou Nature Reserve.

### Cost assessment

To make a simplified cost comparison between traditional visual, capture, and eDNA survey methods (both using cPCR and qPCR) to detect and quantify YFP populations, we used 2015 and 2016 data on visual monitoring efforts by WWF officers at the Tian-e-Zhou Nature Reserve (presence/absence). In reality, the full cost of such sampling would require the transportation of samples and/or sampling personnel to and from the reserve. However, this will vary among practitioners employing these techniques (i.e., whether or not they filter on site, and/or transit distance for visual observers/eDNA collectors) and our purpose is merely to create a streamlined evaluation among monitoring methods. Thus, we have restricted our comparison to average labor costs for this particular region (government recommendations), laboratory consumables, and boat rentals. We also assume eDNA collections will be conducted in laboratories already fully equipped for extraction and analysis, that (species-specific) optimization of genetic analysis is already complete (e.g., this study; Ma et al. 2016), and that amplification failure is negligible given recommendations for detection cutoffs and the proven methodological sensitivity shown here. Cost assessments for eDNA surveys utilizing both cPCR and qPCR were based on 3-1 L water sample across 15 sites (1 site per 1 km of habitable reserve length to reduce potential overlap in the dispersion of eDNA detection sginals; Goldberg et al. 2016), for a total of 45 samples. cPCR costs were calculated based on data presented in Ma et al. (2016), including consumables and extraction kits. Therefore, both visual and genetic methodological comparisons overlap in both year of data collection and geographical distribution.

### Data analysis

We derived equations of the line and slopes for each primer pair's ability to predict known concentrations of synthetic gene DNA (log copies/μL) based on its qPCR critical threshold (Ct). From these correlations, we acquired Pearson's coefficient $R^2$ to calculate the efficiency of each primer pair and direct managers towards the best markers for use in YFP eDNA analysis. To analyze whether DNA quantity (log copies/μL) and primer pair influenced the critical threshold, we also performed a two-way ANOVA. All analyses were performed using JMP v.12.0 (SAS Institute Inc., Cary, NC, 1989–2007).

## Results

For our assays, we did not detect any evidence of inhibition. In general, amplification demonstrated 8/12 and 12/12 primer pairs successfully amplified for cPCR and qPCR respectively, suggesting good species detection *in situ* for the positive control (Tian-e-Zhou) and no amplification for the negative control (Gonghuwan). For cPCR analysis, primer pairs showed low sensitivity, ranging from the lowest at $2.96 \times 10^{10}$ copies/μL for FP97, and the highest at $1.95 \times 10^{8}$ copies/μL for FP147 required for positive detection (Table 1).

Our qPCR amplification demonstrated $R^2$ values ranging between 0.993–0.999 for all primer pairs, and efficiency ranging from 67.02%–128.97%, with only six primer pairs falling within the standard acceptable range (MIQE guidelines) of 90–110% (Table 1). qPCR amplification also demonstrated a significant difference in slope among the 12 primer pairs ($F_{23,301} = 25.459$, $P < 0.001$), with primer ($F_{11} = 2.72$, $p = 0.002$) and DNA quantity (copies/μL) ($F_1 = 549.227$, $P < 0.001$), but not their interaction ($F_{11} = 0.835$, $P = 0.61$), showing an influence on the quantification slope. Accordingly, *post hoc* analysis (Tukey's HSD test) demonstrated primer FP133 to statistically have the steepest slope (*t* ratio =

**Table 1** The amplification sensitivity (LOD; copies/μL) of 12 primer pairs using either cPCR (via DNA tissue dilution) or qPCR (synthetic gene dilutions), including the intercept, slope, and efficiency (%) of qPCR amplifications

| Primer | qPCR | | | | | | | | | cPCR | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | LOD 5 copies/μL | LOD 25 copies/μL | LOD 50 copies/μL | LOD 500 copies/μL | LOD 5000 copies/μL | Intercept | Slope | Efficiency (%) | *In situ* Detection | Max. Sensitivity (copies/μL) | *In situ* Detection |
| FP76 | 31% | 46% | 77% | 66.6% | 100% | 38.10 | −3.27 | 97.18 | + | $1.89 \times 10^{10}$ | + |
| FP90 | 15% | 46% | 85% | 100% | 100% | 40.26 | −3.92 | *128.95* | + | $3.19 \times 10^{9}$ | + |
| FP97 | 23% | 23% | 62% | 100% | 100% | 38.69 | −3.33 | 99.85 | + | $2.96 \times 10^{10}$ | - |
| FP104 | 23% | 23% | 46% | 100% | 100% | 37.61 | −2.67 | *72.85* | + | $2.76 \times 10^{10}$ | - |
| FP133 | 31% | 62% | 85% | 100% | 100% | 39.15 | −3.00 | *85.71* | + | $1.08 \times 10^{10}$ | + |
| FP147 | 15% | 46% | 54% | 100% | 100% | 37.83 | −2.51 | *67.02* | + | $1.95 \times 10^{8}$ | + |
| FP161 | 54% | 39% | 85% | 100% | 100% | 38.73 | −3.37 | 101.66 | + | $1.78 \times 10^{9}$ | + |
| FP162 | 23% | 23% | 46% | 100% | 100% | 37.69 | −2.96 | *84.09* | + | $1.77 \times 10^{9}$ | - |
| FP171 | 23% | 23% | 23% | 100% | 100% | 36.80 | −3.13 | 91.12 | + | $1.68 \times 10^{9}$ | + |
| FP221 | 15% | 15% | 31% | 100% | 100% | 38.39 | −3.42 | 103.95 | + | $6.5 \times 10^{9}$ | - |
| FP233 | 39% | 39% | 70% | 100% | 100% | 37.79 | −2.65 | *72.11* | + | $6.16 \times 10^{9}$ | + |
| FP249 | 39% | 39% | 77% | 33.3% | 100% | 38.42 | −3.33 | 99.85 | + | $5.77 \times 10^{9}$ | + |

Amplification efficiencies outside the optimal range (90–110%) are italicized. LOD percentage is represented as the number of positive amplifications out of 13 replicates. Primer pair eDNA detection for *in situ* analysis is presented as '+' for positive detection and '-' for no detection

3.16, $P = 0.002$) and primer FP171 to statistically have the shallowest slope ($t$ ratio = −3.63, $P < 0.001$) of all primers tested (Supplementary Information). Indeed, for qPCR, primers FP171 and FP221 showed little increase in sample detection with an increase to LOD (5 to 50 copies/μL). All primer pairs however did show 100% detection rates at an LOD of 500 copies/μL with the exception of FP76 and FP249 (Table 1).

## Cost analysis

Visual surveys in Tian-e-Zhou Nature Reserve are conducted once per month, lasting 7 days in total. Costs accrued for visual surveys in Tian-e-Zhou, including boat rental fees, a three-person-team, and 7 days of labor, approximated to 8000 CNY per month—accordingly, 24,000 CNY per season or 96,000 CNY per year from 2015 to 2016. Additionally, once a year, WWF China officers also complete a capture monitoring protocol in which all individuals within the Tian-e-Zhou reserve are corralled for data collection (e.g., demographic, morphological, health inspection). For this process to be achieved, a minimum of 300,000 CNY was spent on a 10 day, 15 boat, 40 personnel endeavor (Table 2).

Environmental DNA sampling, however, including labor, filtering water collections, extractions, amplifications, and sequencing, ranged from 4257 CNY for cPCR to 5686 CNY for qPCR per sampling event (45 samples tested in triplicate) (Table 2). Given eDNA for this species can persist in the aquatic environment for 30 days or more (Ma et al. 2016), sampling events with the aim to demonstrate current population distribution (or relative abundance) could conceivably take place at most once a month, to approximately once per season.

Visual surveying on a monthly basis thus costs 1.88X that of eDNA collections utilizing cPCR (species detection) at the same temporal schedule. If, however, eDNA sampling using cPCR occurred only once per season, visual surveys would approximate 5.64X more expensive. Similarly, visual surveys compared to eDNA sampling utilizing qPCR would equate to 1.41X on a monthly sampling schedule, and 4.22X on a seasonal sampling schedule.

# Discussion

Efforts towards species conservation are only as good as the data used to measure a change in distribution and abundance. Yet, the high cost and substantial workforce required for species detection surveys, especially in developing countries, has hindered our ability to assess the status of species and populations globally. In part, this is reflected in depauperate information accrued in areas of low-governance but high biodiversity (including gaps in data collection), and due to difficult-to-survey habitats (Frazier et al. 2016) causing selection biases towards developed countries in temperate regions (McGeoch et al. 2010; Martin et al. 2012; Hudson et al. 2014) and terrestrial ecosystems (Mittermeier et al. 2011). Although rapidly emerging as a salient and valuable technology for biodiversity data collection, systematic appraisals for when to apply eDNA over traditional methods are scarce (but see Biggs et al. 2015; Davy et al. 2015; Sigsgaard et al. 2015; Smart et al. 2016), and comparisons between different eDNA approaches, to our knowledge, are non-existent to date. Given recent demonstration that traditional survey methods are not always more

**Table 2** Comparison of Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) survey cost estimates (Chinese Yuan; CNY) in the Tian-e-Zhou Baiji National Nature Reserve as of 2015–2016 (Chinese Yuan; CNY) between eDNA protocols (45 samples in triplicate) utilizing conventional PCR (cPCR) or quantitative PCR (qPCR), and traditional methods, such as visual or capture (WWF China; *personal communication*)

| Survey method | Details | Cost (CNY) |
|---|---|---|
| cPCR | eDNA collection labor | 495 |
| | Filter papers+consumables | 180 |
| | Extraction QIAGEN DNEasy blood and tissue kit | 1701 |
| | Amplification | 405 |
| | Confirmation (visualization) | 675 |
| | PCR labor | 486 |
| | Total | 4257 |
| qPCR | eDNA collection labor | 495 |
| | Filter papers+consumables | 180 |
| | Extraction MOBIO DNEasy PowerWater kit | 3701 |
| | Amplification and quantification | 230 |
| | qPCR labor | 1080 |
| | Total | 5686 |
| Visual monitoring | $X$ = 7 days, 1 boat, 3 personnel | |
| | Per month (1$X$) | 8000 |
| | Per season (3$X$) | 24,000 |
| | Per year (12$X$) | 96,000 |
| Capture monitoring | $X$ = 10 days, 15 boats, 40 personnel | |
| | Per year (1$X$) | 300,000 |

Traditional surveying (visual and capture) cost calculations are based on sampling per event ($X$), currently employed once a month for visual monitoring, and once per year for capture monitoring. Sampling events are then multiplied based on time period

expensive than eDNA approaches, particularly for smaller census budgets and small passive sampling tactics (e.g., Smart et al. 2016), providing conservation managers with the most practical tools for their specific goals is imperative to speed data assembly.

For YFP monitoring, our analysis demonstrated cPCR to be the least expensive option for species-specific surveying compared to both qPCR and traditional methods. Nevertheless, as with other studies (e.g., Amberg et al. 2015), cPCR remained a less-sensitive tool compared to qPCR, and thus more prone to false-negatives, making it more useful for data collection in areas where larger populations are expected, or as a supplementary test for when false-negatives can be negated (e.g., when combined with visual surveying efforts). On the other hand, qPCR, a slightly more expensive option than cPCR, but comparatively cheaper than traditional visual surveys for YFP, demonstrated relatively high sensitivity and reproducibility for managers, suggesting high detection accuracy. Typically, thresholds for positive (or negative) detection scoring of samples in qPCR eDNA analyses can vary from study to study, spanning one of three, one of eight (Jerde et al. 2011, 2013; Mahon et al. 2013; Piaggio et al. 2014; Rees et al. 2014), or two of twelve (Schneider et al. 2016) replicates. For our study, we present all scoring data ranging from two in thirteen (LOD of 15% at 5 copies/μL) to all thirteen replicates (LOD 100% at 5000 copies/μL) showing positive amplification, thereby allowing practitioners to establish their own level of confidence for eDNA detection (Table 1). In our study, the primer pairs representing the best efficiency and sensitivity via qPCR (FP76, FP97, FP161, FP171, FP221, and FP249) also demonstrated a minimal detection capability of > 23% positive scoring at an LOD of 25 DNA copies/μL (with the exception of FP221). This may suggest a high likelihood of picking up dilute eDNA signals either as a result of habitat nuances (i.e., large aquatic environments), or relatively small population numbers, with relatively low sampling replicates. Given the multitude of information qPCR could afford managers (distribution combined with relative abundance) while simultaneously increasing eDNA sensitivity, qPCFR may offer the best option for species detection data collection for YFP. However, if managers are faced with financial constraints, either eDNA method may still represent a better alternative than visual monitoring for YFP presence/absence population counts. Despite its ability to accumulate myriad ecological observations (e.g., species demography, co-occurring species, and abiotic parameters such as weather or hydrological patterns), traditional visual surveys within Tian-e-Zhou remain a costly, invasive, and potentially inaccurate (e.g., due to organismal behavior) method for presence/absence data. Indeed, because the visual survey takes place within a reserve, its focus remains on YFP detection, with limited additional information. Whether the presented eDNA monitoring method is applicable to a fast-flowing environment such as the Yangtze River, however, still necessitates testing, though using these techniques in tributaries and lakes where nearly half the species population resides, remains promising.

Although eDNA analysis may require high initial costs (laboratory equipment, primer development, primer validation, and optimization of sampling protocols), our assessment corroborates the few other studies available (e.g., Biggs et al. 2015; Davy et al. 2015; Sigsgaard et al. 2015; Smart et al. 2016) suggesting eDNA methods may still represent the most cost-efficient means of acquiring biodiversity data, especially given budgetary constraints. Moreover, numerous companies are now available for outsourcing eDNA analyses, promising vast reductions to these initial expenses. Costs associated with eDNA protocols are also likely to mirror the observed decrease in DNA sequencing costs in general (Metzker 2010), causing eDNA sampling to become more cost-efficient over time.

Admittedly, our study only analyzed data from a single enclosed site (Tian-e-Zhou Nature Reserve) and because the hydrological dynamics of the Yangtze River are vastly different to that of enclosed lake, the costs required to eDNA

sample the entirety of the Yangtze River (and its tributaries) may exceed the cost of traditional visual methods currently employed. Still, managers are encouraged to consider the likelihood of detecting this taxon across its range with traditional (e.g., visual) protocols given known organismal ecology and behavior. For example, due to their preference for benthic prey (Park et al. 2011; Shirakihara et al. 2009), YFP have been observed to spend approximately 60% of their time on long dives (Beasley and Jefferson 2002) reducing opportunities for visual encounters. Visual surveys also inadvertently increase the probability of accidental harm or death due to boat traffic, either through a disruption in the species' sonar navigation ability or through fatal contact with ship propellers (Chen et al. 1997; Wang 2013). Moreover, collecting water samples for eDNA analysis requires less technical expertise than accurate taxonomic proficiency in morphological identification, a skill suggested to currently be in rapid decline (Wheeler et al. 2004). As the ecology of YFP (spring reproductive bouts, feeding on benthic invertebrates, and seasonal dispersal) may influence detection sensitivities and the amount of eDNA found within the water column (Goldberg et al. 2016), careful consideration of the sinks/sources of this information should remain paramount to managers before applying this methodology. However, this information could also give invaluable insights into the cryptic ecology of the species that may be otherwise difficult to attain. We thus encourage managers to consider the trade-offs between cost and data accuracy when considering which sampling method to employ when collecting valuable biodiversity data.

**Acknowledgments** The authors would like to thank the WWF China officers and Dr. Jinsong Zheng for assistance in data collection and access to the Tian-e-Zhou Nature Reserve.

**Funding information** The authors would like to thank the National Natural Sciences Foundation of China (KAS; 31400468) and the Tongji University State Key Laboratory for Pollution Control and Resource Reuse for financially supporting this research.



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