Comparing conservation monitoring approaches: traditional and environmental DNA tools for a critically endangered mammal

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While conservation management has made tremendous strides in the last few decades, the decision of knowing where and how to invest (often) small surveying budgets for biodiversity data collection remains a central hurdle for impactful conservation decision making. New analytical tools, such as environmental DNA (eDNA), are now facilitating broader biodiversity monitoring to take place at unprecedented scales, in part due to its time-efficient, and presumably cost-efficient, premise. eDNA approaches vary from conventional PCR (detecting presence/absence of species), metabarcoding (community structure), to qPCR (relative DNA abundance), and knowing when to employ these techniques over traditional sampling protocols could enable conservation practitioners to make informed trade-offs between cost, accuracy, and speed of data collection. Using 12 species-specific primers designed for conventional PCR use in eDNA analysis of the Yangtze Finless Porpoise (Neophocaena asiaeorientalis asiaeorientalis), a critically endangered aquatic mammal within the Yangtze River, we validated and optimized these same primers for use in real-time Quantitative PCR (qPCR). We tested the repeatability and sensitivity of primer each to detect YFP eDNA and subsequently compared the cost of traditional visual sampling to both conventional PCR and qPCR eDNA tools. Our results suggest qPCR to be substantially more sensitive than conventional PCR eDNA analysis, although the later remains the least-expensive sampling option. Still, due to a lack of sensitivity causing an increased probability of false negatives, conventional PCR may not be the most robust sampling method for this taxa and should only be employed as a supplementary tool or when large populations are expected to be present. Alternatively, utilizing qPCR for eDNA protocols is still less-expensive than visual surveying and represents a highly repeatable and sensitive method for this behaviorally elusive species. Presenting a cost assessment of eDNA to traditional surveying practices has scarcely been discussed, while contrasting deliverables to the cost of different eDNA methods has, to
date, been ignored. Yet given budgetary constraints, particularly for developing countries where low-governance and high endemism are present, we encourage managers to carefully consider the trade-offs among data accuracy, cost, coverage and speed for biodiversity collections.
Comparing conservation monitoring approaches: traditional and environmental DNA tools

for a critically endangered mammal

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Abstract

While conservation management has made tremendous strides in the last few decades, the decision of knowing where and how to invest (often) small surveying budgets for biodiversity data collection remains a central hurdle for impactful conservation decision making. New analytical tools, such as environmental DNA (eDNA), are now facilitating broader biodiversity monitoring to take place at unprecedented scales, in part due to its time-efficient, and presumably cost-efficient, premise. eDNA approaches vary from conventional PCR (detecting presence/absence of species), metabarcoding (community structure), to qPCR (relative DNA abundance), and knowing when to employ these techniques over traditional sampling protocols could enable conservation practitioners to make informed trade-offs between cost, accuracy, and speed of data collection. Using 12 species-specific primers designed for conventional PCR use in eDNA analysis of the Yangtze Finless Porpoise (*Neophocaena asiaeorientalis asiaeorientalis*), a critically endangered aquatic mammal within the Yangtze River, we validated and optimized these same primers for use in real-time Quantitative PCR (qPCR). We tested the repeatability and sensitivity of primer each to detect YFP eDNA and subsequently compared the cost of traditional visual sampling to both conventional PCR and qPCR eDNA tools. Our results suggest qPCR to be substantially more sensitive than conventional PCR eDNA analysis, although the later remains the least-expensive sampling option. Still, due to a lack of sensitivity causing an increased probability of false negatives, conventional PCR may not be the most robust sampling method for this taxa and should only be employed as a supplementary tool or when large
populations are expected to be present. Alternatively, utilizing qPCR for eDNA protocols is still less-expensive than visual surveying and represents a highly repeatable and sensitive method for this behaviorally elusive species. Presenting a cost assessment of eDNA to traditional surveying practices has scarcely been discussed, while contrasting deliverables to the cost of different eDNA methods has, to date, been ignored. Yet given budgetary constraints, particularly for developing countries where low-governance and high endemism are present, we encourage managers to carefully consider the trade-offs among data accuracy, cost, coverage and speed for biodiversity collections.

Introduction

As a discipline, systematic conservation planning 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 surrounds choices about where to make investments, thus requiring organismal information at scales commensurate with goals and monetary availability for impactful decision making. 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 programmes, 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 collections via DNA sampled straight from the environment (without invasively targeting taxa) known as environmental DNA (or eDNA), has recently revolutionized conservation biology.

Advocated as a time and cost-effective alternative to traditional methods of biodiversity data collection, 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, Ushimaru & Minamoto, 2015; Dougherty et al., 2016;). Yet 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 to data collected via eDNA methodology can range from detecting the presence/absence of species with conventional PCR (e.g. Jerde et al., 2011; Dejean et al., 2012; Thomsen et al., 2012; Mahon et al., 2013; Piaggio et al., 2014; Fukumoto, Ushimaru & Minamoto, 2015) or community constituents via metabarcoding (e.g. Evans et al., 2015; Valentini et al., 2015; Hanfling et al., 2016; Shaw et al., 2016), to quantifying the relative abundance of DNA sequences (proxies for species abundance) via quantitative real-time PCR (qPCR) (e.g. Takahara et al., 2012; Goldberg et al., 2013; Pilliod et al., 2013; Klymus et al., 2015; Laramie, Pilliod & Goldberg, 2015). Still, it is difficult to discern when and how to match appropriate sampling protocols with conservation goals in the most cost-effective manner; yet
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*; henceforth YFP) is the only cetacean species to be found within the Yangtze River (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 and its tributaries, approximately 1890 km in length (Li, 2011). Although a conservation priority for China, not only does the YFP natural history make it difficult to survey, its large distribution makes approaches to population management and data collection costly, a particular concern in this developing country.

Previous research has successfully developed 12 species-specific YFP primer pairs used in conventional PCR for eDNA sampling (Ma et al., 2016). However, this eDNA approach only allows for the identification of presence/absence data to be collected. For this study, we optimize and validate the same species-specific primer pairs for qPCR amplification (quantifying relative DNA abundance), simultaneously testing and comparing the sensitivity of each primer pair for eDNA applications. We additionally conducted a cost-assessment of YFP traditional visual monitoring to conventional and quantitative PCR data collections, ultimately generating management recommendations for conservation practitioners, commensurate with the scale, goal, and financial feasibility of each sampling application available.
Methods

Primer optimization and validation

Using 12 published YFP species-specific primers (Ma et al., 2016), we proceeded to optimize qPCR protocols for use in eDNA surveying by first acquiring a synthetic gene solution as template DNA. This synthetic gene was then used to optimize amplification and explore the sensitivity/limit of detection (LOD; minimum level of target DNA detected in a sample), of these primer pairs. The synthetic cytochrome $b$ (cyt $b$) gene sequence was 1140 bp in length and retrieved from Genbank (accession number KJ472902). Each qPCR reaction contained 10µL of SYBR Premix Ex Taq II (Tli RNaseH Plus) (2×) (TaKaRa.), 0.4µL of ROX Reference Dye II (50×) (TaKaRa.), 6.4µL of RNase-free Water, 0.6µL of forward primer (10µM), 0.6µL of reverse primer (10µM), and 2µL 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 5s and 64°C for 34s), then followed to a melt-curve stage (95°C for 15s, 60°C for 1min, 95°C for 30s, and 60°C for 15s).

We qualified qPCR amplification to be successful if the results met the following requirements: 1) qPCR standards (or samples) displayed expected amplification curve; 2) amplifications displayed an expected melting curve peak; and 3) negative controls showed no amplification. 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 showed no amplification prior to cycle threshold (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, equates to 100% efficiency.

To test the sensitivity of each primer 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 following formula:

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

For this calculation we assumed 650 Daltons for the weight of each base pair and 6.022x10^{23} for the conversion to molecules/mole (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 conventional PCR 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) (for details see Ma et al., 2016).
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Validation via eDNA collection

To authenticate the efficacy of these primer pairs, we tested whether these primers could amplify YFP eDNA from a location where we knew this species to be present. Thus, as a positive control we collected water from the “netted cage” (approximately 15m x 15m enclosure) within Tian e-Zhou Baiji National Nature Reserve (29°51′11″N, 112°35′15″E), a nature reserve for the YFP which runs parallel to the Yangtze River (similar in protocol to Foote et al., 2012). Within the netted cage area, 2 YFP individuals were being temporarily housed for breeding research purposes before being released into the reserve. The water within the netted cage was connected to the rest of the reserve which further housed approximately 58 other YFP individuals at the time of sampling (April 2015). Water was sampled 0.4m 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 Sited-Pro Professional Grade) with 47mm diameter mixed cellulose esters (MCE) filter paper, 0.45μm pore size, utilizing a sterilized, reusable filter holder (Sterifil 47mm 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 conventional PCR protocols (Ma et al., 2016), we collected and filtered approximately 3-1L 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% alcohol. Samples were immediately stored in a -20°C freezer until DNA extraction (Ma et al., 2016).

DNA was extracted from stored, dried, filter paper with the PowerWater® DNA Isolation Kit (Mo Bio Laboratories, Inc) via placing filter paper into a 5mL 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. Netted cage eDNA was also diluted 1:10, 1:20 and 1:30 to check for PCR inhibition (Davy, Kidd & Wilson, 2015) but we found no evidence for qPCR inhibition during our assays.

To minimize contamination risk, we utilized filtered pipette tips, separate clean-rooms for DNA extraction and PCR amplification, and all equipment was sterilized with 20% diluted bleach, rinsed with sterilized water, and then dried under UV light for 30 minutes. We also used latex gloves for each sample collected. After each filtering session, medical-grade sterilized gauze 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, Kidd & Wilson, 2015).

As all samples involved environmental water with no direct contact to YFP individuals, animal collection permits were thus not required, although all collections were ethically conducted with permission from conservation managers and WWF officers affiliated with the Tian e-Zhou Nature Reserve.
Cost analysis

To create a streamlined cost comparison between visual and eDNA survey methods to detect (conventional PCR) and quantify (qPCR) YFP populations, we compiled 2015 and 2016 data on visual monitoring efforts by WWF officers at the Tian e-Zhou Nature Reserve. Veritably, total costs for such sampling would necessitate the transportation of samples and/or personnel to and from the reserve, yet these aspects may vary among practitioners employing these techniques. Thus, for brevity, we have restricted our comparison to average labour costs for this particular region (government recommendations), laboratory consumables, and boat rentals, as well as 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, and that amplification failure is negligible. Price tallies for eDNA surveys utilizing both conventional and quantitative PCR (qPCR) were based on 3-1L water samples across 15 sites within the reserve (1 site per 1km of habitable reserve length). PCR costs correspond to data presented in Ma et al. (2016) and Stewart et al. (2017), including consumables and extraction kits.

Data analysis

We derived equations of the line and slopes for each primer pairs 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 R² to calculate the efficiency of each primer and direct managers towards the best primers 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. All primer pairs demonstrated amplification of the positive control (netted cage sample within Tian e-Zhou) validating these primers for use in eDNA analysis, (mirroring analysis presented in Ma et al., 2016). The ultimate threshold for positive (or negative) detection scoring of samples in qPCR eDNA analysis can vary from study to study, spanning one of three, one of eight (Jerde et al., 2011; Jerde et al., 2013; Mahon et al., 2013; Piaggio et al., 2013; Reese 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). Comparatively, conventional PCR maximum sensitivity or lowest number of DNA copies/µl for each primer pair was retrieved from previous data (Ma et al., 2016) (Table 1).

Real-time 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 6 primers falling within the standard acceptable range 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=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.

Cost analysis

Costs accrued for visual surveys in Tian e-Zhou, including boat rental fees, a three-person-team, and 7 days of labour, approximated to 8,000 CNY per month, 32,000 CNY per season or 128,000 CNY per year from 2015-2016. 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, etc). 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).

eDNA sampling however, including labour, filtering water collections, extractions, amplifications, sequencing, ranged from 4,257 CNY for conventional PCR to 5,686 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 up to 30 days or more (Ma et al., 2016), sampling events with the aim to demonstrate current population distribution or abundance should 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
conventional PCR (species detection) at the same temporal schedule. If however, eDNA sampling using conventional PCR occurred only once per season, visual surveys would approximate 7.52X more expensive. Similarly, visual surveys compared to eDNA sampling utilizing qPCR (population abundance) would equate to 1.41X on a monthly, and 5.63X on a seasonal sampling schedule.

Discussion

Efforts toward biodiversity conservation are only as good as the data used to measure change in distribution and abundance. Yet, the high cost and substantial man-power required for biodiversity surveys, especially in developing countries, has hindered our ability to globally assess the status of species and populations. In part, this is reflected in the depauperate information currently accrued in areas of low-governance but high biodiversity (often with gaps in data collection), but also due to difficult-to-survey habitats such as aquatic environments (Frazier, Longo & Halpern, 2016) causing collection biases towards developed countries in temperate regions (McGeoch et al., 2010; Martin, Blossey & Ellis, 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., 2014; Davy, Kidd & Wilson, 2015; Sigsgaard et al., 2015; Smart et al., 2016), and comparisons between different eDNA approaches, to our knowledge, non-existent to date. Given recent demonstration that traditional survey methods are not always more expensive than eDNA approaches, particularly for smaller
survey budgets (e.g. Smart et al., 2016), providing conservation managers with the most effective tools for their specific goals is imperative to speed data assembly.

Our analysis demonstrated conventional PCR to be the least expensive option for species-specific surveying for YFP populations compared to both qPCR and traditional methods. Nevertheless, as with other studies (e.g. Amberg et al., 2015), conventional PCR remained a less-sensitive tool compared to qPCR and thus more prone to false-negatives making it more effective for data collection in areas where larger populations are expected or as a supplementary test for when false-negatives can be negated.

On the other hand, qPCR, a slightly more expensive option than conventional PCR but comparatively cheaper than traditional visual surveys for YFP, demonstrated high sensitivity and reproducibility for managers, suggesting high detection accuracy. Indeed, the primers representing the best efficiency and sensitivity (FP76, FP97, FP161, FP171, FP221, and FP249) also demonstrated a minimal detection capability of > 23% positive scoring at an LOD of 5 DNA copies/µl (with the exception of FP221), showing a high likelihood of picking up dilute eDNA signals within large aquatic environments even with relatively low population numbers and sampling replicates. Given the multitude of information qPCR could afford managers (distribution combined with relative abundance), it may offer the best option for biodiversity data collection. However, if managers are faced with financial constraints, either eDNA method may still represent a better alternative than visual monitoring. Traditional visual surveys remain a costly, invasive, and potentially inaccurate (e.g. due to organismal behavior) method for presence/absence data for YFP, in effect missing important demographic information that is
likely impactful for meaningful conservation decisions.

Although eDNA analysis may necessitate high initial costs (laboratory equipment, primer development, primer validation, and optimization of sampling protocols), our analysis corroborates the few other studies available (e.g. Biggs et al., 2014; Davy, Kidd & Wilson, 2015; Sigsgaard et al., 2015; Smart et al., 2016) suggesting eDNA methods may still represent the most cost-efficient means of acquiring biodiversity data. 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 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 taxa across its range with visual protocols given known organismal ecology. For example, due to their preference for benthic prey (Park et al., 2011; Shirakihara et al., 2009), Finless Porpoise have been observed to spend approximately 60% of their time on long dives (Beasley & 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, Liu & Wang, 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, Raven & Wilson, 2004). We thus encourage managers to consider the trade-offs between cost and data
accuracy when considering which sampling method to employ when collecting important biodiversity data for YFP populations.

Acknowledgments

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References


Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L,


**Tables**

<table>
<thead>
<tr>
<th>Primer</th>
<th>LOD 5 copies/µl</th>
<th>LOD 25 copies/µl</th>
<th>LOD 50 copies/µl</th>
<th>LOD 500 copies/µl</th>
<th>LOD 5000 copies/µl</th>
<th>qPCR Intercept</th>
<th>qPCR Slope</th>
<th>qPCR Efficiency (%)</th>
<th>PCR Max. Sensitivity (copies/µl)</th>
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</table>
### Table 2: Comparison of Yangtze Finless Porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) survey cost estimates in the Tian e-Zhou Baiji National Nature Reserve as of 2015-2016 (CNY) between eDNA protocols (45 samples in triplicate) utilizing conventional PCR (PCR) or quantitative PCR (qPCR), and traditional methods such as visual or capture methods (WWF China; *personal communication*).

<table>
<thead>
<tr>
<th>Survey Method</th>
<th>Details</th>
<th>Cost (CNY)</th>
</tr>
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<tbody>
<tr>
<td>PCR</td>
<td>eDNA collection labour</td>
<td>495</td>
</tr>
<tr>
<td></td>
<td>Filter papers + consumables</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Extraction QIAGEN DNEasy Blood and Tissue Kit</td>
<td>1,701</td>
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<tr>
<td></td>
<td>Amplification</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td>Confirmation (visualization)</td>
<td>675</td>
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<tr>
<td></td>
<td>PCR labour</td>
<td>486</td>
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<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>4,257</strong></td>
</tr>
<tr>
<td>qPCR</td>
<td>eDNA collection labour</td>
<td>495</td>
</tr>
<tr>
<td></td>
<td>Filter papers + consumables</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Extraction MOBIO DNEasy PowerWater Kit</td>
<td>3,701</td>
</tr>
<tr>
<td></td>
<td>Amplification and quantification</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>qPCR labour</td>
<td>1080</td>
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<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>5,686</strong></td>
</tr>
<tr>
<td>Visual</td>
<td>7 days, 1 boat, 3 personnel</td>
<td></td>
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<tr>
<td></td>
<td><strong>Total per month</strong></td>
<td><strong>8,000</strong></td>
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<tr>
<td></td>
<td><strong>Total per season</strong></td>
<td><strong>32,000</strong></td>
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<tr>
<td></td>
<td><strong>Total per year</strong></td>
<td><strong>128,000</strong></td>
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<tr>
<td><strong>Capture Monitoring</strong></td>
<td>10 days, 15 boats, 40 personnel</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total per year</strong></td>
<td><strong>300,000</strong></td>
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