Assessing the cost-efficiency of environmental DNA sampling

Adam S. Smart¹, Andrew R. Weeks¹,², Anthony R. van Rooyen², Alana Moore¹,³, Michael A. McCarthy¹, Reid Tingley¹

¹ School of BioSciences, The University of Melbourne, Parkville, Victoria 3010, Australia
² cesar Pty Ltd, 293 Royal Pde, Parkville, Victoria 3052, Australia
³ Unité de Mathématiques et Informatique Appliquées (MIAT), Toulouse INRA, Auzeville, France

Abstract
1. Environmental DNA (eDNA) sampling can be a highly sensitive method for detecting aquatic taxa; however, the cost-efficiency of this technique relative to traditional methods has not been rigorously assessed.
2. We show how methods that account for imperfect and stochastic detection can be used to: (i) determine the optimal allocation of survey effort with eDNA sampling for a fixed budget (i.e., identify the optimal combination of water samples vs. site visits); and (ii) assess the cost-efficiency of eDNA sampling relative to traditional survey techniques. We illustrate this approach by comparing eDNA sampling and bottle-trapping for an exotic newt species (Lissotriton v. vulgaris) recently detected in Melbourne, Australia.
3. Bottle traps produced much lower detection rates than eDNA sampling, but the cost-efficiency of the two methods can be similar because bottle-trapping is cheaper per sample. The relative cost-efficiency of the two sampling methods was sensitive to the available survey budget, the costs of eDNA primer/probe development and sample processing, and the number of positive quantitative PCR assays (qPCRs) used to designate a water sample as positive for newt DNA. Environmental DNA sampling was more cost-efficient than bottle-trapping for small-intermediate budgets when primer/probe development and sample processing costs were low, and 1/4 or 2/4 positive qPCRs was used to label a water sample as positive for newt eDNA. However, bottle traps were generally more cost-efficient than eDNA sampling when primer/probe development and sample processing costs were high, regardless of qPCR threshold or survey budget.
4. Traditional sampling methods may achieve lower detection probabilities compared to eDNA sampling, but the totality of costs can make eDNA sampling less efficient than traditional techniques in some circumstances. Our approach provides a quantitative framework for determining how many water samples and site visits are required to maximize detection probabilities with eDNA sampling, and can calculate the cost-efficiency of any sampling method.

**Key words**: cost-effectiveness, detectability, detection probability, detection rate, eDNA, invasive species, optimal survey design

**Introduction**
Management of aquatic biodiversity requires reliable survey methods for determining species distributions. Survey methods that are sensitive (i.e., achieve high probabilities of detecting target species when they are present) have strategic value for biodiversity monitoring applications. Environmental DNA (eDNA) sampling, which relies on the detection of species-specific genetic material in the environment, can be a highly sensitive technique for monitoring biodiversity (Ficetola et al. 2008). This approach was originally developed to detect mammal, bird, and plant DNA in glacial sediment cores (Willerslev et al. 2003). More recently, eDNA sampling has been used to detect the presence of contemporary biodiversity in water samples (Goldberg et al. 2011, 2013; Thomsen et al. 2012b; Schmidt et al. 2013; Rees et al. 2014).

Quantitative comparisons with conventional survey methods (e.g., trapping, visual surveys) indicate that eDNA sampling can achieve superior detection rates in aquatic systems (Ficetola et al. 2008; Jerde et al. 2011; Dejean et al. 2012; Smart et al. 2015), yet there has been little discussion of the cost-efficiency of eDNA sampling relative to traditional methods. Conservation budgets are limited, so monitoring methods must be both sensitive and cost-efficient. Like many technical sampling methods (e.g., electrofishing, automated recording stations, unmanned aerial vehicles, radio-telemetry), eDNA surveys can entail high start-up costs (e.g., for primer development and testing, and sequencing non-target species), potentially hindering adoption of this technique under stringent survey budgets. Nonetheless, several studies suggest that eDNA sampling can reduce total survey costs. For example, Sigsgaard et al. (2015) found that it was cheaper to detect a highly endangered fish species (Misgurnus fossilis) with eDNA sampling than with a combination of traditional survey methods (electrofishing, traps, and nets). Similarly, Huver et al. (2014) estimated that using eDNA sampling to survey single sites for trematodes (Ribeiroia ondatrae) was two times cheaper than using a traditional sampling approach (host necropsy), whereas Biggs et al. (2015) estimated that eDNA sampling was ten times cheaper for detecting newts (Triturus cristatus). However, previous studies have merely estimated the total cost of surveying a site (or all sites) with eDNA sampling vs. traditional survey methods; they have not combined cost estimates with site-specific detection rates to evaluate the cost-efficiency of both survey methods across a range of budgets and scenarios.

A related limitation of eDNA studies to date is that they have not considered how best to allocate survey effort with eDNA sampling in order to maximize the probability of detecting a species at an occupied site. Should an entire budget be spent taking water samples on a single visit (in which case travel costs are minimized), or should efforts be staggered to account for stochastic variation in detection over time? Although optimal survey design protocols have been established for many traditional survey methods (Harrison 2006; Lyra-Jorge et al. 2008; Shannon et al. 2014; Sanderlin et al. 2014), most previous analyses have failed to account for temporal variation in detection.
probabilities (cf. Moore et al. 2014), and no studies have considered how best to allocate survey effort with eDNA sampling across a range of budgets.

Here, we show how methods that account for imperfect and stochastic detection can be used to allocate survey resources with eDNA sampling under a fixed budget, and use this method to contrast the cost-efficiency of eDNA sampling and a traditional survey method under optimal sampling designs. Our method uses the recent work by Moore et al. (2014), who developed an optimization method for investigating the allocation of sampling effort at a site when detection varies through time. We illustrate this method by applying it to a European newt (Lissotriton vulgaris vulgaris, Linnaeus 1758) invasion in Victoria, Australia (Tingley et al. 2015). Previous work in this system (Smart et al. 2015) has shown that eDNA sampling is more sensitive than a traditional sampling method (bottle traps), but this analysis did not consider costs of either method, or how best to allocate survey resources.

Materials and methods

OVERVIEW

To compare the cost-efficiency of eDNA sampling to bottle-trapping, we considered a situation in which the objective is to detect a species at a single site (Moore et al. 2014). Briefly, the model minimizes the probability of failing to detect a target species given a fixed survey budget and set of financial constraints, whilst incorporating variability in detection rate. Using this model one can estimate the optimal number of visits to a site, the corresponding survey effort per visit (e.g., the number of water samples to take each visit), and the resulting expected detection probability. Full details of the model are provided by Moore et al. (2014), but we provide an overview of the approach below.

THE MODEL

We assumed that detections result from a Poisson process. If the species is detected during survey \( i \), by survey method \( j \), at rate \( \lambda_{i,j} \), then the probability of failing to detect the target species with a given survey method and number of samples \( k_{i,j} \) is \( \exp(-\lambda_{i,j} k_{i,j}) \). If we assume the detection rate \( \lambda_{i,j} \) varies independently among surveys, then the probability of failing to detect the species during \( n \) surveys using method \( j \) is:

\[
Q_j = \prod_{i=1}^{n} \exp(-\lambda_{i,j} k_{i,j}) = \exp\left(-\prod_{i=1}^{n} \lambda_{i,j} k_{i,j}\right) = \exp(-A_j)
\]

where \( A_j = \sum_{i=1}^{n} (\lambda_{i,j} k_{i,j}) \) is the expected number of detections for the entire survey period with method \( j \).

Our objective was to identify the sampling regime (combination of samples/traps and independent site visits) that maximizes the detection probability for a fixed budget. First, we found the number of surveys that minimizes the expected probability of failed detection \( E[Q] \). If \( X \) is a normal random variable with mean \( m \) and variance \( v \), the cumulative density function of \( Q_j = \exp(-A_j) = \exp(-\exp(X)) \) is given by,

\[
F(q_j) = \Pr(Q_j < q_j),
= 1 - \frac{1}{2} \left(1 + \frac{m \ln(-\ln[q_j])}{\sqrt{2v}}\right),
\]

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FIELD SAMPLING

Six of the seven surveyed sites were linear roadside drains ~1 m wide x 135 m in length. The seventh roadside drain, which we use in our analyses below, was smaller and less linear than the others. Sites were surveyed using bottle traps constructed from 2 L soda bottles (Griffiths 1984) baited with 10 × 100 mm glow sticks. Each site was surveyed for four consecutive nights once per month (one survey cycle), over a four month period (September–December 2013). Sites were surveyed with 11 bottle traps each night and checked the following morning, whilst eDNA sampling (three 500 mL water samples per visit) occurred on the first and last day of each survey cycle. Previous eDNA studies have achieved high detection rates under natural conditions with as little as 15 mL of water (Thomsen et al. 2012b). Given the shallow nature of our sites and the size of the filters used to process water samples (0.45 µm), we deemed 500 mL as appropriate for our system. A total of 1006 traps were set over 16 separate trapping days and 190 water samples were taken over eight days. As the seventh site was...
considerably smaller than the other six sites, we only set four traps and took a single 500 mL water sample at that site during each sampling period. Field sampling and eDNA analysis protocols are described in Tingley et al. (2015) and Smart et al. (2015).

Multiple quantitative PCRs (qPCRs) are typically run on each water sample to reduce the probability of false negatives. For our analyses, we ran four qPCRs on each water sample. Studies have considered water samples positive for the presence of a target species if any one of three to eight qPCR replicates was positive (Rees et al. 2014). However, the possibility of false positive results means that some studies have required >1 qPCR replicate to test positive. Here we explore how the assigned qPCR threshold (1/4, 2/4, 3/4 or 4/4 positive qPCR replicates) affects the cost-efficiency of eDNA sampling by re-running our models using each threshold.

ESTIMATING DETECTION RATES

Estimates of the mean and coefficient of variation θ in detection rates for each survey method were estimated with a hierarchical Bayesian generalized linear model in OpenBUGS v 3.0.0 (Thomas et al. 2006). Details of the model can be found in Smart et al. (2015) and R-code (R Development Core Team 2015) and associated data can be found in Appendix S1 (Supporting Information). Estimates of the mean detection rate and θ were modelled using data from the two sites with the highest and lowest detection rates (Smart et al. 2015). Detection rates of both sampling methods at the other five sites monitored by Smart et al. (2015) were very similar to those at the low detection site. We estimated θ by summing the temporal variance modelled at the daily and monthly level (Smart et al. 2015). Modelled mean detection rates per bottle trap were 0.016 and 0.086 at the lowest and highest detection sites, respectively (θ = 1.68 in both cases). Assuming a water sample was positive when 1/4 qPCR replicates tested positive for L. v. vulgaris DNA, per-sample detection rates were 0.58 and 2.99 (θ = 2.07). These parameter estimates were used to determine the most cost-efficient survey technique for a range of budgets, given that fixed and variable costs, mean detection rates, and variation in those rates were unique to each survey method. All analyses were conducted in R 3.2.2 (R Development Core Team 2015).

COST ANALYSIS

Costs required to conduct a survey with each sampling method were based on our previous fieldwork (Smart et al. 2015). For ease of presentation, we break costs down into per-sample, travel, and site setup costs (Table 1). Per-sample equipment costs for trapping included consumables (e.g., bottle traps, stakes, glow sticks), whereas per-sample personnel costs for trapping included preparing equipment, constructing, setting, checking, and replacing traps, and disposing of waste materials. We assumed that eDNA samples were processed by a fully-equipped genetics laboratory; hence we did not include the costs of non-perishables or laboratory equipment (Sigsgaard et al. 2014). Per-sample field equipment costs for eDNA sampling included items such as sample bottles and sterilizing agents. Per-sample personnel costs for eDNA sampling included preparing/sterilizing equipment, collecting water samples at a site, and disposing of waste products. Personnel costs for fieldwork and genetic analyses were based on a rate of $85 AUD per hour.

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Travel costs for trapping and eDNA sampling included travelling to and from a site (one hour each way). However, because traps needed to be set and then checked the following day, travel costs for trapping were double those of eDNA sampling. For eDNA sampling, travel costs included dropping samples off at the laboratory (an additional 10 minutes). Travel costs were based on hourly rates elicited from a local consultant (M. West, pers. comm.) and included the $85 consultant rate plus the additional cost of fuel, insurance, and vehicle maintenance (an extra $36 per hour based on a per km charge and the distance to our sites). Thus, total travel costs were $111 per hour. Setup costs for both methods included a one-off expense to delimit and mark out the survey sites. For eDNA sampling, an additional setup cost for primer/probe development was included (see below).

Equipment, personnel, and setup costs associated with traditional survey techniques such as bottle-trapping should be relatively consistent between studies and are unlikely to decrease over time. Costs associated with eDNA sampling, on the other hand, can be quite variable depending, for example, on the number of water samples processed, whether sequence data are available for the target species, and the richness and phylogenetic relatedness of non-target species in the study area (because designing species-specific primers/probes becomes more of a challenge as the number of closely-related taxa increases). Furthermore, costs associated with genetic analyses decrease rapidly over time (Metzker 2010; Stein 2010). We therefore explored high- and low-cost scenarios for eDNA sampling. These expenses were based on our previous work on *L. v. vulgaris* (in which primer/probe specificity using a TaqMan® assay [Life Technologies, Australia] was not an issue due to the fact that *L. v. vulgaris* has no close relatives in Australia), and a similar study on native fishes (*Galaxilla pusilla* and *G. fusca*) (Weeks, A.R. and Van Rooyen, A.R., unpublished data). For the high-cost scenario, we assumed that 24 samples were processed at a time (per-sample cost = $86.06), and that primers/probes needed to be tested against DNA from closely related species at an additional cost of $1000 (galaxid scenario). For the low-cost scenario, we assumed that samples were run in batches of 100 (per-sample cost = $62.29), and that non-target DNA testing was not required due to phylogenetic distinctiveness (*L. v. vulgaris* scenario). Thus, the high-cost scenario assumed that per-sample and primer/probe development costs were 1.38 and 2.76 times higher than in the low-cost scenario, respectively. See Appendix S2 in Supporting Information for a finer-scale breakdown of costs associated with each method.

Finally, we ran an additional analysis, assuming that the sampling site had already been established (i.e., that there were no costs associated with delimiting and marking out the site for either bottle-trapping or eDNA sampling) and that suitably specific primers/probes had already been developed. Thus, in this revised analysis, only per-sample costs differed between the high- and low-cost eDNA scenarios.

**Results**

The optimal number of repeat visits to a site increased linearly with the available survey budget for both bottle-trapping and eDNA sampling, illustrating the importance of accounting for stochastic variation in detection rates. The slope of this relationship was much steeper for eDNA sampling, however, due to the lower per-sample cost and higher travel cost associated with bottle-trapping (Fig. 1).

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Comparing the costs and detection probabilities of optimal trapping and eDNA sampling regimes enabled us to directly compare the cost-efficiency of the two sampling methods across a range of fixed budgets. This analysis revealed that the most cost-efficient sampling method was sensitive to the survey budget, the costs of eDNA primer/probe development and sample processing, and the number of positive qPCRs used to designate a water sample as positive for *L. v. vulgaris* DNA. Environmental DNA sampling was more cost-efficient than bottle-trapping across a range of small to intermediate budgets when primer/probe development and sample processing costs were low (i.e., low-cost scenario), and when 1/4 or 2/4 positive qPCRs was used to designate a water sample as positive for *L. v. vulgaris* DNA (Fig. 2). This disparity in cost-efficiency was greater when detection rate differed more markedly between the two sampling methods (Fig. 3). Conversely, when eDNA sampling involved extensive testing of primers/probes and processing fewer water samples (i.e., high-cost scenario), bottle traps were more cost-efficient than eDNA sampling across all survey budgets and qPCR thresholds at both high and low detection sites (Figs. 2 & 3). Removing site setup and primer/probe development costs from the analysis slightly increased the cost-efficiency of eDNA sampling relative to when these costs were included (Figs. S1 & S2; Appendix S3 in Supporting Information); however, bottle-trapping was still more cost-efficient than eDNA sampling when >2 qPCRs were used to designate a water sample as positive, except under very small budgets.

**Discussion**

Our analysis represents the first attempt to explore how the mean and temporal variation in site-level detection rates, different cost scenarios, and the qPCR threshold chosen to designate a water sample as positive for a target species’ DNA can influence the cost-efficiency of eDNA sampling relative to a traditional sampling technique. We have shown that primer/probe development and sample processing costs, in conjunction with the chosen qPCR threshold, crucially influence whether eDNA sampling is worthwhile compared to more traditional methods.

A wide range of techniques have been used to collect, preserve, filter, and extract DNA from water samples in the literature, and each of these techniques has different financial costs (Goldberg *et al.* 2011; Lodge *et al.* 2012; Rees *et al.* 2014; Barnes & Turner 2015). Rather than conduct an exhaustive comparison of the cost-efficiency of various methods, we explored high- and low-cost scenarios parameterised from previous research. This scenario approach was justified by the fact that travel and setup costs had a much greater influence on the cost-efficiency of eDNA sampling than per-sample costs. Under the low-cost scenario, in which the primers/probe were clearly unique to the target species (i.e., low setup costs), eDNA sampling was more cost-efficient than trapping for small to intermediate budgets (at least when 1/4 or 2/4 qPCRs were used as thresholds). Conversely, in the high-cost scenario, we assumed that a large portion of the available survey budget was used to ensure that the primers/probe were suitably specific to the focal species, reducing the available budget to collect water samples. As a result, bottle-traps were more cost-efficient than eDNA sampling across all combinations of qPCR threshold and detection rate under the high-cost scenario. Across all modelled scenarios, differences in cost-efficiency between trapping and eDNA sampling were greatest for small survey budgets; imperfect detection was generally not an issue with either sampling method given a large enough budget.

The other parameter that had a large influence on the cost-efficiency of eDNA sampling was the qPCR threshold used to designate a water sample as positive for *L. v. vulgaris* DNA. We found that the use of more stringent qPCR thresholds drastically reduced the cost-efficiency of eDNA sampling relative to bottle-trapping, due to marked decreases in eDNA sensitivity at higher qPCR thresholds.
Rather than choosing an arbitrary qPCR threshold, an alternative approach would be to extend the hierarchical models used here to simultaneously estimate detection rates at both the water sample level and the qPCR level (Schmidt et al. 2013). The estimated number of qPCRs needed to obtain a high detection rate could then be used to inform an appropriate threshold (under the assumption of no false positive errors). The decision as to how many positive qPCR runs should be used to denote a water sample as positive will clearly depend on the decision context, and on system-specific factors that influence false positive rates, such as primer and probe specificities, sample collection and preparation protocols, and eDNA degradation rates (Ficetola et al. 2014; Lahoz-Monfort et al. in press). However, removing qPCR detections under the suspicion that they represent false positive errors can bias occupancy and detectability estimation (because some of the omitted detections may represent true positives: Lahoz-Monfort et al., 2016). Integrating cost-efficiency analyses such as ours with site occupancy detection models that explicitly account for false positive errors (Chambert et al. 2014) represents a promising avenue for further research.

Our case study illustrates that eDNA sampling can be less cost-efficient than a traditional sampling method in some circumstances, but our analyses should be viewed as conservative for several reasons. First, in addition to primer and probe development costs, our high-cost scenario incorporated more expensive sample processing (because fewer samples were analyzed simultaneously). However, per-sample costs of eDNA sampling are likely to mirror the observed decrease in the cost of DNA sequencing over the last decade (Metzker 2010; Stein 2010). Thus, the cost-efficiency of eDNA sampling will likely increase over time. Second, using eDNA sampling in a multi-species framework (e.g., metabarcoding: Thomsen et al. 2012a; Ji et al. 2013) could further increase the cost-efficiency of the eDNA approach, especially when sampling multiple taxonomic groups that require different survey methods. An interesting avenue for further research, then, is to extend the optimization approach presented here to multi-species management objectives (e.g., minimizing the number of undetected species: McCarthy et al. 2010). Third, we investigated a relatively inexpensive traditional sampling method (bottle-trapping), but in situations where more elaborate methods are required (e.g., electro-fishing), eDNA may prove more cost-efficient. Finally, it is important to note that the objective of our optimization function was to minimize the probability of failed detection at a single site. Such situations might arise, for example, when conducting environmental impact assessments for a threatened species, or attempting to protect sites from an invasive species. In many other cases, however, more than one site will need to be surveyed. In these cases, primer/probe development and testing costs will simply scale inversely with the number of surveyed sites. Therefore, as the number of sites in a management portfolio increases, the cost-efficiency of eDNA sampling too will increase.

This inverse scaling means that eDNA sampling holds great promise for large-scale monitoring programs (e.g., using citizen-science: Biggs et al. 2015). Future studies could use optimisation methods that account for travel costs between sites to examine multi-species objectives explicitly (Moore & McCarthy, in press).

The above caveats demonstrate that the cost-efficiency of eDNA sampling will depend on a variety of factors. However, our goal was not to make an absolute assessment of the cost-efficiency of eDNA sampling, which would require investigating a prohibitively large range of study systems, traditional sampling approaches, and eDNA methodologies. Instead, we have presented a framework for assessing how such factors can influence the cost-efficiency of eDNA sampling for particular study systems.
The optimization approach presented here also addresses the question of how researchers should stagger the collection of water samples for eDNA analysis over time. In our case study, significant temporal variation in eDNA and bottle-trapping detection rates meant that it was optimal to conduct repeat visits to a site. The optimal number of site visits increased linearly with the available survey budget for both sampling methods, even after accounting for additional travel costs. Given the sensitivity of eDNA degradation to environmental conditions (Pilliod et al. 2014; Strickler et al. 2014), future studies should consider collecting water samples on repeat visits to account for stochastic temporal variation in the probability of detection.

CONCLUSION
Determining which sampling method is optimal for a given application will depend not only on the monitoring objective, but also on the sensitivity, ease, and cost-efficiency of each approach. Our results illustrate that the latter consideration can be critical. We show that although a method may achieve a low detection probability, the totality of costs associated with a more sensitive technique may mean that the inferior technique is more efficient. The approach presented here allows researchers and managers to assess such trade-offs between sampling methods by quantifying how different parameter values and assumptions affect the cost-efficiency of each sampling technique. Importantly, this approach can be applied to any sampling method or study system where information on detection rates and survey costs are available. This information could come from pilot studies, prior research, or simulated using sensitivity analyses (where plausible bounds on parameters can be obtained). Our modelling approach could usefully be extended to other emerging technologies such as unmanned aerial vehicles, camera traps, and automated recording devices. Such applications will enable researchers and managers to determine the circumstances in which it is worthwhile to adopt novel, but uncertain techniques in lieu of more established sampling methods.

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Data Accessibility
R scripts and corresponding data: uploaded as online Supporting Information (Appendix S1).

References


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### Table 1. Costs of sampling a site for *L. vulgaris* (based on Smart et al. 2015). All values are in $AU (2014). The high-cost scenario for eDNA sampling assumes 100 water samples are processed at a time and extensive primer/probe development and testing costs are necessary. The low-cost scenario assumes 24 water samples are processed at a time and low primer/probe development costs. See Materials and methods for details of cost categories and scenarios, and Appendix S2 for a finer breakdown of costs.

<table>
<thead>
<tr>
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<th>Environmental DNA sampling</th>
<th>Bottle-trapping</th>
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<tbody>
<tr>
<td></td>
<td>High Cost</td>
<td>Low Cost</td>
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Figure legends

**Fig 1.** Optimal number of repeat site visits for eDNA sampling and bottle-trapping at a high detection site over a range of survey budgets. In this example, one out of four qPCR replicates had to test positive for *L. v. vulgaris* DNA to deem the species as present in a water sample. Panel (a) shows the results of the optimisation using actual costs, mean detection rates, and the coefficient of variation (CV) in those rates. The remaining panels explore how the optimal number of site visits changes when eDNA sampling is given the same travel costs (b), per-sample costs (c), and CV (d) as bottle-trapping.

**Fig 2.** Cost-efficiency of eDNA sampling and bottle-trapping at a high detection site over a range of survey budgets. Panels show how the cost-efficiency of eDNA sampling changes with the number of positive qPCR replicates used to deem a water sample as positive for *L. v. vulgaris* DNA. The dotted line in each panel represents a detection probability of 0.95.

**Fig 3.** Cost-efficiency of eDNA sampling and bottle-trapping at a low detection site over a range of survey budgets. Panels show how the cost-efficiency of eDNA sampling changes with the number of positive qPCR replicates used to deem a water sample as positive for *L. v. vulgaris* DNA. The dotted line in each panel represents a detection probability of 0.95.

**Supporting Information**

**Appendix S1.** R scripts and data for running the optimization.

**Appendix S2.** Costs associated with using bottle-traps and eDNA sampling for detecting *L. v. vulgaris*.

**Appendix S3.** Optimization results assuming that the sampling site had already been established and that suitably specific primers/probes had already been developed.
(a) Observed costs and variation in detection rates

- eDNA
  - Low Cost
  - High Cost
- Trapping
  - Static Cost

(b) eDNA travel costs = trapping travel costs

(c) eDNA sample costs = trapping sample costs

(d) eDNA CV = trapping CV

Optimal number of site visits vs. Budget ($AU)