Applications and limitations of measuring environmental DNA as indicators of the presence of aquatic animals

Helen C. Rees¹, Kevin C. Gough², David J. Middleditch³, James R. M. Patmore⁴, & Ben C. Maddison¹.

¹ADAS UK Ltd, School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD.
²School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD.
³ADAS UK Ltd, ADAS Boxworth, Boxworth, Cambridgeshire, CB23 4NN.
⁴REC Ltd, Osprey House, Pacific Quay, Broadway, Manchester, M50 2UE.

Emails: helen.rees@adas.co.uk, Kevin.Gough@nottingham.ac.uk, david.middleditch@adas.co.uk, jpatmore@recltd.co.uk, ben.maddison@adas.co.uk.

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Corresponding Author: Helen C. Rees, ADAS UK Ltd, School of Veterinary Medicine and Science, The University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD. Email: helen.rees@adas.co.uk. Telephone: +44 (0)115 951 6747. Fax: +44 (0)115 951 6415
Summary

1. In Rees et al. (2014) we reviewed the current status of eDNA to monitor aquatic populations. Our aim was to focus on discussion of methodologies used, application of eDNA analysis as a survey tool in ecology, and include some innovative ideas for using eDNA in conservation and management.

2. Roussel et al. (2015) claim that analysis of Rees et al. (2014b) and other publications highlights the downsides of the method and they suggest that some conclusions should be toned down. Many of their arguments were covered in our original paper (Rees et al. 2014b) however, they make the point that modelling approaches should be encouraged and we fully agree with this suggestion.

3. Roussel et al. (2015) also claim that we neglected to recognise that there are two sources of imperfect detection (at the field level and the laboratory level), we would dispute this fact.

4. Synthesis and applications. Roussel et al. (2015) re-iterate many of the points made in the original paper but do cover some additional areas that improve the debate on the use of eDNA. Both the comment (Roussel et al. 2015) and our reply clearly highlight that detailed laboratory protocols and rigorous field sampling design are crucial factors which require sufficient reporting in the literature to allow for experimental comparison and replication.

Any development of a new method for eDNA detection should be compared directly with established “gold standard” methods for the detection of the species/habitat under investigation. None of the issues raised in Roussel et al. (2015) would alter our main conclusions.
Keywords

eDNA, false positive, limit of detection, PCR, sampling design, species detectability, study
comparison, water sampling

Introduction

eDNA analysis in the context of monitoring aquatic and semi-aquatic species via species-specific DNA
from water bodies is still in its relative infancy. Because of this there is much still to be done in terms
of optimising and improving eDNA analysis. Our review intended to discuss the state of both the
field sampling and laboratory analysis of these samples and to suggest areas which required
improvement, we also discussed innovative ideas for how eDNA can be applied to conservation and
management. It was not our intention to produce an exhaustive review of every aspect of the field,
we aimed instead to produce a review with specific emphasis on environmental water sampling, the
PCR analysis itself, and then the specific areas where this technique has been applied: eDNA
persistence; biomass estimation; species composition; and invasive and rare or threatened species;
along with a comparison of eDNA versus traditional methods. There was no intention to neglect
what are deemed to be important areas of this type of work such as the use of modelling for
sampling design. In this respect we welcome the endeavour of Roussel et al. (2015, hereafter
'Roussel') in providing comment on our review paper (Rees et al. 2014b). We respond to the five
main points below.

Reply to Roussel

The first point made by Roussel states that it is ‘premature to imply that eDNA will be an efficient
tool for detection all aquatic animal species’. We agree with this statement and also that from
Ficetola et al. (2008) who stated that eDNA is ‘useful for studying secretive aquatic or semi-aquatic
species, which release DNA into the environment through mucus, faeces, urine and remainst’ . In our
review we do not imply that eDNA can be used for ‘all’ aquatic species we merely point out that: 1)
eDNA analysis has been used as an accurate indicator of species presence in a range of environments; 2) that techniques such as eDNA analysis are of great interest in the management and conservation of vulnerable species and would be of great use in these areas; and 3) that eDNA is particularly useful for those species which are difficult to detect using conventional methods. We go on to state that the success rate of eDNA analysis is not always 100% illustrating that it is not always an efficient tool for the detection of aquatic animal species.

Roussel rightly chose one of their own publications (Treguier et al. 2014), which was not available at the time of writing Rees et al. (2014b), as an example of eDNA being an inefficient tool on its own for the detection of the red-swap crayfish, Procambarus clarkia. They showed that when crayfish abundance was estimated to be low by conventional trapping, eDNA detection efficiency was poor illustrating a limitation of eDNA detection for this species/habitat combination. However, Roussel did not mention that this same study found that eDNA had a better overall detection efficiency than conventional trapping (73% versus 65% respectively) and that experimental evidenced suggested that false positives were unlikely in this case so would not account for the higher detection efficiency. This result would suggest that in this case eDNA was more efficient than traditional survey. A further example of an invertebrate which has been successfully detected using eDNA analysis is the tadpole shrimp, Lepidurus apus (Thomsen et al. 2012) which was detected at 100% success rate in 10 ponds known to contain this species. Both studies illustrate that detection of invertebrate species is possible and that with continuing developments in PCR technologies and sampling methods the detection efficiency of invertebrate species could be improved making eDNA a more efficient tool for their detection.

In Rees et al. (2014b) we stated ‘The probability of detection will vary for the chosen target species and will be dependent on the taxa, the density of organisms, and also the type of water body.’ therefore, we completely agree with Roussel that when studying a new target species, preliminary tests to estimate the detectability of the target DNA should be a pre-requisite for surveying. We would go further and suggest that any such development of a new method for eDNA
detection should be compared directly with any established “gold standard” method for the
detection of the species/habitat under investigation. As mentioned in the original review, given the
developmental stage of applying eDNA to ecological surveys of aquatic animals it is vital that new
emerging methodologies are correlated directly with the established survey methods for a particular
species/habitat type, enabling a robust comparison and assessment of new eDNA survey methods.

The second point made by Roussel is again correct in that it is often perceived that eDNA analysis is
more efficient than traditional surveys. As we stated in Rees et al. (2014b) eDNA has ‘the potential of
greater sensitivity over traditional survey methods’ we do not state that it is more efficient. eDNA
analysis has been shown to be more efficient in a few studies (Dejean et al. 2012; Takahara,
Minamoto & Doi 2013) and latterly (Treguier et al. 2014) but our review points out that the eDNA
success rate was not always 100% when the species was known to be present (Thomsen et al. 2012)
illustrating that it is not always more efficient.

Roussel is again correct to state that the abundancies of the species in question is not always stated
within the literature and that it is therefore ‘difficult to understand how the method might perform
across a gradient of target species density’ an important point which does add to our review. We
agree that each survey method (eDNA versus traditional) is imperfect and we devoted a whole sub-
section in Rees et al. (2014b) to ‘eDNA versus traditional survey methods’ where we compared the
use of eDNA analysis and traditional survey methods. We agree that there should be more
comparisons between eDNA and traditional census methods, so much so that this is exactly what we
did in a recent study on great crested newts, Triturus cristatus in the UK (Rees et al. 2014a). Likewise
we agree that it would be very useful for method comparison if species abundance were included in
these studies and feel that this is an important point which has not necessarily been mentioned
elsewhere.
The third point made by Roussel concentrates on the reporting of experimental details, and false positives. We agree with the comments made on reporting of experimental details and in fact the point should be made here that consideration of the MIQE guidelines (Bustin et al. 2009) (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) should be done before publication of any study involving real-time PCR a point which both our Rees et al. (2014b) review and Roussel neglected to make. The background to these guidelines is that there is very little consensus on ‘how best to perform and interpret real time PCR (qPCR) experiments’ and that this problem is exacerbated by the fact that little experimental information is included in published studies. Not only do these guidelines encourage the inclusion of the complete reaction/thermocycling conditions and limits of detection as suggested should be included in publications by Roussel, but it also covers many other items that should be reported to allow for useful comparison or replication of experiments (provided in the form of a useful checklist). Also encouraged is the correct use of nomenclature which can often be confused in publications.

In terms of false positives Roussel makes the point that we also made in that false positives ‘occur when there is no target DNA in the sample’. Roussel is correct that the term ‘false positive’ should be carefully and unambiguously defined and of course in terms of positive PCR signals you need to consider the possibility, as was stated the review, that ‘there is target DNA in the sample but no viable/live organisms in the system’. During the peer-review process of our review paper (Rees et al. 2014b) we were asked to be careful about the use of the wording ‘false positive’. We therefore suggested that a ‘false positive’ could also be termed a ‘false detection’. Perhaps our definitions should have been made more clearly to state that a ‘false positive’ occurs when there is no target DNA in the sample and a ‘false detection’ occurs when there is target DNA in the sample but no viable/live organisms in the system. It will be important for future publications to stipulate whether they are referring to a ‘false positive’ or a possible ‘false detection’. Roussel goes on to state that ‘false detections’ need more attention if we want to understand what an eDNA detection represents. We would like to emphasise the point that in some species that are transiently present...
in water and for example move between ponds, an eDNA detection would still be valid whether the
species was present or not at the time of sampling especially when trying to define species
distribution. Of course, as suggested by Roussel consideration should be made to the fact that DNA
in a water sample could originate from predator faeces or dead animals which we also stated in Rees
et al (2014b). We agree with Roussel that care should be taken to avoid the inclusion of sedimentary
material when taking water samples as sediment could contain eDNA from past site occupancy
rather than recent (dependent on eDNA degradation rates) or present occupancy. We would also
point out that in our experience the presence of sediment can act to inhibit the PCR reactions. The
use of an inhibition control (Biggs et al. 2014) can help to monitor for this and other inhibitors which
may be present in a sample. In fact, the inclusion of an inhibition control is now carried out as
standard in our laboratories for every sample being tested.

The fourth point made by Roussel relates to sampling design, and we agree that rigorous sampling
design is important and that tools such as site occupancy models for example the program
(MacKenzie et al. 2002)] can be used. Roussel stated that in Rees et al. (2014b) we failed to
recognise that there are two sources of imperfect detection: at the field level and in the laboratory.
We would dispute this comment as we have a large section devoted to sampling in which we discuss
the numerous different methods employed for sampling in the literature. We discuss how groups
have improved the sampling coverage of the water systems and noted that methods for sample
collection will vary depending on the type of water body. Additionally we recognised that the
probability of detection will depend on several factors including the taxa, the density of organisms,
and the type of water body. We also note that there is considerable variation in the processing of
the water samples prior to the extraction step for which we state that ‘a direct comparison of these
methods to monitor the effects of different processing methods on eDNA extraction and detection
would be useful’. This implies that there are sources of imperfect detection at the field level,
although we may not have stated so in so many words, the citations in Roussel that list the studies (Schmidt et al. 2013; Ficetola et al. 2014) which illustrate this imperfect detection are useful and should be considered when designing sampling regimes.

The fifth point made by Roussel deals with the application of eDNA detection to running water, an area in which there has been few studies (Roussel indicates 9 papers have been published on this subject). We chose to discuss the sampling regimes used so far and concluded that ‘methods for sample collection will vary’ depending on the environment which is to be studied. We did not discuss the issue of downstream transportation of DNA as there was little information on this within the published studies. Roussel is correct that the implications of discharge variation and dendritic organization will need to be considered when studying these environments along with DNA degradation (which was discussed within Rees et al. (2014b)). This again goes back to the point that Roussel made about the importance of the study design and the use of modelling approaches such that all these factors are taken into consideration prior to study commencement.

**Conclusions**

For the most part we agree with the comments of Roussel and in several instances the comments made do not correspond to what was said in our review paper but may correspond to the other papers as analysed by Roussel. We note that many of the points raised were already covered in our original review paper although there are some additional valid considerations, which are discussed above. Additionally, we dispute the fact that we neglected to discuss the potential for errors in the field sampling although we did not included discussion on the use of modelling during the study design phase, which was an important omission. We feel that our original conclusions are valid: eDNA analysis is fast becoming an important tool in the study of aquatic species; alternative methods of PCR or novel methodologies could be used to provide rapid on-site detections; the fact that there does not appear to be ‘a single study which could be used as a template for
standardization of this technique’ due to the very different natures of the environments being studied, the taxa, the density of organisms, and the disparity in the way samples are scored as positive; the fact that next generation sequencing of eDNA samples could become a powerful tool for monitoring species diversity; and finally that the technique has been used as an ‘accurate indicator of the presence of species in a range of aquatic environments’.

In agreement with the conclusions of Roussel, we feel that eDNA analysis is a ‘promising method’, that more investigations are required, and as with any method users should be aware of potential weaknesses within its use. Roussel concludes that non-detection of target species DNA does not mean the absence of that species, nor does a positive detection mean that the species is present. This is of course true for the reasons already discussed above. However, it is also the case that traditional field survey methods are not always accurate when recording the absence of a particular species, particularly in cases where a secretive or elusive species is being surveyed. This points to the fact that neither field survey nor eDNA analysis is perfect, which supports a statement made in the original review and is our concluding remark here, that ‘eDNA should not be used to replace or disregard the knowledge and expertise of experienced field ecologists and taxon specialists, but should become an important tool to enhance limited conservation resources’.

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Not applicable.

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