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Letter to the editor

Monitoring of animal abundance by environmental DNA — An increasingly obscure perspective: A reply to Klymus et al., 2015

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In recent years analysis of environmental DNA (eDNA) has been presented as a convenient techno-fix to zoological surveys and monitoring. In particular aquatic organisms have received much attention. Following a number of proof-of-concept papers, Klymus et al. (2015) are among the first of much needed studies using elaborate experiments to understand the nature of environmental DNA from aquatic animals. Klymus et al. (2015) examined eDNA as a function of temperature, feeding, and biomass of two species of carp in aquaria. Similar to previous studies they conclude "Our results demonstrate that quantification of eDNA may be useful for predicting carp density, as well as densities of other rare or invasive species." With this comment we contend to the positive interpretation of the data at hand. In particular we feel the need to comment on it since Klymus et al. (2015) represents a pertinent example of the general problem that overly optimistic proclamations are building up in the literature on this novel method. This is concerning because eDNA is already finding its way into applied science and is rapidly being adopted to various aspects of nature management.

Contrary to the authors, we find that their data contribute substantially to the increasing documentation of complications with eDNA as a measure of animal density. Klymus et al. (2015) demonstrate firstly, it is not possible to make robust quantitative estimates of DNA excretion, and secondly such estimates are in any case weakly related to biomass or individual density.

The first experiment by Klymus et al. (2015) tested whether DNA shedding of a single individual can be quantified robustly by replicate sampling over 6 weeks. DNA shedding rates had standard deviations approximately equal to or higher than the mean (mean copies/h ± sd; 1 L/h tank: 50,000 ± 43,000; 2 L/h tank: 62,000 ± 61,000; 3 L/h tank: 42,000 ± 71,000). Thus, the mean eDNA shedding in samples from the same animal under constant condition can range between 0 and hundreds of thousands of copies, and the authors conclude: **"**We found that quantification of eDNA samples can be highly variable even when sampling from the same individual under controlled conditions". Interestingly they continue stating: "Nevertheles**s** our preliminary study showed that similarly sized fish shed eDNA at similar rates under the same conditions, suggesting that we should be able to detect differences in shedding rates caused by different conditions".

We question the validity of the argument by the authors; that there are no differences in shedding rates between individuals, while they are unable to estimate the shedding rates of a single individual. We argue that if you cannot quantify with reasonable accuracy a certain parameter for one individual, then you cannot say anything about differences between individuals. This premise will also invalidate the conclusion of the latter statement. Studying conditional effects on shedding rates require that shedding rates of a single individual can be quantified with reasonable accuracy.

Assuming that it is possible to measure shedding rates of an individual fish in a robust manner, the authors move on to find a correlation between shedding rates and total fish biomass. Such correlations lead to the conclusion: "Our work extends previous studies, by estimating actual eDNA shedding rates relative to fish biomass. Estimating shedding rate per gram of fish may be useful for future modeling of eDNA distributions in natural settings."A fundamental assumption behind this statement is that shedding rates are constant per unit body mass across the range of body mass. There is little evidence in the literature to support this assumption. In contrast, shedding rates relative to fish body mass have been shown to be higher for juvenile than adult fishes (Maruyama et al., 2014). Thus, in practice the authors will not be able to distinguish between measured eDNA concentration generated by a high number of juveniles (with a low total biomass) from that of a low number of sub-adults (with a higher total biomass). For example, the authors quantify the relationship between the absolute shedding rate and body mass on a log–log scale by the linear function: shedding rate = 0.94 × mass + 4.23 (Fig. 2 in Klymus et al., 2015). A shedding rate of 9 log10(copies/h), could hypothetically come from one specimen with a shedding rate of 1,000,000,000 copies/h or two specimens, each with a shedding rate of 500,000,000 copies/h, or 8.7 log10(copies/h). In the first case the shedding rate is related to 119,000 g body mass (10^((9–4.23)/0.94)), in the second case the shedding rate is related to a 56,900 g body mass per specimen (10^((8.7–4.23)/0.94)) or a total biomass of 114,000 g.

Although we commend Klymus et al. (2015) for a notable contribution to our general understanding of eDNA monitoring, we caution against the application of quantitative data in conservation and nature management until the factors discussed above are adequately addressed.

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