Biological Conservation 192 (2015) 481–482

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

Letter to the Editor

A reply to Iversen et al.'s comment "Monitoring of animal abundance by environmental DNA — An increasingly obscure perspective"

We appreciate the conversation put forward by Iversen et al. (2015) in their response to our article "Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*" in the 2015 environmental DNA special issue of Biological Conservation.

We agree with Iversen et al.'s concern about overly optimistic conclusions that could be drawn from the current eDNA literature. One hope for eDNA technology is that it can be used in estimating abundance or population density. Evidence suggests that eDNA measurements correlate with total biomass (Takahara et al., 2012) rather than abundance. We demonstrate a similar relationship between biomass and eDNA shedding rates. Nevertheless, without field testing of these methods and specific survey protocols, we cannot make strong conclusions regarding the technique's field applicability. In our manuscript, we attempted to point out areas in which more research is needed.

Specific to our study, Iversen et al. contend that 1: we cannot make robust estimates of shedding rates, given the variability in our data; and 2: our estimates are weakly correlated with biomass. We do not agree with these conclusions.

1- Our preliminary flow rate study found that similarly sized fish released similar amounts of DNA (copies/h) independent of flow conditions. Because our estimates of eDNA concentration via qPCR are not normally distributed, the standard deviations of untransformed data are sometimes greater than the mean. The large variance and skewed distribution of our data, however, does not mean that we cannot observe an effect of a changing variable.

Replicate eDNA samples can help decrease the effects of large variance and outliers on data. The number of replicates per group (n_j) needed to detect a difference can be quantified through power analysis (Oris and Bailer, 1993). Using the data from the flow rate experiment (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) we can estimate the population mean at 51,300 copies/h and the variance at (58,300 copies/h)^2. Using equation 2 from Oris and Bailer (1993) and setting type I error $\alpha = 0.05$, type II error $\beta = 0.20$, the relative variance of the compared group, $c = 1$, and the difference between groups, $\delta = 50{,}000$ copies/h:

$$n_j = \frac{(z_\alpha + z_\beta)^2(1+c)\sigma_0^2}{\partial^2}$$

$$n_j = \frac{(1.645 + 0.842)^2(1+1)(58,300)^2}{(50,000)^2}$$

$$n_j \cong 17.$$

Thus, we predict that 17 × 50 mL water samples would be required to detect a difference of 50,000 copies/h between treatment groups. Conversely, we can rearrange the power analysis equation to predict that the smallest difference between groups detectable with 95% confidence using 8 × 50 mL water samples per group is 72,000 copies/h. Applying power analysis to the log transformed biomass data, the composite standard deviation is 0.581 log10(copies/h) and the smallest detectable difference between groups with 8 × 50 mL water samples per group is 0.72 log10(copies/h), corresponding to 0.77 log10(g total biomass), or a 5.9-fold difference in total biomass. Variation may be greater in a field setting, in which more case samples will be needed for discriminatory power.

2- Our study shows a strong correlation between biomass and eDNA shedding rates ($R^2 = 0.91$) in controlled experiments. This result agrees with a similar study on common carp (Takahara et al., 2012).In contrast, work in the field with hellbenders has not found a strong correlation (Spear et al., 2015). Future studies addressing system and species-specific eDNA release, movement, and degradation will be vital in testing the applicability of this data to real world situations.

Iversen et al. also disagree with our assumption that shedding rates are constant per unit body mass, based on a previous study demonstrating a difference in shedding rates between adults and younger fish. Our data, however, shows a constant eDNA shedding rate per unit body mass for juvenile and subadult fish. The effects of developmental stage on eDNA shedding rate should be addressed by future research and likely differ among species.

Furthermore, Iverson et al. state that we cannot distinguish between the number of fish based on shedding rate. This is correct, as shedding rate correlates with total biomass and not abundance. Regardless, biomass may be useful in estimating abundance in the field. For instance, Spear et al. (2015) suggest that the binning of eDNA quantification estimates as a way to characterize relative abundance.

Finally, it should be noted that imprecision in abundance measurements is also observed in traditional fisheries methods. However, we should not abandon these methods, or cease to make improvements on their implementation. We feel the same way for eDNA methods. Although there remains much to learn about eDNA detection and quantification, we believe that eDNA technology has great promise for fisheries research and management. A better understanding of the capabilities and limitations of eDNA technology will be necessary to productively apply this technique in the field.

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http://dx.doi.org/10.1016/j.biocon.2015.09.025

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