MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 2565–2573 doi: 10.1111/j.1365-294X.2011.05418.x

FROM THE COVER

Monitoring endangered freshwater biodiversity using environmental DNA

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Abstract

Freshwater ecosystems are among the most endangered habitats on Earth, with thousands of animal species known to be threatened or already extinct. Reliable monitoring of threatened organisms is crucial for data-driven conservation actions but remains a challenge owing to nonstandardized methods that depend on practical and taxonomic expertise, which is rapidly declining. Here, we show that a diversity of rare and threatened freshwater animals—representing amphibians, fish, mammals, insects and crustaceans—can be detected and quantified based on DNA obtained directly from small water samples of lakes, ponds and streams. We successfully validate our findings in a controlled mesocosm experiment and show that DNA becomes undetectable within 2 weeks after removal of animals, indicating that DNA traces are near contemporary with presence of the species. We further demonstrate that entire faunas of amphibians and fish can be detected by high-throughput sequencing of DNA extracted from pond water. Our findings underpin the ubiquitous nature of DNA traces in the environment and establish environmental DNA as a tool for monitoring rare and threatened species across a wide range of taxonomic groups.

Keywords: biological diversity, molecular detection, pyrosequencing, threatened species, wildlife conservation

Received 19 August 2011; revision received 8 November 2011; accepted 17 November 2011

Introduction

Monitoring of plant and animal biodiversity is conventionally based on visual detection and counting. Such data collection is nonstandardized and dependent on practical and taxonomic expertise, which is rapidly declining (Hopkins & Freckleton 2002; Wheeler *et al.* 2004). Freshwater ecosystems are among the Earth's most threatened habitats in terms of anthropogenic impact as well as global and local species loss (Revenga & Mock 2000; Sala *et al.* 2000; Dudgeon *et al.* 2006; Vié *et al.* 2009; Hambler *et al.* 2011). Worldwide, more than 4600 freshwater animal species are threatened or recently extinct—representing more than a quarter of all freshwater animals assessed so far (IUCNredlist 2011).

DNA obtained directly from environmental samples (environmental DNA) as a method to assess the diversity of macro-organism communities was first applied to ancient sediments, revealing the past of extinct and extant mammals, birds and plants (Willerslev *et al.* 2003). Subsequently, the approach has been successfully used on several different modern and ancient environmental samples including terrestrial sediments, lake and ice cores, and freshwater lakes and rivers (Hofreiter *et al.* 2003; Haile *et al.* 2007, 2009; Willerslev *et al.* 2007; Ficetola *et al.* 2008; Matisoo-Smith *et al.* 2008; Jerde *et al.* 2011). Faeces, urine and epidermal cells are believed to be the predominant sources of environmen-

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tal DNA (Lydolph *et al.* 2005; Haile *et al.* 2009), which may survive from hours to thousands of years depending on the environmental setting (Willerslev *et al.* 2004). Although of great potential for contemporary biodiversity monitoring, environmental DNA detection in wild populations has so far only been applied to a few common or invasive species of amphibians and fish (Ficetola *et al.* 2008; Goldberg *et al.* 2011; Jerde *et al.* 2011). The potential for monitoring rare and threatened species of direct interest in a conservation context remains unreported. It also remains untested i) whether environmental DNA concentrations reflect species abundance in natural freshwater systems and ii) whether the approach is broadly applicable across taxonomic groups. Answers to these questions are crucial for the relevance and reliability of environmental DNA detection to applied conservation biology.

To address the potential of environmental DNA as a tool for monitoring rare and threatened freshwater species, we conducted comparative surveys in natural lakes, ponds, streams and temporary pools in Europe. We used conventional monitoring methods in parallel with environmental mitochondrial DNA-based species detection and quantification, by applying quantitative PCR (qPCR) to DNA extracted from water samples. We specifically surveyed six animal species representing different taxonomic groups: the amphibians common spadefoot toad (*Pelobates fuscus*) and great crested newt (*Triturus cristatus*), the fish European weather loach (*Misgurnus fossilis*), the mammal Eurasian otter (*Lutra lutra*), the dragonfly large white-faced darter (*Leucorrhinia pectoralis*) and the crustacean tadpole shrimp (*Lepidurus apus*). All species are locally rare and occur in low abundance in their natural environments (Helsdingen *et al.* 1996; Conroy & Chanin 2000; Edgar & Bird 2006; Eggert *et al.* 2006; Brendonck *et al.* 2008; Hartvich *et al.* 2010). All except the tadpole shrimp are listed in the EU Habitat Directive (Council of the European Union 1992) as requiring strict protection in their natural habitats and substantial monitoring efforts in the EU. We further examined our findings from wild populations in a controlled mesocosm experiment and explored the potential of DNA detection by 454 pyrosequencing of PCR amplicons from environmental water samples targeting entire faunas of fish and amphibians.

Materials and methods

Field sampling and surveys

3×15 mL water samples were collected in 98 natural ponds, lakes and streams in Europe between 2009 and 2011, following Ficetola *et al.* 2008 (Fig. 1 and Table S1, Supporting information). The three samples from each site were taken to improve coverage of the extent of the freshwater systems and species detection probability (Fig. S1, Supporting information). All samples were stored at −20°C until processed. A proxy for population density was calculated for the amphibians *Pelobates fuscus* and *Triturus cristatus* using conventional monitoring (based on active dip-netting and counting larvae one person-hour pr. pond) and assuming a reverse cone shape for the estimation of pond water volume using direct measures. Qualitative occurrence data were supplied by taxon specialists based on fresh tracks or scat for the Eurasian otter (*Lutra lutra*), electrofishing with active dip-netting for the European weather loach (*Misgurnus fossilis*) and active dip-netting for the large white-faced darter (*Leucorrhinia pectoralis*) larvae and the tadpole shrimp (*Lepidurus apus*).

Mesocosm experiment

Outdoor experiments in aquatic mesocosms were set up (at the Natural History Museum of Denmark) in a full factorial design for the two amphibian species at larval densities 0, 1, 2 or 4 specimens pr. 80 L. Two weeks prior to the introduction of experimental animals, all containers were filled with tap water and inoculated with identical quantities of water plants, nonpredatory invertebrates, filamentous algae, phytoplankton and zooplankton to simulate natural biotic complexity. Newt larvae and tadpoles were fed *ad libitum* with zooplankton and algal pellets (Tetra GmBH Plecomin) during the experiment. Water samples of 15 mL were taken before introduction of animals and after 2, 9, 23, 44 and 64 days. Hereafter, animals were removed and samples were taken after 2, 9, 15 and 48 days (Table S2, Supporting information).

DNA extraction, PCR and sequencing

DNA extraction and post-PCR work were performed in two separate laboratories assigned for these purposes. All water samples were centrifuged (35 min, 6°C, 5000 ***g***), and DNA from the pellet was extracted using Qiagen DNeasy Blood & Tissue kit (spin-column protocol). Extraction blanks were included for all DNA extractions and were tested negative in subsequent PCRs.

TaqMan qPCRs were performed on a Stratagene Mx3000P using 3 μL of template DNA, 15 μL of TaqMan® Environmental Master Mix 2.0 (Life Technologies), 4 μL of ddH_2O, 1 μL of each primer (10 μM) and 1 μL of probe (2.5 μM) under thermal cycling 50°C for 5 min and 95°C for 10 min, followed by 55 cycles of 95°C for 30 s. and 50–60°C for 1 min. Species-specific primers and minor groove binding probes targeting

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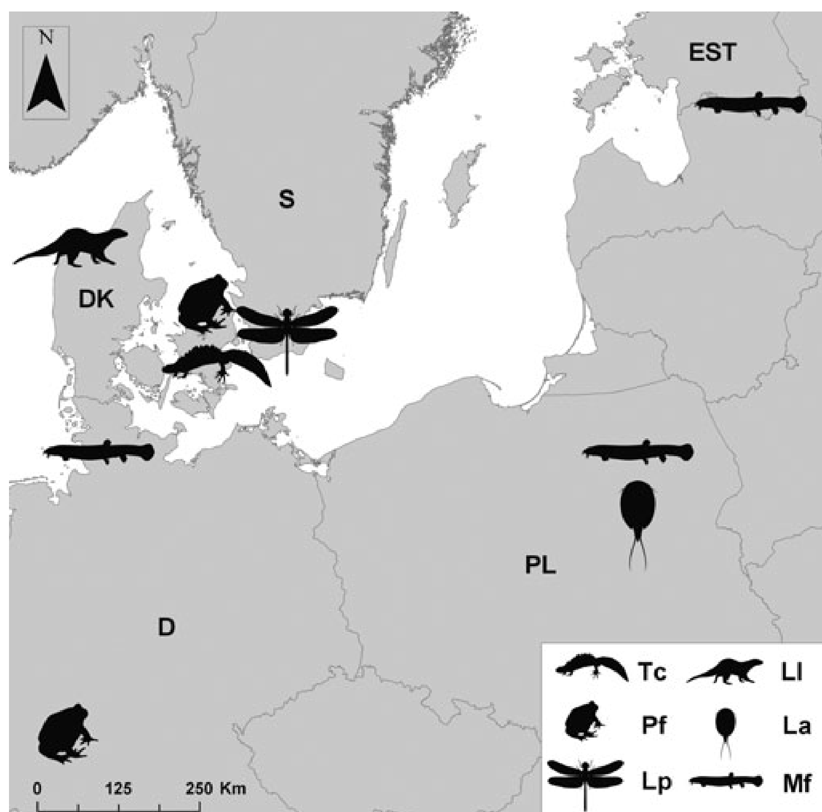

Fig. 1 Sampling locations of the 90 European natural freshwater systems targeted in this study. Samples were taken in Denmark (DK), Sweden (S), Germany (D), Poland (PL) and Estonia (EST) and covers Tc (*Triturus cristatus*, 11 ponds), Pf (*Pelobates fuscus*, 17 ponds), Lp (*Leucorrhinia pectoralis*, 11 ponds), Ll (*Lutra lutra*, 15 streams and lakes), Mf (*Misgurnus fossilis*, 11 ponds and 15 streams) and La (*Lepidurus apus*, 10 temporary pools). An additional six ponds were sampled as controls and two additional ponds were sampled for 454 pyrosequencing (all in Denmark), giving a total of 98 freshwater systems sampled. For exact positions of all 98 localities see Table S1 (Supporting information).

mitochondrial genes (cytochrome oxidase I and cytochrome b) were validated with relevant species occurring in the area. The amphibian primers/probe systems were tested negative for all amphibian species occurring in the sampled area *Pelophylax* kl. *esculentus*, *Rana arvalis*, *R. temporaria*, *R. dalmatina*, *Bufo bufo* and *Lissotriton vulgaris*. The system for the fish *M. fossilis* was tested negative for *Cobitis taenia, Anguilla anguilla*, *Tinca tinca*, *Carassius carassius*, *Rutilus rutilus* and *Cyprinus carpio*; the system for the dragonfly *L. pectoralis* was tested negative for *L. dubia*, *L. ribicunda*, *Anax imperator* and *Cordulia aenea*; the system for the crustacean *L. apus* was tested negative for *Daphnia pulex, C. aenea* and *Dorcus parallelipipedus*; and the system for the mammal *L. lutra* was tested negative for *Mustela vison, Neomys fodiens* and *Homo sapiens*. All primers and probes used and developed in this study are listed in Table S3 (Supporting information). Negative controls were included for all PCRs and showed no amplification.

qPCR standards for the amphibian species were prepared as a dilution series (10^{-5}–10^{-11}) of purified PCR products on tissue-derived DNA with concentration measured on a Nanodrop ND-1000. Three independent qPCR replications were performed for each sample.

For all species, 25–60% of the positive field samples and 20–25% of the positive mesocosm samples were validated as authentic by cloning using Topo TA cloning kit (Invitrogen), followed by purification and sequencing of the inserted PCR fragment (Macrogen, Europe) (Table S4, Supporting information). Final concentrations in DNA molecules/15 mL of water sample were calculated from the standards setting the molecular weight of DNA as 660 g/mol/base pair. Efficiency of all qPCRs with standards was 80–100%.

454 Pyrosequencing

Roche GS FLX 454 sequencing was performed on PCR products pooled from six PCR replicates performed on DNA extracts from each pond. DNA extraction was identical to the rest of the study. However, 3×15 mL of water samples were used for the fish community and a pooled DNA extraction of 20×15 mL subsampled from a 1.5-L water sample for the amphibian communi-

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ties. Conventional PCRs were performed using 5 μL of DNA, 25 μL of TaqMan® Environmental Master Mix 2.0 (Life Technologies), 16 μL of ddH_2O and 2 μL of each primer (10 μM) under thermal cycling conditions: 95°C for 10 min followed by 45 cycles of 94°C for 30 s, 45–48°C for 30 s, 72°C for 30 s with a final 72°C for 5 min. For primer details see Table S3 (Supporting information). PCR products were tested on 2% agarose gels stained with ethidium bromide and purified using a Qiagen QIAquick PCR purification kit or QIAquick Gel extraction kit. Library builds were carried out using custom Y-shaped adaptors with MID barcode identifiers, and all reactions were performed according to protocol using NEBnext DNA Sample Prep Master Mix Set 2 (New England Biolabs, Ipswich, MA). Sequencing was carried out in accordance with manufacturer's guidelines. A total of 524 027 sequences were generated on three-quarters of an XLR70 PTP (Roche, Basel, Switzerland). GS FLX light intensity files were sorted per combination of primer and MID in separate files and trimmed accordingly before being used as input for AmpliconNoise and Perseus to remove sequencing errors and PCR chimeras (Quince *et al.* 2011). Given the length of the amplicons, the original procedure that keeps only reads where the first noisy flow occurred on or after 360 was relaxed to flow number 100. Parameters σ_p and c_p were set at the values 1/60 and 0.01, respectively. Data were analysed using a custom-made Perl script (available on request) and compared to the nt database using BLAST with 7 as word size and 0.001 as a maximal expect value and only considering sequences with 100% identity in full sequence length.

Statistical Modelling

To describe DNA concentration in water through time, a differential equation model was constructed assuming (i) DNA is generated at constant rate (i.e. secreted from the animal) but depends on the size of the animal(s), here taken to be linear over time $\alpha{\cdot}t + \beta$ in the interval of observation, and (ii) DNA degradation occurs at a constant rate (Fig. S2, Supporting information). Here, $\gamma{\cdot}x(t)$, where $x(t)$ is the amount of DNA present at time t. The unit of the parameter γ is per molecule per day.

This leads to an equation for the concentration of DNA present at time t:

$$\frac{dx}{dt} = \alpha \cdot t + \beta - \gamma \cdot x(t) \text{and}\, x(0) = 0 \qquad \text{(eqn 1)}$$

It has the following solution $x(t)$:

$$x(t) = \frac{\alpha}{\gamma}t + \frac{1}{\gamma}\left(\frac{\alpha}{\gamma} - \beta\right)\exp(-\gamma \cdot t) - \frac{1}{\gamma}\left(\frac{\alpha}{\gamma} - \beta\right) \qquad \text{(eqn 2)}$$

When the animals have been removed from the containers, only DNA degradation occurs:

$$x(t) = x(t_R)\exp(-\gamma(t - t_R)) \qquad \text{(eqn 3)}$$

where t_R is the time of removal. Hence, there are three parameters, α, β and γ, to be estimated from the data (Fig. S3, Supporting information). TC4.2 at $t = 73$ days was omitted in parameter estimation as it was not possible to replicate in qPCR.

The observations y_{ij} are assumed to be independent of each other. Here, $j = 1,\ldots, n(t_i)$ denotes the jth sample obtained at time t_i. The parameters are estimated using ordinary least square, weighted according to the number of observations available from each container and time point. Confidence intervals on parameter estimates are obtained from the likelihood curve assuming data are Gaussian distributed. The explained variance is calculated as follows:

$$r^2 = 1 - \frac{\frac{1}{n-3}\sum_{j,i}(y_{ij} - x(t_i))^2}{\frac{1}{n-1}\sum_{i,j}(y_{ij} - \bar{y})^2} \qquad \text{(eqn 4)}$$

where n is the total number of observations and $\bar{y}$ the mean of all observations.

We used a linear mixed model to describe the relationship between DNA concentration, and time and density, respectively. Time and density were set as fixed effects, while individual containers were set as random effect. Two separate models (one with interaction between the factors time and density and one without interaction) were compared by a likelihood ratio test. Data were log10-transformed for Pearson's product–moment correlation to meet the assumption of normality (Fig. S4, Supporting information). All statistics were performed using R version 2.13.1.

Results and discussion

The success rate of the DNA-based species detection by qPCR in ponds with known occurrence of the targeted species was 100% for the fish, 91–100% for the amphibian species, 82% for the dragonfly and 100% for the tadpole shrimp (Fig. 2). Using the same strategy, negative results were recovered for each of the six species from three control ponds where the respective species are known to be absent. Interestingly, for an additional eight sampled ponds with recent historical records of *P. fuscus*, the species was not found during conventional surveys. However, using the DNA detection approach, the presence of the species was confirmed in

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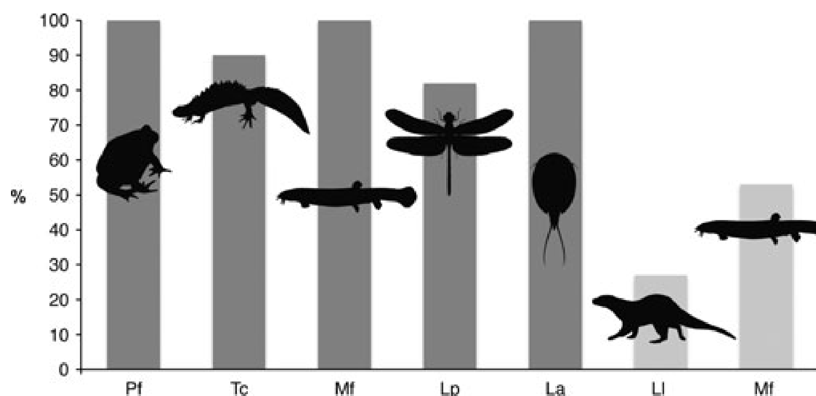

Fig. 2 Environmental DNA detection rates by qPCR in natural freshwater ponds with 100% occurrence of the species confirmed in the field (dark grey) or larger freshwater systems with known occurrence in the area (light grey). Detection rates are given in percentage positive localities out of the total number of localities surveyed for each species. Data covers amphibians: Pf (*Pelobates fuscus*, $n = 9$) and Tc (*Triturus cristatus*, $n = 11$); fish: Mf (*Misgurnus fossilis*, $n = 11$ ponds and $n = 15$ streams—light grey); insects: Lp (*Leucorrhinia pectoralis*, $n = 11$); crustaceans: La (*Lepidurus apus*, $n = 10$) and mammals: Ll (*Lutra lutra*, $n = 15$ streams and lakes).

five of these sites, suggesting that the DNA approach may in some cases be more sensitive. Supporting this view, the respective five sites had lower average DNA concentration than the sites where the presence of *P. fuscus* was confirmed by expert surveys ($P < 0.05$, Mann–Whitney *U* test). For the amphibians, where environmental DNA was quantified, we find positive correlation between DNA concentration and estimated population density based on conventional monitoring (*P. fuscus*: $P < 0.01$, $R^2 = 0.68$; *T. cristatus*: $P < 0.05$; $R^2 = 0.40$, Pearson's product–moment correlation) (Fig. 3).

To examine the performance of environmental DNA detection in running water, the fish *M. fossilis* was furthermore targeted in independent water samples taken throughout a continuous 225 km^2 ditch system of running water that is known to be inhabited by the species. The 54% success rate obtained (Fig. 2) was comparable to the results of a conventional expert survey in the area. Considering water volume per individual and water retention time, the difference between detection probability in running and stagnant water systems is expected. Similarly, we tested the performance of environmental DNA detection in large water volumes using streams and lakes inhabited by the Eurasian otter and confirmed presence of species-specific DNA in 27% of the sampled sites (Fig. 2). The semiaquatic lifestyle and large territorial range of this mammal can account for the low detectability compared to the other investigated organisms. Nevertheless, for Eurasian otter, the

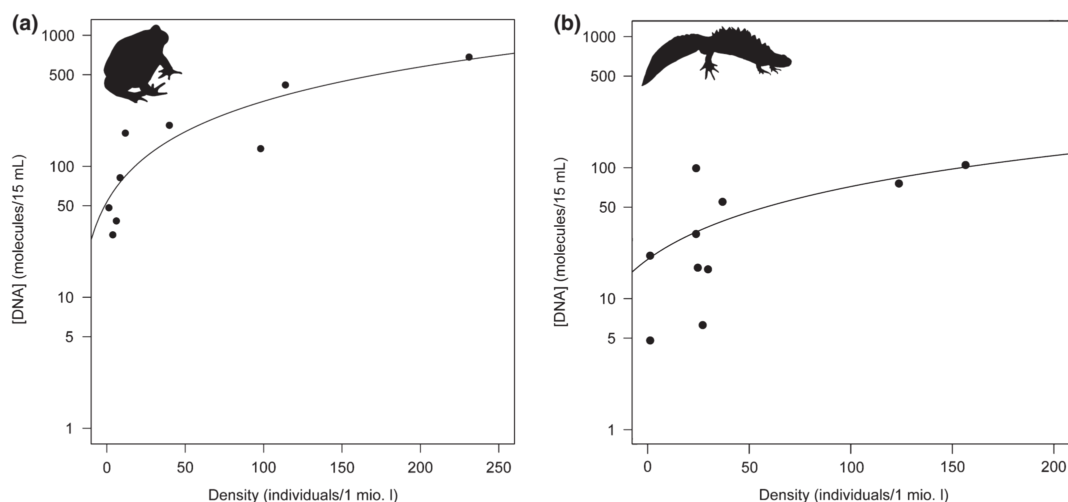

Fig. 3 Environmental DNA quantification in natural ponds with *Pelobates fuscus* ($n = 9$) (a) and *Triturus cristatus* ($n = 10$) (b). Pearson's product moment correlation between average number of DNA molecules and estimated population size in each pond. The line shows linear regression, a: $R^2 = 0.68$, $P < 0.01$; b: $R^2 = 0.40$, $P < 0.05$.

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environmental DNA approach may still be a valuable complement to conventional monitoring (based on the identification of tracks and faecal remains), which is both resource demanding and error prone (Hansen & Jacobsen 1999; Davison *et al.* 2002).

While our population density estimates based on conventional monitoring methods are robust and comparable relative to each other, they serve only as proxies for true population densities. We therefore investigated the consistency of the observed quantitative trend in the relationship between DNA concentration and population density of the two amphibian species under semi-natural conditions, allowing control of absolute animal density through time. We quantified DNA concentrations by repeated water sampling from freshwater mesocosms with densities of 0, 1, 2 or 4 larvae in 80 L of water, respectively. We sampled at 2, 9, 23, 44 and 64 days after introduction of animals to freshwater containers. All animals were removed from the containers after 64 days when metamorphosis initiated, and DNA concentration was quantified after additional 2, 9, 15 and 48 days to investigate DNA persistence (Table S2, Supporting information).

For both species, we observe a highly significant effect of animal density and time on DNA concentration quantified from the freshwater mesocosms as well as an interaction of the two factors (*P. fuscus*, $P < 0.001$; *T. cristatus*, $P < 0.001$; linear mixed model). This confirms our field observations in an experimental setting. Interestingly, DNA concentrations were consistently higher for *P. fuscus* than for *T. cristatus* in both the controlled experiment and the field survey (Figs 3 and 4), likely due to the fact that the herbivorous tadpole is substantially larger and more active than the carnivorous newt larvae. Immediately after the animals were removed, we observed a rapid and continuous decrease in DNA concentration, until it could no longer be detected only 1–2 weeks after removal (Fig. 4). These results suggest that DNA traces are near contemporary with the presence of the species, in agreement with previous studies observing rapid degradation of DNA in freshwater (Kim *et al.* 1996; Matsui *et al.* 2001; England *et al.* 2005; Douville *et al.* 2007; Dejean *et al.* 2011).

We speculate that the ability to detect and quantify DNA from a given freshwater animal species is determined as a simple relationship between DNA excretion depending on animal density and size, and degradation of this DNA owing to both microbial/enzymatic attack and spontaneous chemical reactions such as hydrolysis and oxidation (Lindahl 1993). Based on this general assumption, we integrated the observed DNA degradation in the examination of the quantitative relation between animal density and DNA concentration in a simple differential equation. This model was constructed assuming that DNA is generated at a rate dependent on the animal density and growth and degraded by a constant rate. We find that the model parameters estimated from the data are in concordance with each other across both species showing constant degradation and increasing excretion of DNA with increased density of animals and animal growth (Fig. S3, Supporting information).

The observed trends in both the field and controlled experiments support the conclusion that, despite rapid

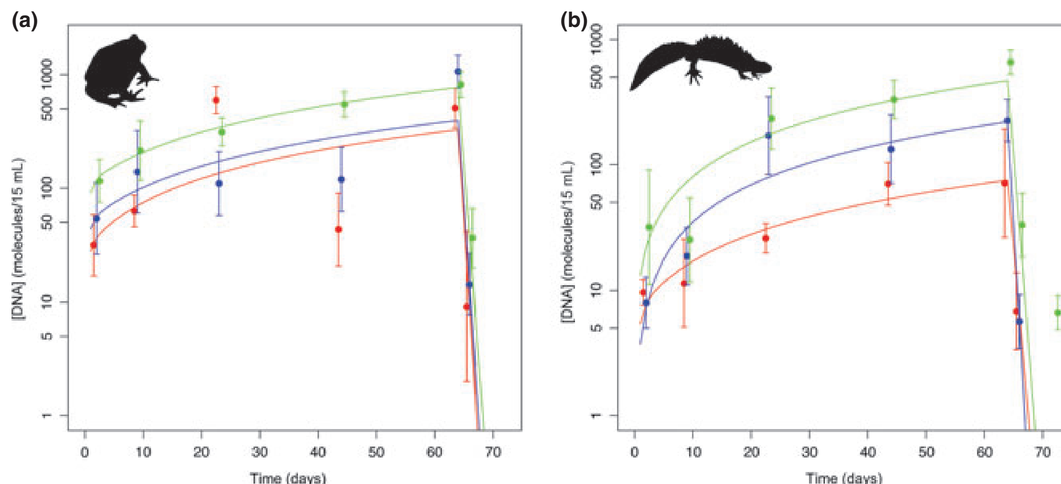

Fig. 4 Environmental DNA quantification in controlled mesocosm experiment with *Pelobates fuscus* (a) and *Triturus cristatus* (b). Means + 2 × SE of DNA molecules in water samples from freshwater containers with 1 (red), 2 (blue) or 4 (green) individuals in 80 L. After a control sample was taken, animals were introduced at time $t = 0$ and samples were taken at 2, 9, 23, 44, 64, 66, 73, 79 and 112 days. Animals were removed at $t = 64$ (after sampling). The lines show a differential equation model fitted to the data (see Materials and methods section), a: $R^2 = 0.29$ (red), 0.50(blue), 0.61(green); b: $R^2 = 0.49$ (red), 0.67 (blue), 0.62 (green).

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DNA degradation processes, there is a consistent quantitative relation between the density of animals and DNA molecules, which can be measured and accounted for through time (Fig. 4). Overall, these findings constitute promising evidence that DNA may be not only applied as an efficient tool to detect species in the environment but also used to estimate population densities. However, this will necessitate rigorous species-specific comparative studies to fine-tune model parameters and further validate the approach in natural freshwater environments. Moreover, the effect of factors such as temperature, pH, conductivity and microbial community composition should be further investigated as these are likely to influence DNA decay and detectability. Also, the exact cellular origin of environmental DNA in freshwater and the relative contribution of different states (e.g. free, cellular or particle-bound DNA) remain unclear, and clarification of this may focus future sampling strategies. Precipitation, as used in this study, recovers DNA independent of state but is limited to small sample volumes compared to filtering methods (e.g. Jerde *et al.* 2011), which accommodate larger samples but may fail to recover free DNA.

Finally, to explore the broad-scale potential of environmental DNA-based species detection, we investigated the extent to which complete species diversity can be documented by environmental DNA screening. We used water samples from four ponds with well-known amphibian or fish faunas (updated occurrence data from the Danish freshwater fish atlas project and Amphi-Consult Aps national amphibian monitoring data) and targeted DNA from these groups with a combination of specific and generic primers (Table S3, Supporting information). PCR products were sequenced using the Roche GS FLX 454 platform and Sanger sequencing, generating a total of 524 027 sequences.

We recovered species-specific DNA fragments with 100% sequence match for all species of amphibians or fish previously recorded from each of the ponds (Table 1 and Table S4, Supporting information). Interestingly, we furthermore recovered DNA sequences from species living in close proximity to the water, including birds: Eurasian coot (*Fulica atra*), wood pigeon (*Columba palumbus*) and marsh warbler (*Acrocephalus palustris*) and red deer (*Cervus elaphus*). These results suggest that the success of DNA detection is largely independent of animal species and abundance, as long as DNA is excreted into the water. Furthermore, this illustrates that DNA is homogeneously distributed in pond water, in stark contrast to recent observations of animal DNA in soil, characterized by a patchy distribution (Andersen *et al.* 2011). The ability to exhaustively recover all species in the investigated faunas of fish and amphibians probably relies on the design of generic primers specifically targeting the taxonomic groups in question. It is inherent to the use of generic primers that there is a trade-off between targeting higher taxonomic levels and detecting rare sequences.

Table 1 Species of amphibians and fish detected by species specific DNA in pond water samples. In each of the four ponds DNA fragments with 100% sequence match were recovered from all species known to occur, respectively. Sequences were obtained through Roche 454 GS FLX sequencing using generic primers except P. fuscus, T. tinca, P. fluviatilis and L. delineatus, which were recovered through PCR using species specific primers with subsequent cloning and Sanger sequencing. For the former three because the applied generic primers do not amplify tissue derived DNA of these species. JD11 (N55.79799, E12.58399), HEL56 (N55.98929, E12.20933), ELL1 (N55.842498, E12.534903), BOT1 (N55.68651, E12.57432) (Datum: WGS84)

	Species	Pond
Amphibians	*Lissotriton vulgaris*	JD11, HEL56
	Triturus cristatus	JD11
	Pelophylax kl. *esculentus*	JD11, HEL56
	Rana temporaria	HEL56
	Rana arvalis	HEL56
	Pelobates fuscus	HEL56
Fish	*Carassius carassius*	ELL1, BOT1
	Carassius auratus	ELL1, BOT1
	Cyprinus carpio	ELL1, BOT1
	Scardinius erythrophthalmus	ELL1, BOT1
	Tinca tinca	ELL1, BOT1
	Leucaspius delineatus	ELL1
	Perca fluviatilis	BOT1

Conclusion

Faced with a global decline in biodiversity that is 100–1000 times faster than prehuman rates (Pimm *et al.* 1995; Barnosky *et al.* 2011), there is an urgent need for data-driven prioritization of conservation actions, which relies heavily on fast and effective monitoring of threatened species. Environmental DNA monitoring cannot replace field observations by experienced ecologists and taxon specialists, who retrieve information beyond quantitative and qualitative records. However, monitoring of threatened species through environmental DNA may be a quick, cost-effective and standardized way to obtain basic data on distribution and abundance, enabling efficient deployment of limited conservation resources and taxonomic expertise. Further research on environmental DNA in relation to conservation of rare and threatened species should focus on large-scale comparative validation and optimization including additional organismal groups and applying the approach beyond freshwater ecosystems. However, our findings highlight a vast potential for integrating DNA detection

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in the tool set of biodiversity field research and conservation. With DNA sequencing technology advancing at rapidly dropping costs (Metzker 2009; Anonymous 2010), environmental DNA research is set to change from being merely a scientific curiosity to become an important tool in applied field biology.

Acknowledgements

We thank Dr. P.R. Møller (Natural History Museum of Denmark), A. Drews (LLUR, Schleswig-Holstein), A. Linnet (Danish Nature Agency, Thy) and Martin Hesselsøe (Amphi-Consult Aps) for generously supplying monitoring data. All work involving live animals was conducted under permit number SNS-441-00116 of the Danish Nature Agency. This study was supported by the University of Copenhagen, The Natural History Museum of Denmark and the Danish National Research Foundation.

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P.F.T, J.K (Centre for GeoGenetics, Copenhagen University) and L.L.I (Freshwater Biology Section, Copenhagen University) are developing methods for monitoring biodiversity using environmental DNA. C.W (Bioinformatics Research Center, Aarhus University) is working with statistical modeling of biological systems. M.R, M.T.P.G, L.O and E.W (Centre for Goe-Genetics, Copenhagen University) are developing methods for analyzing ancient and fragmented DNA from various environmental samples.

Data accessibility

Sequences from pyrosequencing are uploaded to NCBI SRA: ERP000988.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Overview of the results of field experiments.

Table S2 Overview of the results of controlled mesocosm experiments.

Table S3 Primers and probes designed and used in this study.

Table S4 Summary of DNA sequences recovered in this study.

Fig. S1 Accumulated probabilities of detecting the targeted species in the field studies when taking 1, 2 or 3 samples.

Fig. S2 Model fit on the DNA concentration in individual containers of the mesocosm experiment through time.

Fig. S3 Modeled parameter estimates.

Fig. S4 QQ-plot for mesocosm experiments.

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