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Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions

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Abstract

Environmental DNA (eDNA) promises to ease noninvasive quantification of fish biomass or abundance, but its integration within conservation and fisheries management is currently limited by a lack of understanding of the influence of eDNA collection method and environmental conditions on eDNA concentrations in water samples. Water temperature is known to influence the metabolism of fish and consequently could strongly affect eDNA release rate. As water temperature varies in temperate regions (both seasonally and geographically), the unknown effect of water temperature on eDNA concentrations poses practical limitations on quantifying fish populations using eDNA from water samples. This study aimed to clarify how water temperature and the eDNA capture method alter the relationships between eDNA concentration and fish abundance/biomass. Water samples (1 L) were collected from 30 aquaria including triplicate of 0, 5, 10, 15 and 20 Brook Charr specimens at two different temperatures (7 °C and 14 °C). Water samples were filtered with five different types of filters. The eDNA concentration obtained by quantitative PCR (qPCR) varied significantly with fish abundance and biomass and types of filters (mixed-design ANOVA, $P < 0.001$). Results also show that fish released more eDNA in warm water than in cold water and that eDNA concentration better reflects fish abundance/biomass at high temperature. From a technical standpoint, higher levels of eDNA were captured with glass fibre (GF) filters than with mixed cellulose ester (MCE) filters and support the importance of adequate filters to quantify fish abundance based on the eDNA method. This study supports the importance of including water temperature in fish abundance/biomass prediction models based on eDNA.

Keywords: conservation genetics, fish, qPCR, salmonid, species detection, water sampling

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Introduction

Environmental DNA (eDNA) refers to DNA noninvasively extracted from environmental samples (e.g. soil, water, air). The genetic traces of eukaryote organisms may be composed of free eDNA and/or DNA within cell or organelles derived from skin, urine, faeces, mucus or extracellular DNA resulting from cell death (Taberlet *et al.* 2012). In addition to collecting data without capturing organisms, recent studies have shown that tracing eDNA instead of using classical sampling method may increase the power of species detection and the spatial coverage and frequency of aquatic wildlife data sampling, leading to an increasing interest of integrating eDNA within aquatic conservation and fisheries management strategies (Lodge *et al.* 2012; Bohmann *et al.* 2014). For example, eDNA collection can successfully monitor the presence/absence of rare, endangered, indicator and invasive species, assess biodiversity and determine species historical patterns of distribution, population dynamics, ecosystem health and trophic interactions (Ficetola *et al.* 2008; Jerde *et al.* 2011; Mahon *et al.* 2013; Díaz-Ferguson & Moyer 2014; Mächler *et al.* 2014; Piaggio *et al.* 2014; Rees *et al.* 2014).

Relationships between eDNA concentration and species abundance/biomass have previously been reported (Takahara *et al.* 2012; Thomsen *et al.* 2012b; Pilliod *et al.* 2013a; Kelly *et al.* 2014; Klymus *et al.* 2015). Recent studies from natural fish populations showed also that eDNA concentration in water samples may provide similar fish abundance index of invasive capture methods used in fisheries management, but the latter was limited to similar environmental conditions among sampled bodies of

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water (Lacoursière-Roussel *et al.* 2016). However, inconsistency among studies and large eDNA variance between samples are observed in the overall eDNA studies, raising an ongoing debate about the ability of predicting fish abundance/biomass based on the eDNA concentration (Iversen *et al.* 2015). Estimating fish abundance from eDNA would greatly reduce the costs associated with data collection and avoid negative consequences on studied organisms (Lodge *et al.* 2012; Taberlet *et al.* 2012). However, limited knowledge about how environmental factors alter eDNA concentration currently restraints our ability to predict population size based on eDNA concentration, which contributes to delay the efficient integration of this new molecular tool for management and conservation purposes.

eDNA concentration in water samples reflects the rate of eDNA release and degradation, both of which may strongly be altered by environmental conditions. High temperatures significantly alter DNA degradation by denaturing DNA molecules and increasing enzyme kinetics and microbial metabolism (Dejean *et al.* 2011; Barnes *et al.* 2014). In parallel, water temperature may have a major influence on metabolism, growth, physiology and immune function in fish (Engelsma *et al.* 2003; Person-Le Ruyet *et al.* 2004; Takahara *et al.* 2011), which may increase the excretion of mucus and shedding of epithelial cells of aquatic species (Jobling 1994). However, little is known about temperature's effect on the amount of eDNA released. The lack of understanding of how water temperature affects eDNA concentration limits our ability to predict fish abundance based on models built on similar environmental conditions (Lacoursière-Roussel *et al.* 2016). In one of the few studies addressing this problem, Takahara *et al.* (2012) found a significant positive relationship between eDNA and water temperature in their field surveys. In parallel, they did not find significant differences in eDNA concentrations originating from common carp (*Cyprinus carpio*) among water temperatures (7 °C, 15 °C and 25 °C) in aquaria and the authors concluded that the significant effect of temperature was likely due to the fish clustering at locations with higher water temperature. However, the pronounced variation in eDNA concentration among replicate aquaria could have altered the conclusions about their *in situ* experiment.

In temperate regions, seasonal, longitudinal and latitudinal variation may cause a large range of water temperature among lakes, as well as temporal variation within lakes. Knowledge about the effects of water temperature on eDNA concentration is thus needed to improve the predictive power of eDNA-based, fish abundance models in a wide range of natural habitats. More specifically, clarifying the rate of eDNA released at different temperatures may help elucidate the nature of the eDNA captured (i.e. excretion material released) and how eDNA concentration varies among species and developmental stages. The nature of the secretion captured, including the physiological source, the state (i.e. intra- or extracellular) and fate (e.g. suspension time) of aqueous macrobial eDNA, may influence eDNA quantification (Turner *et al.* 2014a,b, 2015; Deiner *et al.* 2015).

From a technical standpoint, the method used to capture eDNA may also have impact on the ability to reliably quantify the amount of eDNA in water samples. Mitochondrial DNA is typically targeted because there are a greater number of copies compared to nuclear DNA, its effectiveness in identifying organisms to the species level by means of DNA barcoding (Hebert *et al.* 2003), including in fish (Hubert *et al.* 2008; April *et al.* 2011) and its accessibility via universal sequence databases on public servers (e.g. GenBank and BOLDSYSTEMS). The amplified mitochondrial eDNA may originate from extracellular DNA fragments, mitochondria, cells, excretions or eggs, and the amount of eDNA quantified is likely to vary as a function of the genetic matter being targeted. Determining which eDNA capture methods provide the most accurate estimation of fish abundance is thus crucial towards developing better predictive population size models.

Two methods to capture eDNA are currently used for water samples: filtration (Jerde *et al.* 2011; Minamoto *et al.* 2012; Takahara *et al.* 2012; Wilcox *et al.* 2013; Turner *et al.* 2014b; Deiner *et al.* 2015) and precipitation (Valiere & Taberlet 2000; Ficetola *et al.* 2008; Dejean *et al.* 2011; Thomsen *et al.* 2012a,b; Collins *et al.* 2013). The precipitation method is a nonsize selective method to capture eDNA usually applied to smaller volumes of water (i.e. 15 mL vs. litres of water). With regard to filter pore size, Eichmiller *et al.* (2015) suggest that eDNA retention is greater with smaller pore size filters. Turner *et al.* (2014a) used filters sequentially through decreasing pore sizes, and the sum of eDNA material captured was greater at the smallest pore size and decreases with an increase in pore size. They showed that genetic traces are detected in water samples when capturing eDNA with pore size ranging from 0.02 to 180 μm. In addition to size, the material of the filter may play a role in capturing eDNA from water samples (Eichmiller *et al.* 2015). Moreover, the efficacy of the extraction method may vary among types of filter, and it is also unclear whether the eDNA capture is additive through the filtration process. As the type of filter may influence studies comparison and alter conclusions of studies, guidelines on how to address this variance are needed.

With a general goal of improving our understanding of the relationship between eDNA concentration and fish abundance/biomass, this study uses the economically important salmonid, Brook Charr *Salvelinus fontinalis*, in

order to (i) evaluate how the relationship between eDNA concentration and fish abundance is affected by water temperature and (ii) evaluate the efficacy of different types of filters in assessing this relationship.

Materials and methods

Target species

Brook Charr is a sportfish highly valued for anglers and susceptible to overfishing (Johnston & Post 2009), with a well-known ecology. In Québec in particular, Brook Charr supports the most important freshwater angling fishery involving 500 000 fishermen and 4 000 000 angling days per year (Department of Fisheries and Oceans Canada (DFO) 2012 and unpublished provincial government statistics 2013). Despite such a high fishing pressure, little data exist to determine the current status of Brook Charr populations and their temporal dynamics, and there is no standardized method to compare population data. Brook Charr live in clear and well-oxygenated rivers, lakes and ponds and prefer water temperatures between 11 °C and 13 °C, but the annual range of temperature the species is exposed to spans approximately 0–20 °C (Power 1980). As for other fish species, increased water temperature has been documented to result in higher metabolic rate in Brook Charr (Selong *et al.* 2001; Gale *et al.* 2013).

Experimental design

The relationship between Brook Charr abundance/biomass and eDNA concentration was evaluated at two different temperatures by collecting water from aquaria and then comparing results among eDNA capture methods. Brook Charr fingerlings (age 0+) were obtained from a fish hatchery (Pisciculture de la Jacques-Cartier Inc, Cap-Santé (Qc), Canada). Brook Charr fingerlings were placed in aquaria at two different temperatures whereby 15 aquaria (plastic 20-L containers filled with 15 L of water) were kept at 7 °C and 15 others at 14 °C (Fig. 1). These water temperatures roughly correspond to limits most likely to be encountered by Brook Charr during their growing season corresponding also to the period when eDNA monitoring would most likely be performed for management purposes. For each temperature, 0, 5, 10, 15 and 20 fish were placed in each aquarium with triplicate for each fish abundance (30 aquaria in total). The aquaria without fish (abundance: 0; Fig. 1) were used as negative controls (i.e. three aquaria were used as negative controls for each temperature). The total biomass for each aquarium was measured using the difference in weight between a water bucket with and without fish. Fish were acclimated to the experimental temperature for at least 5 days prior to the onset of the experiment to reduce changes in the rates of various physiological processes associated with stress due to abrupt alteration in temperature (Jobling 1994). Fish were then transferred to aquaria with sterilized instruments and left in aquaria in the dark and without stimulus for 48 h. They were then removed prior to water collection using sterile instruments, and the water has been rapidly collected to limit the effect of eDNA degradation. To ensure stable temperature among aquaria, the 20-L containers were placed in a larger pool with controlled water temperature. All fish were held in accordance with Laval University's Institutional Animal Care (CPAUL; protocol number: 2013033-1).

eDNA collection and capture

For each of the 30 aquaria, five separate 1-L samples of water (i.e. 1 L for each filter; Fig. 1) were collected in sterilized bottles after the water was well mixed using a sterilized stick. The water bottle was kept on ice and in the dark until filtration, which was performed 1–2 h after the water collection. Water samples were filtered using five different types of filters: mixed cellulose ester filters (MCE, a combination of cellulose nitrate and cellulose acetate; Advantec) with a nominal pore size of 0.2 μm or 0.45 μm and glass microfibre filters (GF, borosilicate glass) with a nominal pore size of 0.7 μm (Whatman GF/F), 1.2 μm (Whatman GF/C) or 3 μm (PALL Type A/D). All filters were frozen at −20 °C immediately after DNA filtration. In addition to filtration material composition and pore size, the filter thickness also varied: 133 μm of thickness for the MCE 0.2 filter, 145 μm for the MCE 0.45 filter, 420 μm for the Whatman GF/F filter, 260 μm for the Whatman GF/C filter and 660 μm for the PALL Type A/D filter. Filtration tools were sterilized after each filtration with a 10% bleach solution. Bottles of water were all collected at the same time for each temperature treatment and filtered in a random order by two simultaneous water pump systems.

Extraction and amplification

DNA was extracted using the salt extraction method (Aljanabi & Martinez 1997). A 139-bp fragment of the *cytochrome b* gene (cyt b) was amplified using the BRK2 primers and probes designed by Wilcox *et al.* (2013): forward primer (5′- GCCAAGTAATATAGCTACAAAACCTAATAGATC -3′), reverse primer (5′- GCCAAGTAATATAGCTACAAAACCTAATAGATC -3′), probe (5′- ACTCCGACGCTGACAA -3′). The eDNA concentration was quantified using real-time Taq-Man® PCR with a 7500 Fast

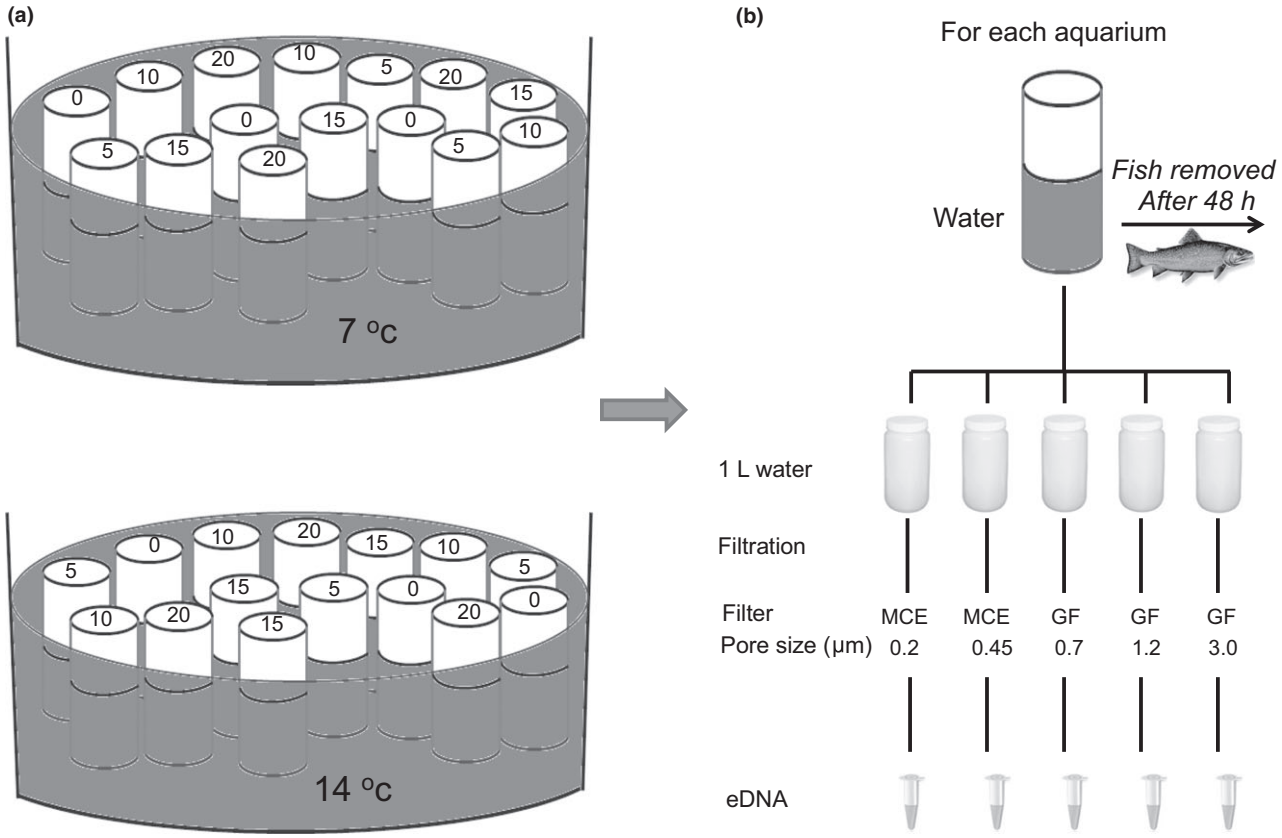

Fig. 1 (a) Experimental design of Brook Charr exposed to 7 °C and 14 °C and (b) eDNA collection procedures. Fish abundance for each water temperature was randomly assigned. eDNA was captured via filtration (MCE, mixed cellulose ester filters; GF, glass fibre).

Real-Time PCR system (Life Technologies). For each sample, the qPCR was performed six times on separate plates (3 replicates per plate). The final reaction volume was 20 μL, including 1.8 μL of each primer (10 μM), 0.5 μL probe (10 μM), 10 μL Environmental Master Mix 2.0 (Life Technologies), 3.9 μL diH_20 and 2 μL DNA. The qPCR amplification was performed under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 70 cycles of 15 s at 95 °C and 60 s at 60 °C. Each qPCR plate included three wells that contained all reagents, but no DNA template, to serve as a negative control (N_{total} = 60 qPCR negative controls). Each qPCR plate also contained a 7-point qPCR standard curve ranging from 4.0–2.6 10^{-4} ng/reaction. The R^2 values for the qPCR standard curve ranged from 0.99 to 1.00, and the efficiency ranged from 89 to 98%. Filtration, extraction, preparation of the qPCR plates and qPCR cycling were all performed in different rooms to avoid potential laboratory cross-contamination. TaqMan® Exogenous Internal Positive Control Reagents (Life Technologies) were also used to detect deviation of the amplification curves potentially caused by inhibitors (Pilliod *et al.* 2013a). The effect of inhibitors on the deviation of the Ct values was evaluated from the comparison of the Ct values between amplifications containing positive exogenous internal control in sterile nuclease-free water (i.e. inhibitor free). A Ct shift of ≥3 cycles beyond the water blank was considered inhibitory (Hartman *et al.* 2005).

Statistical analyses

Mixed-design analysis of variance was performed with *R* 3.0.3 using the function *Anova()* of the CAR library to evaluate the differences in eDNA concentration captured using different types of filters, water temperatures and fish abundance/biomass per tank. The tank replicates were also included as a random variable. Because the normality and homoscedasticity assumptions were not satisfied, differences in eDNA concentration among filters for each fish abundance were assessed by the non-parametric Kruskal–Wallis one-way ANOVA followed by a Tukey's honest significant differences (HSD) test on ranks. To test for significant differences in the average eDNA concentration between 7 °C and 14 °C for each type of filter and fish abundance, Mann–Whitney test was performed. To evaluate the relationship between

abundance/biomass and eDNA concentration, the quantification of the amount of eDNA was obtained by averaging amplification technical replicates (Ellison *et al.* 2006). The relationships between eDNA concentrations and fish abundance or biomass were initially examined in bivariate scatterplots. Linear and exponential regression models were plotted only for significant relationships ($P \leq 0.01$ and $R^2 > 0.40$) when the necessary assumptions (normality and homoscedasticity) were satisfied. The Shapiro–Wilks test was used to verify the normality of distributions of the regression residuals, and the Levene's test was applied for assessing the equality of variances of the residuals. A Cook's distance test was used to detect outliers and influential points to increase the robustness of the relationship between abundance/biomass and eDNA concentration. The latter statistical analyses were all performed using JUMP 9.0.0 (SAS Institute Inc., Cary, NC, USA), and the figures were drawn using SigmaPlot 10.0 (Systat Software Inc., CA, USA). The significance threshold used was $P < 0.05$. To inform about the dose response between fish density and the amount of eDNA released (e.g. activity increasing at high fish density (Guénard *et al.* 2012)), the goodness of fit for models selection was conducted using the corrected Akaike's information criterion (AICc). AICc differences (ΔAICc) were obtained by the AIC of the exponential fits minus the AIC of the linear fits. Models with the smallest AICc values gave the most parsimonious description of the data. In general, ΔAICc >10 suggests strong evidence for a difference between models (Burnham & Anderson 2002). Regressions statistics and AICc values were calculated using R version 3.0.3.

Results

eDNA from Brook Charr was detected in all samples when fish were present. In each control sample ($n = 15$ for each temperature studied), the qPCR reactions (performed six times on separate plates in triplicate) of the negative controls showed no significant eDNA detection (0.009 ng/L and 0.06 ng/L on average for aquaria at 7 °C and 14 °C, respectively), indicating successful limitation of contamination during the fish exposure, collection and filtration of samples. No evidence of significant PCR inhibition was found: ΔCq ranged from 0.03 to 1.31 cycles for all data (SD = 0.24).

For the analyses of fish abundance, the concentration of eDNA varied significantly with fish abundance and filter type, and the effect of the temperature depended on both the types of filters and fish abundance (a significant interaction between temperature, fish abundance and the types of filters; Table 1). Similar to fish abundance, the concentration of eDNA varied significantly with fish biomass and filters, and the effect of the temperature depended also on the types of filters and fish biomass (Table 1).

Effects of types of filters

The different types of filters differed in their efficiency in capturing eDNA. In general, higher levels of eDNA were captured with GF than were captured using MCE filters. At 7 °C, the filter GF of 0.7 μm yielded the highest eDNA concentrations for 10, 15 and 20 fish per tank (Fig. 2). At 14 °C, GF of 1.2 μm yielded the highest eDNA concentration at densities of 5, 10 and 15 fish per tank and GF of 3.0 μm at 20 fish per tank (Fig. 2). Overall, the GF of 1.2-μm filter had the highest eDNA collection efficiency (average of eDNA concentration for all fish densities and both temperatures = 115.28 ng/L, median = 38.55 ng/L), followed by GF of 0.7 μm (average = 81.12 ng/L, median = 4.57 ng/L) and GF of 3.0 μm (average = 67.16 ng/L, median = 22.49 ng/L). In contrast, MCE of 0.2 μm captured very low levels of eDNA (average = 9.04 ng/L, median = 3.74 ng/L) and, despite a larger pore size, MCE of 0.45 μm captured more eDNA than MCE of 0.2-μm filters (MCE 0.45 μm: average = 42.77 ng/L, median = 11.70 ng/L).

Effect of temperature

A single aquarium exposed at 7 °C (10 fish) showed extremely high eDNA concentration (eDNA > 1500 ng/L). Therefore, the effect of temperature on eDNA concentration was assessed with (Fig. 3) and without this aquarium (Appendix S1). For both analyses, when considering each type of filter separately, water temperature significantly altered eDNA concentration except for the MCE of 0.2-μm and GF of 0.7-μm filters (Fig. 3 and Appendix S1). Filters with low levels of eDNA capture (i.e. MCE of 0.2 μm) did not detect the difference between the eDNA concentration at the two different water temperatures. Higher eDNA concentrations were found at 14 °C than at 7 °C at all levels of fish abundance, and the difference was consistently significant at all fish abundance for the GF of 3.0-μm filter (Fig. 3).

Relationship between fish density and eDNA

Relationships were found between the eDNA concentration and fish density. Importantly, the predictive power of quantifying fish abundance/biomass varied between temperatures studies and among eDNA capture methods. The relationships between the eDNA concentration and fish abundance/biomass were stronger at 14 °C than at 7 °C (Table 2; Figs 4 and 5; see Table S1 and Appendices S2 and S3 for results including outliers). Filter type and filter pore size also influenced the power of predict-

Table 1 Differences in eDNA concentration captured using different types of filters for two different water temperatures (7 °C and 14 °C) and fish abundance and biomass (fish) per tank

	Abundance			Biomass		
	SS	*F*	*P*	SS	*F*	*P*
Models including all filters						
Fish	499 840	28.54	<0.001	712 211	10.19	<0.001
Filter	718 847	10.26	<0.001	543 478	31.11	<0.001
Temperature	2117	0.12	0.728	61 296	3.51	0.061
Temperature X Fish	34 487	1.97	0.161	22 881	1.31	0.253
Temperature X Filter	748 426	10.68	<0.001	669 596	9.58	<0.001
Fish X Filter	127 878	1.83	0.122	170 417	2.44	0.046
Temperature X Fish X Filter	269 540	3.85	0.004	235 689	3.37	0.009
MCE 0.2						
Temperature	119.1	1.06	0.305	416.2	3.68	0.057
Fish	1551.0	13.77	<0.001	1495.2	13.22	<0.001
Temperature X Fish	23.4	0.21	0.649	0.0	0.00	0.995
MCE 0.45						
Temperature	11 641	3.85	0.051	1502	0.48	0.488
Fish	56 348	18.61	<0.001	55 047	17.74	<0.001
Temperature X Fish	12 080	3.99	0.047	117	0.04	0.846
GF 0.7						
Temperature	578 427	10.46	0.001	688 906	12.57	0.001
Fish	173 202	3.13	0.079	128 398	2.34	0.128
Temperature X Fish	95 387	1.72	0.191	234 290	4.28	0.040
GF 1.2						
Temperature	92 206	3.83	0.052	27 034	1.11	0.294
Fish	181 731	7.54	0.007	200 593	8.22	0.005
Temperature X Fish	65 769	2.73	0.100	72	0.00	0.957
GF 3.0						
Temperature	72 919	14.98	<0.001	72 919	15.06	<0.001
Fish	216 045	44.39	<0.001	326 898	67.50	<0.001
Temperature X Fish	130 767	26.87	<0.001	24 091	4.97	0.027

ing fish abundance/biomass based on the eDNA captured, with strongest relationships observed with the GF of 0.7- and 3.0-μm filters (Table 1; Figs 4 and 5). Except for the GF of 3.0-μm filter, which showed a significant evidence for a linear fit (fish biomass ΔAICc = 10.8; fish abundance ΔAICc = 6.9), no strong evidence was found for a difference between biomass/abundance and linear/exponential dose response ($|\Delta AICc|$ 4.9 for all other filter types; Table 1).

Discussion

The concentration of eDNA varies as a function of the rate of eDNA release from the organism and the rate of degradation in the environment, both of which are expected to be the result of complex interactions between environmental conditions, metabolism and the ecology of the targeted species (Barnes *et al.* 2014; Strickler *et al.* 2015). High temperature is known to increase DNA degradation, decreasing therefore the eDNA detectability in water sample (Dejean *et al.* 2011; Pilliod *et al.* 2013b). Here, by leaving fish in aquaria only for 48 h and rapidly collecting water, our experimental design aimed to minimize the effect of water degradation and better underline the effect of eDNA released. Many environmental conditions and technical methods are likely to integrate more or less variance in DNA concentration between samples, which is likely to explain in part some of the inconsistency among studies, thus contributing to the ongoing debate about the relationship between eDNA concentration and species abundance and limit the extrapolation of *in situ* experiments and empirical observations. Here, our experimental data show that the rate of eDNA release into the water column from Brook Charr significantly increases at higher water temperatures (14 °C vs. 7 °C) and that the ability to predict Brook Charr abundance and biomass based on eDNA concentrations is stronger at higher temperatures.

As water temperature increases, so do fish mobility (Petty *et al.* 2012) and fish metabolic rate (Xu *et al.* 2010) until their upper limit of physiological tolerance is reached. Here, we show that temperature also influences on the amount of eDNA released from fish into the water. We hypothesize that this is because the increase

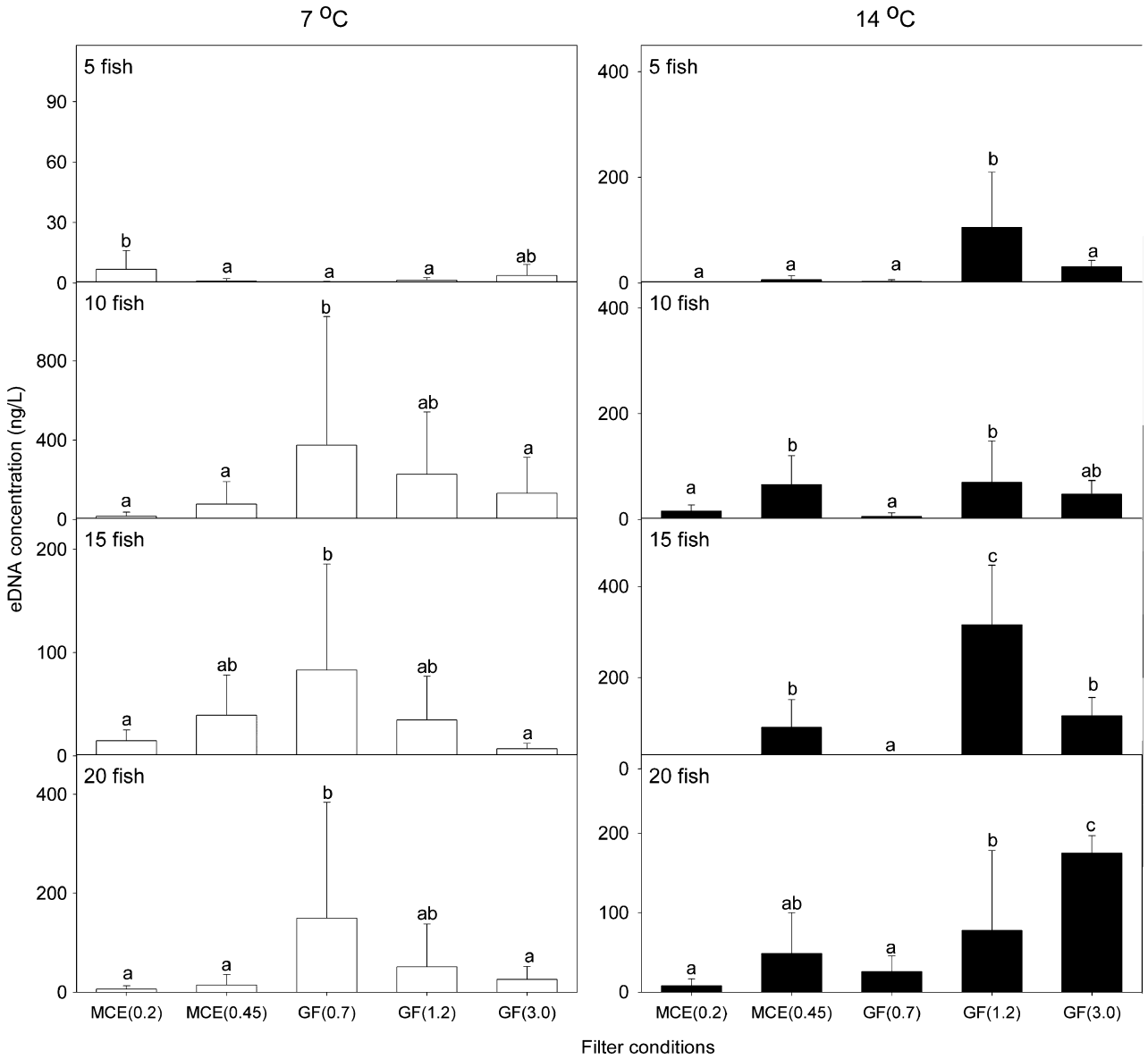

Fig. 2 Mean Brook Charr eDNA concentration (ng/L) for each filter type at various types of fish abundance at 7 °C (white bars) and 14 °C (black bars). Error bars are standard deviations. Different letters indicate a significant difference; similar letters depict no significant differences ($P < 0.05$).

in metabolic rate with water temperature also increases the release of epidermal cells and other secretions, as well as increases digestive rates, leading to increased excretion of faeces and urine (Selong *et al.* 2001; Gale *et al.* 2013). Therefore, in temperate regions, the large spatial and temporal variations in water temperature will most likely influence eDNA concentration. Consequently, water temperature needs to be considered when predicting fish abundance/biomass with this method. Moreover, trout juvenile stage may have higher shedding rates than adult fish (Maruyama *et al.* 2014). The eDNA concentration generated by a high number of juveniles (with a low total biomass) may thus be similar than by a low number of subadults (with a higher total biomass) (Iversen *et al.* 2015). Here, only juveniles were targeted and more studies are therefore needed to inform about the effect of the developmental stages and biomass on the relationship between water temperature and eDNA release rate.

The filter characteristics also have a significant influence on the yield of the eDNA being captured. The source of the eDNA captured (i.e. from eggs, whole cells, mitochondria) is likely to vary as a function of the filter material. Here, the amount of captured eDNA was com-

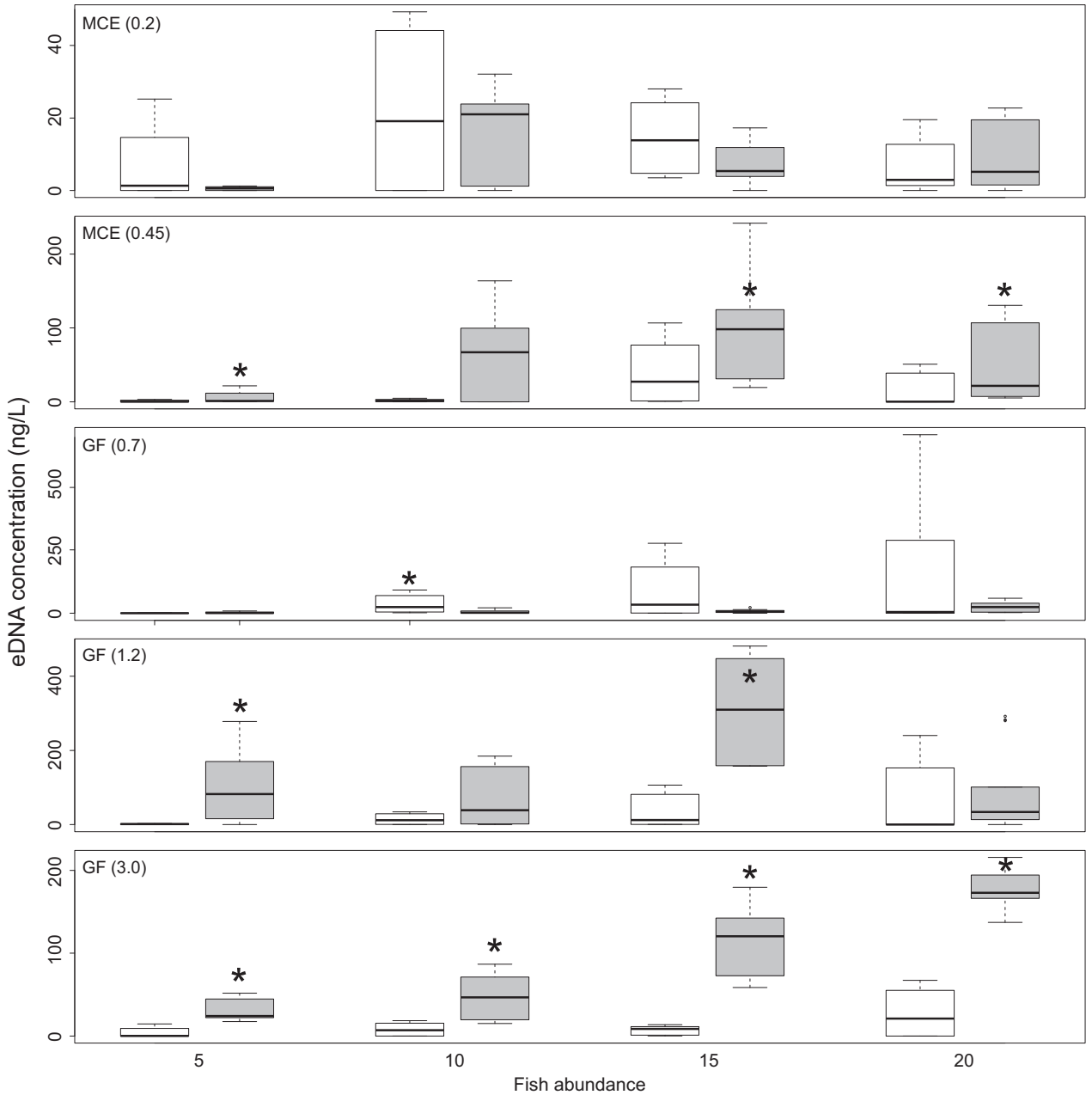

Fig. 3 Boxplots comparing Brook Charr eDNA concentrations (eDNA; ng/L) at 7 °C (white) and 14 °C (grey) for each fish abundance and various types of filters. The lines inside the boxes represent the median values, the top and bottom of the boxes represent the 75% and 25% quartiles. *Represents a significant differences ($P < 0.05$) for a given fish abundance.

pared between five commonly used filters, including mixed cellulose ester (MCE) and glass microfibre (GF) filters. MCE filters have been shown to recover about threefold more copies of plasmid DNA than polyethersulfone filters (Liang & Keeley 2013), whereas GF Whatman filters were shown to be highly effective for eDNA capture, but are only available for large pore sizes (i.e. >0.7 μm) (Eichmiller *et al*. 2015). In this study, the use of GF (glass microfibre) filters captured the most eDNA from Brook Charr and best predicted fish abundance/biomass based upon eDNA concentration. In contrast to regular pore size matrix filters (e.g. polycarbonate track-etch, PCTE) that retain only those particles that are larger than the pore size, the nonuniformity and thickness of the GF matrix filters likely embed particles in the filter matrix and retain particles smaller than the pore size (for micrographs of the pore structures commonly used, see Appendix in Turner *et al*. 2014a). The matrix of GF filters

Table 2 Statistical results for models testing the relationship between eDNA concentration and fish abundance and biomass at both temperatures studied

	7 °C				14 °C			
	Linear		Exponential		Linear		Exponential	
Filters	R^2	AIC_c	R^2	AIC_c	R^2	AIC_c	R^2	AIC_c
Abundance (the number of fish)								
MCE 0.2	0.05	116.12	0.21	116.57	0.17	110.60	0.45**	111.93
MCE 0.45	0.04	164.73	0.15	165.28	0.25	163.51	0.48**	166.45
GF 0.7	0.15	196.82	0.25	197.74	0.47**	106.85	0.65***	104.72
GF 1.2	0.13	175.59	0.37	175.67	0.13	184.77	0.37*	186.43
GF 3.0	0.27	120.00	0.33*	118.73	0.85***	148.31	0.80***	155.18
Biomass (total weight of fish)								
MCE 0.2	0.03	116.39	0.16	116.64	0.21	109.91	0.48**	112.05
MCE 0.45	0.07	164.38	0.18	165.18	0.17	164.91	0.39*	167.39
GF 0.7	0.04	185.58	0.19	186.13	0.53**	95.67	0.72***	90.74
GF 1.2	0.08	134.11	0.07	134.88	0.10	195.65	0.32*	197.19
GF 3.0	0.19	121.45	0.28*	122.33	0.82***	151.12	0.70***	161.92

P values: *** ≤0.001, ** ≤0.01, * ≤0.05.

also reduces filter clogging for field water samples (Eichmiller *et al.* 2015). At a similar pumping power, we recorded that the MCE filters took about double the time to filter when compared to GF filters (i.e. when MCE of 0.2 and 0.45 μm took approximately 16 and 14 min, respectively, GF of 0.7, 1.2 and 3 μm took about 11, 7 and 6 min, respectively). In addition to its high retention capacity, the GF matrix reduces filter clogging, has a faster flow rate and a high loading capacity, which makes this filter more preferable for eDNA studies.

As suggested by Turner *et al.* (2014a,b), eDNA yields may be high despite large pore size. Despite the fact that our experimental design does not allow us to compare pore size between the two filter materials *per se*, our results, nevertheless, suggest that an effective sampling strategy may be to use large pore sizes. Surprisingly, there are lower levels of eDNA in the two smaller pore sizes for both filter materials (i.e. MCE of 0.2 μm and GF of 0.7 μm), which may underline that eDNA capture may not be additive (i.e. DNA dislodgement occurring during the filtration due to the filter retention ability) and/or that extraction method extracts differently among the types of filters and pore size. However, our data are consistent with Turner *et al.* (2014a), where high concentration carp eDNA (*Cyprinus carpio* L.) in water samples was detected in the 1- to 10-μm particle size fractions using PCTE filters. As extraorganelle DNA molecules are too small, only intraorganelle DNA is likely to be captured on a filter (Deiner *et al.* 2015). Targeting mitochondrial and intraorganelle genetic material might become a reference for study aiming to evaluate the rate of eDNA released in different environmental conditions or estimate abundance/biomass. Here, we used a cost-effective extraction method (i.e. salt DNA extraction), which may have limited our ability to extract the embedded eDNA in the filter matrix with smaller pore sizes. Thus, different combinations of different types of filters (e.g. PCTE, cellulose nitrate, polyethersulfone; Renshaw *et al.* 2014) and extraction methods should be tested in future.

In natural ecosystems, fish eDNA is less likely to be as homogeneously distributed as our experimental design due to lower fish density and larger water volumes. The detection of low eDNA levels is thus likely to be an important constraint in natural system. Here, we showed that GF filters represent the best analytical strategy to both capture the most eDNA and generate the best predictions of fish abundance for Brook Charr. In a study performed on another salmonid (lake trout, *Salvelinus namaycush*) in its natural environment, Lacoursière-Roussel *et al.* (2016) showed that eDNA concentration was related to fish abundance in lakes during the spring (5.0 °C–9.0 °C) using glass microfibre filters of 1.2 μm (Whatman GF/C). In our experimental approach, at the density of 15 fish per aquarium, GF of 1.2-μm filter captured the highest level of eDNA, but we observed a lower eDNA concentration for 20 fish. Stress could hypothetically limit fish activity rate at high fish density (e.g. movement and growth) and reduce the release of eDNA. However, the latter is unlikely because Marchand & Boisclair (1998) showed that juvenile Brook Charr display more aggressive behaviour, execute more movements and swim faster at high density. An alternative and perhaps more likely explanation could be that, with respect to the GF of 1.2-μm filter, the capture eDNA efficiency at high fish density might have been limited by its

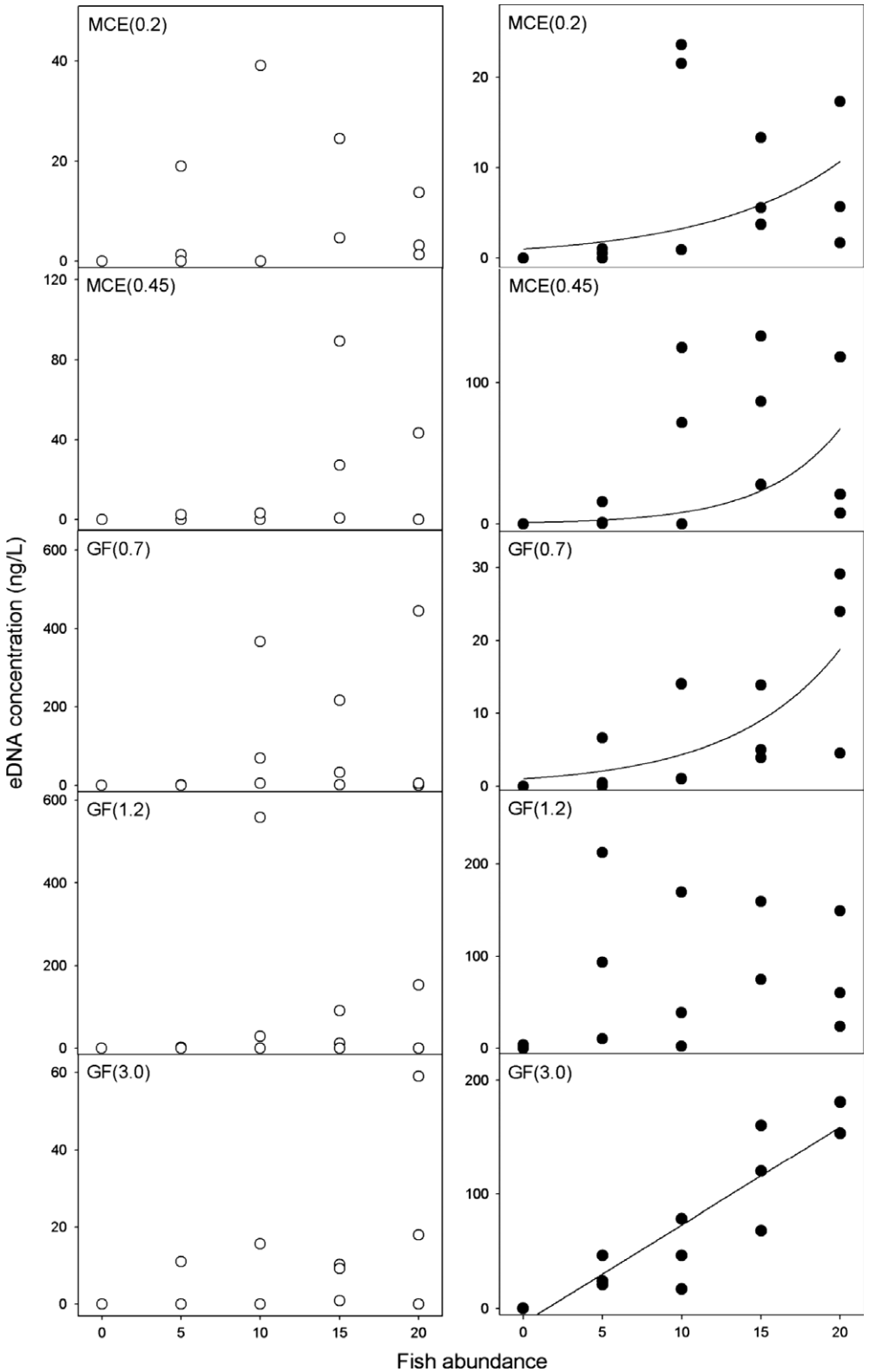

Fig. 4 Relationship between mean (±SD) Brook Charr eDNA concentration (ng/L) and fish abundance for each type of filter (MCE, mixed cellulose ester filters; GF, glass fibre) and pore size (μm; left upper corner) at 7 °C (unfilled points) and 14 °C (filled points). Linear and exponential regression models were plotted only for significant relationships when normality and homoscedasticity assumptions were satisfied.

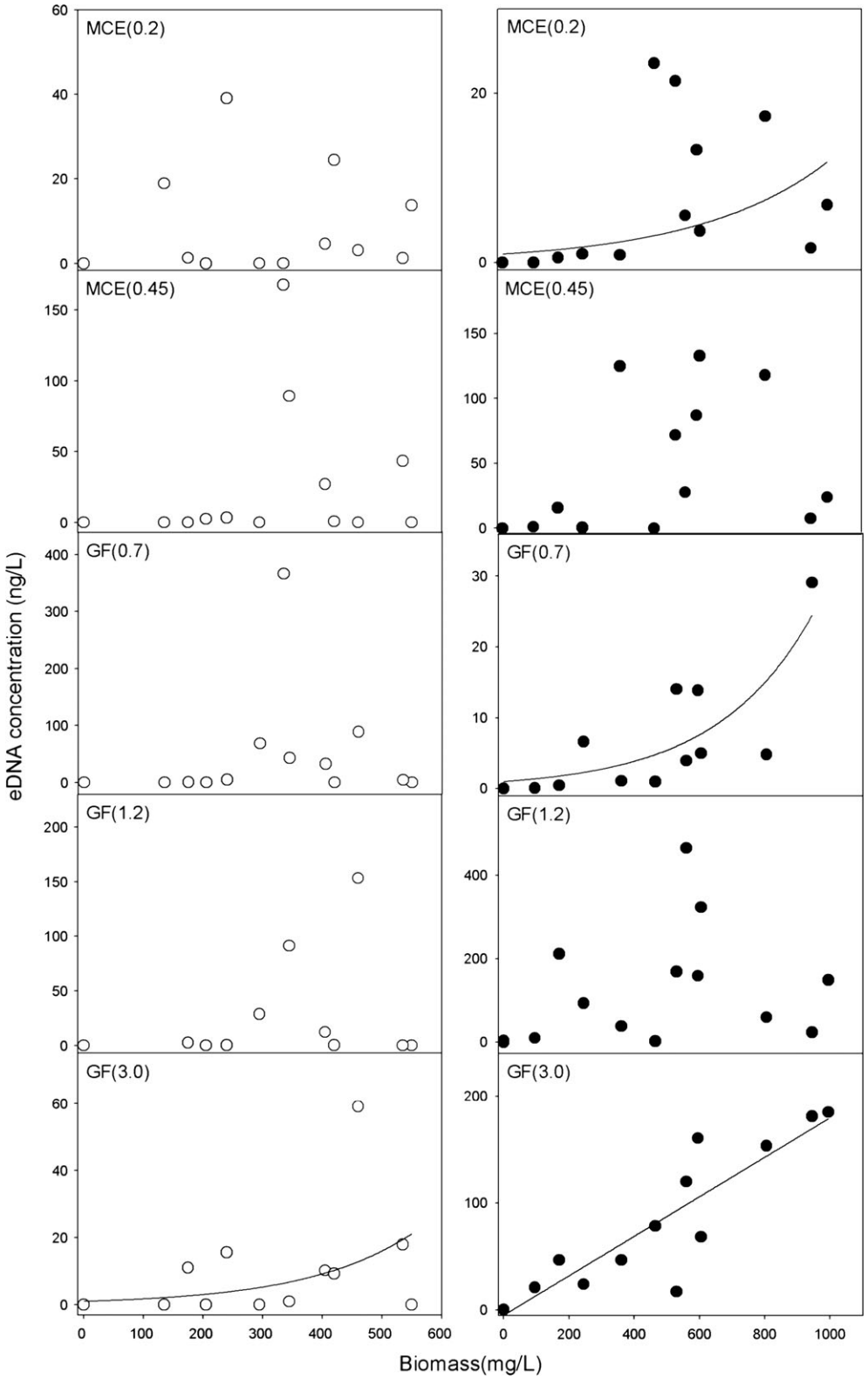

Fig. 5 Relationship between mean (±SD) Brook Charr eDNA concentration (ng/L) and fish biomass (mg/L) for each type of filter (MCE, mixed cellulose ester filters; GF, glass fibre) and pores size (μm; left upper corner) at 7 °C (unfilled points) and 14 °C (filled points). Linear and exponential regression models were plotted only for significant relationships when normality and homoscedasticity assumptions were satisfied.

decreasing DNA retention capacity (i.e. reducing eDNA capture with filter saturation). In both empirical and experimental studies, the eDNA concentration measured in a water sample reflects a recent proximity with fish. Similar to other studies in ponds, aquarium and natural systems (Takahara *et al.* 2012; Thomsen *et al.* 2012b; Pilliod *et al.* 2013a; Kelly *et al.* 2014; Klymus *et al.* 2015; Lacoursière-Roussel *et al.* 2016), our results indicate that eDNA concentration can be a good indicator of fish abundance when using the proper filtering procedure.

By overcoming issues related to the cost and difficulty of sampling, eDNA-based approaches are likely to significantly improve the collection of spatial and temporal information for aquatic populations. Optimizing standardized eDNA capture methods is critical to allow large spatial and temporal population size comparisons and is also necessary to generate predictive eDNA models to estimate fish population abundance/density. Here, we show that eDNA concentrations are significantly correlated with both fish abundance and biomass, but that the strength of the relationship depends on environmental parameters, such as water temperature, and technical parameters, such as the filter being used for capturing eDNA. The significant interactions observed between temperature and filters (Table 1) may imply that the variation in eDNA captured by filters is determined not only by the filter characteristics (e.g. size, material) but also by the nature and eDNA amount released from the secretion/degradation process, where temperature plays an important role. Macroimaging analyses could help to clarify the nature of eDNA captured at various temperatures (e.g. excretion materials or tissue loss due to increased motion). Species biology, environment and filtration methods and other factors (e.g. extraction and fish ecology and spatial distribution) are likely to interact and significantly influence eDNA concentration variation. Caution is therefore needed when interpreting the patterns of eDNA concentration in practical contexts. Parameters such as detection limits in water samples, influence of microbial activities on eDNA degradation, sampling design, seasonal conditions, nature of eDNA and fish ecology should be considered in future studies before predicting fish abundance from eDNA in natural conditions.

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Data accessibility

The eDNA concentration per amplification are available on Dryad doi: 10.5061/dryad.46sm5

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Boxplots comparing the environmental DNA concentration at both temperatures for each fish abundance and various types of filters including the aquarium showing extreme values (N = 10 fish).

Table S1 Statistical values for the relationship between eDNA concentration and fish abundance at both temperature studied without excluding outliers.

Appendix S2 Relationship between eDNA concentration and fish abundance for each filter used at both temperature excluding outlier points.

Appendix S3 Relationship between eDNA concentration and fish biomass for each filter used at both temperature excluding outlier points.