ENVIRONMENTAL BIOTECHNOLOGY 8 (1) 2012, 7-14

Ascertaining optimal protocols for DNA extraction of different qualities of pike (*Esox lucius*) tissue samples – a comparison of commonly used solid phase extraction methods

Erik Eschbach

Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Department of Biology and Ecology of Fishes, Mueggelseedamm 310, 12587 Berlin, Germany
Phone: +49(0)30-64 181 766, E-mail: eschbach@igb-berlin.de

Received in March 2012. Published in June 2012.

ABSTRACT

High quality DNA extractions are a prerequisite for genetic studies of a variety of organisms including fish. The current study focused on the applicability of different commercially available solid phase extraction (SPE) methods as the easiest and fastest methods for DNA extraction and their efficiency with different tissue qualities. These were represented by different kinds of pike tissues (fins, muscle, scales) preserved with different methods and stored at different temperatures over different periods of time (0.5 to 10.0 years). All DNA extractions were analysed according to their yield, purity, integrity and functionality in PCR based downstream analysis. Additionally mechanical pre-treatment of poor quality tissues (e.g. old or aged tissues) and efficient ethanol preservation of frozen bulk fin tissue were investigated. All SPE methods yielded functional DNA from very different qualities of pike tissues as shown by PCR analysis of small nuclear (microsatellite) and large mitochondrial (complete D-loop) DNA fragments. DNA from poor quality tissue can be extracted using single column SPE and in some cases mechanical pre-treatment even improved the yield. Good quality tissue as obtained e.g. from commercial fishermen as frozen bulk material is more efficiently preserved by thawing in ethanol at room temperature than at 2-8°C. DNA from these and air dried tissues was very efficiently prepared by applying reverse SPE in 96-well format, allowing for fast processing of a multitude of samples for high throughput analysis.

INTRODUCTION

For population genetic studies in fish (and other organisms) extraction of DNA presents the first essential step for all subsequent genetic analysis, which are frequently PCR based methods. Different tissues may contain varying amounts of DNA (e.g. scales vs. muscle tissue), which may not be equally dealt with by different DNA extraction methods. Also the quality of samples may be different depending on the type of preservation as well as the technique and duration of storage of tissues. Qualities of tissue may thus vary from good, e.g. fresh, frozen or ethanol preserved material, to poor, e.g. formalin fixed (De Bruyn et al. 2011) and aged tissue (this study).

Nowadays a number of commercial kits for DNA extraction are available. Despite higher costs of purchase as compared to most hand-made lab methods, kits are preferred, due to their ease of use and efficiency in terms of time requirements and costs for personnel. Moreover, routine protocols have been developed for many different tissues of many different species. Still it is often necessary to find out empirically, which is the most appropriate DNA extraction method for a certain tissue in a given research context. As a result a method may be either a more time consuming process for old samples (e.g. museum specimens) employed in studies that might need historical information (Hansen et al. 2009; Larsen et al. 2005; Quinn and Seamons 2009) or alternatively a high throughput routine with good quality samples (e.g. freshly preserved tissue) required for screening and monitoring programs (Beacham et al. 2010).

This study focused on different, commercially available solid phase extraction (SPE) methods for DNA-extraction, because these are the easiest and least time consuming methods as compared to others. The principle is basically a four step procedure consisting of tissue lysis, binding of DNA to a silica membrane column, washing of the column to remove impurities and subsequent elution of the purified DNA from the column. Two of the SPE methods employed are single column methods, which are compared to a differential precipitation method (DPM), proven to be an

efficient method for a poor quality samples (e.g. scales, Lucentini et al. 2006a). Additionally two 96-well SPE formats were compared, in order to test their potential for high throughput routine analysis of large quantities of good quality pike tissues. The two are, however, based on different absorption principles. The common 96-well method, which has been frequently applied (LaHood et al. 2008), works like the single column procedures as described above. The reverse SPE 96-well method is a two step method and works the other way round by binding impurities while DNA passes through the columns. This feature makes it even less time consuming and therefore very attractive for applications requiring high sample throughput.

Different sample qualities for DNA extraction were represented by different pike tissues, preserved differently and stored at different temperatures for different periods of time. The single column extraction methods were especially investigated with regard to their efficiency in dealing with poor quality and aged samples. In this context a simple mechanical pre-treatment was tried out as an alternative to the time consuming and hazardous liquid nitrogen treatment to further increase DNA yields. To provide good quality samples for high throughput routine analysis with 96-well SPE formats different sample preservation protocols were investigated.

Quality and functionality of DNA extractions are most sensitively tested by applying the envisaged downstream analysis, which is often PCR based. Therefore, firstly total and specific DNA yields as well as purity were analysed. Secondly, the integrity, i.e. presence of high molecular genomic DNA, was determined. Finally, functionality was tested by PCR amplification of small nuclear DNA sequences (approximately 100-300bp microsatellites) and a large mitochondrial DNA sequence (approximately 1.4kb control region).

The aim of this study was to investigate, if commercially available SPE methods are able to efficiently deal with extremely different qualities of pike tissue, to yield the DNA that can be used for subsequent PCR-based genetic analysis for different research purposes.

MATERIAL AND METHODS

Samples

Pike tissue samples were provided by commercial fishermen and the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB). In order to simulate different quality of tissues the collection included scales, as well as fin and muscle tissue preserved in different ways and stored for various periods of time at different temperatures for subsequent DNA extractions and genetic analysis (details summarized in Table 1). The dried out muscle tissue, as an example of an aged specimen, was originally ethanol preserved, but the ethanol has evaporated due to inappropriate storage at room temperature.

Ethanol preservation of frozen bulk fin tissue

This experiment was performed to explore the best way of transferring frozen bulk fin tissue into ethanol preservation to allow storage at ambient temperature with a significantly reduced volume. Tissues were sampled and short-term stored at -20°C by commercial fishermen. After transportation on dry ice, the samples were stored again at -20°C at the IGB. For ethanol preservation bulk fin material was either thawn in ethanol at room temperature or at 2-8°C in pre-cooled ethanol (minimum 99.5% absolute ethanol, Thomas Geyer, Renningen, Germany).

DNA extraction

For DNA extraction five commercially available kits based on different separation principles were employed. PeqGOLD Tissue DNA Mini Kit (Peqlab Biotechnologie GmbH, Erlangen, Germany) and DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) are single column applications using solid phase extraction to bind and subsequently release purified DNA (methods referred to as SPE1 and SPE2, respectively). The Wizard® Genomic DNA Purification Kit (Promega GmbH, Mannheim, Germany), also a single sample extraction method, uses differential precipitation to separate DNA from

Table 1. Preservation, storage conditions and age of different pike tissues.

Preservation type	Tissue					
	Fin		Muscle		Scales	
	°C	Years	°C	Years	°C	Years
Ethanol	RT	0.5	RT	0.5	–	–
Frozen	-20°C	1.5	-20°C	1.5	–	–
Dried	RT	0.5	–	–	RT	10
Dried out	–	–	RT	2.5	–	–

RT = room temperature
– = not measured

Table 2. Comparative performance of different DNA preparation methods on different qualities of pike tissue.

DNA extraction method	Samples[1]	Yield				Purity		Integrity	Functionality	
		total μg ± SD		specific μg·mg^{-1} ± SD		OD260/280 ± SD		Gel electro-phoresis[2]	PCR, MiSat (nuDNA)[3]	PCR, D-Loop (mtDNA)[3]
SPE1	fr-m	7.9	6.6	0.3	0.3	1.36	0.21	+	++	-
	fr-f	10.5	4.8	0.9	1.1	1.65	0.19	-	++	-
	eth-m	3.3	1.5	0.1	0.0	1.46	0.27	++	++	++
	eth-f	25.8	15.8	1.1	0.5	1.71	0.21	++	++	++
	eth/dr-m	3.9	3.6	0.2	0.2	1.81	0.46	++	++	+
	dr-f	30.1	13.9	3.0	2.0	1.81	0.09	++	++	-
	dr-s	2.1	2.4	0.8	1.1	1.04	0.17	-	+	-
SPE2	fr-m	7.4	2.6	0.4	0.2	1.54	0.14	+	++	-
	fr-f	8.7	4.7	0.9	0.8	1.47	0.15	-	++	-
	eth-m	8.9	2.5	0.3	0.1	1.63	0.11	++	++	+
	eth-f	17.0	11.9	0.9	0.4	1.94	0.30	++	++	++
	eth/dr-m	7.2	5.2	0.3	0.2	1.39	0.17	++	++	+
	dr-f	10.9	5.6	1.0	0.6	2.09	0.47	++	++	-
	dr-s	4.7	3.0	0.8	0.8	1.14	0.16	-	+	-
DPM	fr-m	7.1	4.5	0.4	0.3	1.36	0.22	+	++	-
	fr-f	23.1	9.1	2.0	1.0	1.62	0.34	-	++	-
	eth-m	1.3	0.7	0.1	0.0	2.14	0.90	++	++	+
	eth-f	67.2	44.4	2.8	1.7	1.66	0.19	++	++	++
	eth/dr-m	19.8	24.6	0.9	1.0	1.73	0.27	+	++	-
	dr-f	63.5	21.8	5.2	2.1	1.68	0.18	++	++	-
	dr-s	4.1	4.2	0.9	1.1	1.55	0.46	-	+	-
SPE-96	dr-f	36.2	13.9	5.6	2.3	1.93	0.03	++	++	-
	eth-f	21.6	11.2	1.6	1.3	1.83	0.05	++	++	- / + / ++
	eth-m	8.3	10.3	0.5	0.7	1.58	0.25	++	++	- / +
revSPE-96	dr-f	25.8	15.6	4.0	3.4	1.30	0.12	++	++	-
	eth-f	33.6	15.3	1.8	1.0	1.50	0.10	++	++	- / + / ++
	eth-m	17.5	4.1	1.1	0.2	1.32	0.05	++	++	- / +

1: fr-m = frozen muscle, fr-f = frozen fins, eth-m = ethanol preserved muscle, eth-f = ethanol preserved fins, eth/dr-m = ethanol preserved and subsequently dried out muscle, dr-f = dried fins, dr-s = dried scales

2: ++ = high molecular band without smear, + = high molecular DNA with smear, - = smear or no DNA band

3: ++ = clear bands, + = faint bands, - = no bands. MiSat = microsatellite, D-loop = mitochondrial control region, nuDNA = nuclear DNA, mtDNA = mitochondrial DNA. More than one symbol indicates variable results from multiple replicate

other tissue components (method referred to as DPM). Additionally two different DNA extraction methods for high sample throughput in 96-well formats were tested. While the DNeasy® 96 Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) corresponds to the respective single column format (SPE2), the nexttec™ DNA isolation system (Biozym Scientific GmbH, Hess. Oldendorf) uses the opposite technique, i.e. after tissue lysis all components of the tissue bind to the column, while DNA passes through (96-well methods referred to as SPE-96 and revSPE-96 respectively). All extractions were performed according to the manufacturer instructions using approximately 20mg of tissue per extraction. For dried scales and dried out muscle tissue mechanical pre-treatment with a Tissue Lyser™ (Qiagen GmbH, Hilden, Germany) was tested as an alternative to grinding with mortar and pestle in the presence of hazardous liquid nitrogen in order to increase DNA yields. For this purpose the pieces of tissue were transferred together with a stainless steel bead in the respective lysis buffer in a 2ml safe-seal reaction cup and vigorously shaken for two times 90s. After this step DNA extraction was performed according to the respective manufacturer's instruction. Altogether, approximately 500 pike tissue samples were prepared using the different extraction and treatment methods, i.e. 10 samples for each tissue type and each single sample extraction and 16 samples for each tissue type and each 96-well method (Table 2). Replicates were prepared either from the same piece of tissue or, if the piece was not large enough, from a different one.

DNA analysis

DNA concentrations in $\mu g \cdot \mu l^{-1}$ were measured by photometry at 260nm (OD 260) with an Eppendorf Bio Photometer (Eppendorf, Hamburg, Germany). Total DNA yield was calculated as the DNA amount in μg in the complete extraction volume. Specific DNA yield was calculated from total DNA yield in μg divided by tissue weight in mg.

Purity was determined by measuring additionally at 280nm (OD 280nm) and calculating the ratio between the two values (OD 260/280). Although not recommended by the manufacturer, these methods were also applied to the revSPE-96 method, but checked additionally with agarose gel electrophoresis.

DNA integrity was tested in a 1.5% agarose gel by separating approximately 50ng of DNA at 90V for 60 minutes. Ethidium bromide stained gels were photographed, visually inspected and grouped in three categories of integrity: high molecular DNA without smear, high molecular DNA with smear and smear or no DNA visible (Table 2).

Functionality of DNA extractions was tested with two types of PCR. Microsatellites Elu64 (Miller and Kapuscinski 1996) and B422 (Aguilar et al. 2005) were simultaneously amplified with the Qiagen® Multiplex PCR Kit (Qiagen, Hilden, Germany) from approximately 25ng genomic DNA according to the manufacturer's instructions, but reducing the reaction volume to 25μl. PCRs were performed with a Thermocycler T Gradient machine (Biometra, Goettingen, Germany) using the following settings: 15min of initial activation at 95°C, 35 cycles with 30s of denaturation at 94°C, 90s of annealing at 60°C and 90s of extension at 72°C, followed by a 10min final extension at 72°C.

The second type of PCR aimed at amplifying the complete D-loop of pike mitochondrial DNA. Primers were designed from a single pike sequence taken from Genbank database (accession number AP004103 in Ishiguro et al. 2003) using Primer 3 software (Rozen and Skaletsky 2000): forward primer EluDL-F with sequence 5´-tagagcgccggttttgtaat-3´, reverse primer EluDL-R with sequence 5´-aaggtcaggaccaagccttt-3´. The PCRs were performed using approximately 25ng genomic DNA with Maxima® Hot Start PCR Mastermix according to the manufacturer's instruction (Fermentas GmbH, St. Leon-Rot, Germany), but reducing the reaction volume to 25μl. PCRs were performed with the same PCR machine as used for microsatellite amplification using the following settings: 15min of initial activation at 95°C, 35 cycles with 30s of denaturation at 95°C, 90s of annealing at 59°C and 90s of extension at 72°C, followed by a 15min final extension at 72°C.

PCR products were subsequently separated with agarose gel electrophoresis as described above. To prove further the identity of some of the PCR fragments obtained with D-loop, specific primers sequence analysis was performed by a sequencing service (GATC Biotech AG, Konstanz, Germany). Afterwards the sequences were compared by BLAST analysis (Altschul et al. 1997) with the sequences in Genbank database. New sequences were deposited in Genbank database (accession numbers JQ312115 - JQ312116).

RESULTS

Total and specific DNA yields

As summarized in Table 2, best total DNA yields were obtained from dried and ethanol preserved fin tissues (10.9 to 63.5μg and 17.0 to 67.2μg respectively) with all extraction methods. Highest total yields were obtained with the DPM method, followed by the 96-well methods. The lowest total yields were observed from dried scales using single extraction methods (2.1 to 4.7μg) and from ethanol preserved muscle (8.3 to 17.5μg) using 96-well extraction formats (scales were not tested here). The DPM method was the most efficient method in terms of total yield showing the highest yields in 4 of 7 tissue types, followed by the SPE2, SPE1 and revSPE-96 methods with highest yields in one tissue type each.

The best specific DNA yields were obtained with the SPE-96 followed by the DPM method, in both cases with dried fin tissue. From this type of tissue the highest DNA yields have been obtained with all methods (1.0 to 5.6$\mu g \cdot mg^{-1}$) whereas from ethanol preserved muscle tissue the lowest yields were obtained (0.1 to 1.1$\mu g \cdot mg^{-1}$). Overall the DPM method was also the most efficient method in terms of specific DNA yields with the highest yields in 5 of 7 tissue types, followed by the SPE2, SPE-96 and revSPE-96 methods with highest yields in one tissue type each.

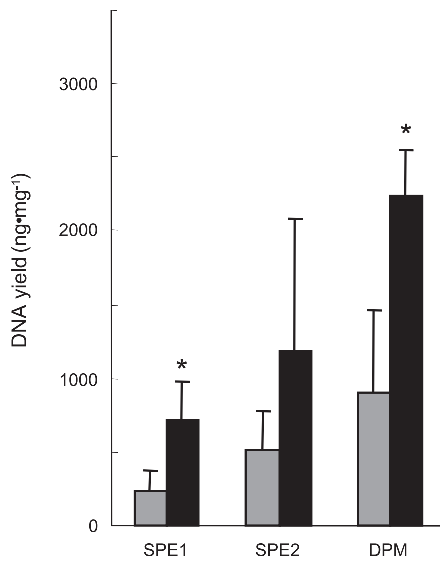

Figure 1. Specific DNA yields of a poor quality sample (ethanol fixed dried out muscle tissue) prepared with (black column) and without (grey column) mechanical pre-treatment (*p≤0.01).

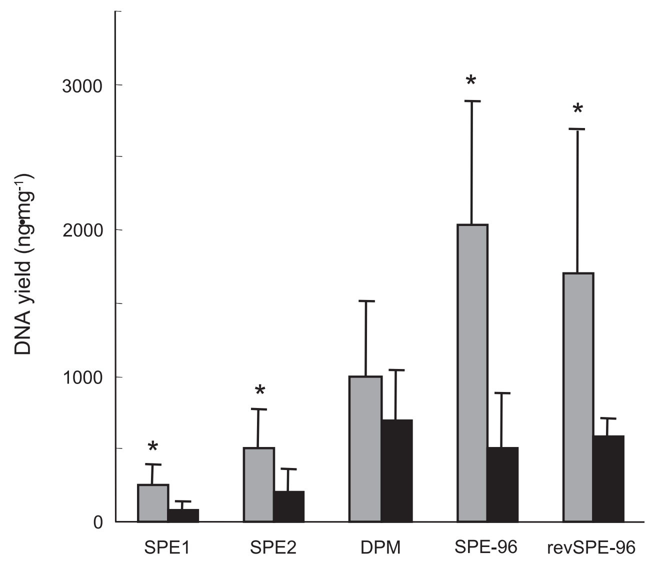

Figure 2. Specific DNA yields prepared from bulk fin tissue thawn in ethanol at room temperature (grey columns) or at 2-8°C (black columns) (*p≤0.006).

To account for the manufacturer´s recommendation not to use photometric measurements to calculate the DNA yield prepared with the revSPE-96 method, DNA prepared by the two 96-well methods was additionally compared with agarose gel electrophoresis. Loading comparable amounts of DNA as calculated from photometric measurements onto the gel did not reveal substantial differences between the two methods (data not shown).

Mechanically pre-treated dried out muscle tissue increased the specific DNA yield more than twice with all single extraction methods (Figure 1). The results with two of the methods (DPM and SPE2) proved to be significant (p≤0.01). Mechanical pre-treatment had no effect on the specific DNA yield of 10 year old scales (data not shown).

Thawing tissues in ethanol at room temperature resulted in higher specific DNA yields than thawing at 2-8°C (Figure 2). The differences were highly significant for all methods (p≤0.006) except for the DPM method.

Purity

Purest DNA was obtained with the SPE1 and DPM methods, showing a OD260/280 ratio ≥1.65, which corresponded to a purity of >80% of the genomic DNA extractions, in 4 of 7 tissue types, followed by the SPE2 method with 2 of 7 tissue types. Of the 96-well methods the SPE-96 method with 2 of 3 tissue types above 1.65, was superior over the revSPE-96 method with all tissue types below this value. Dried fin and ethanol preserved fins resulted in the purest DNA extraction with all methods applied ranging from 1.66 to 2.09 (Table 2).

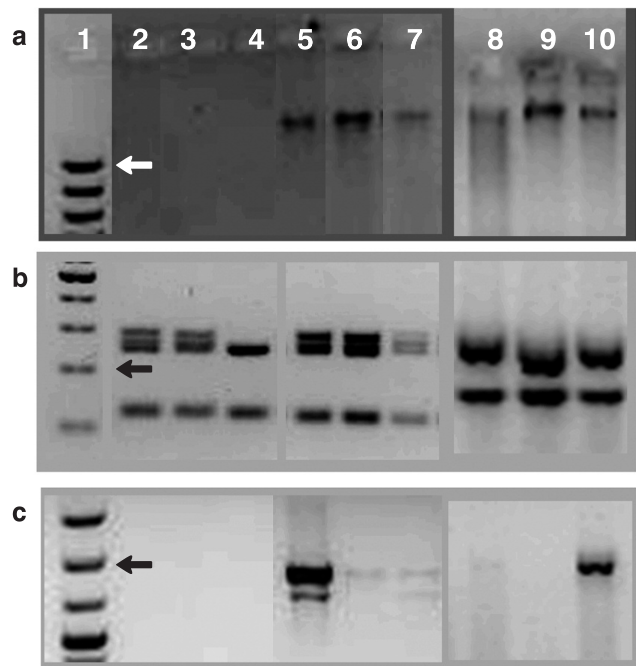

Figure 3. Gel electrophoresis of (a) genomic DNA extractions, (b) multiplex-PCR amplified microsatellites Elu 64 (lower band) and B422 (upper bands), (c) PCR-amplified mitochondrial D-loop region from different pike tissues preserved in different ways: frozen fins (lanes 2-4), ethanol preserved muscle (lanes 5-7) and fins (lanes 8-10). Lanes 2-7 show examples from different single DNA extractions, lanes 8-10 represent results from 96-well DNA extractions. Lane 1 contains size markers. Arrows indicate band sizes of 3.0kb in (a), 0.2kb in (b) and 1.5kb in (c).

Integrity

Integrity of genomic DNA was comparable for all DNA extraction methods, but varied for the different tissue types (Table 2, Figure 3). High molecular DNA with no smear was obtained from ethanol preserved fin and dried out muscle specimen. DNA from frozen muscle tissue showed high molecular DNA with smear and smear only or no DNA was detected in extractions from frozen fin tissue and dried scales.

Functionality

The two microsatellite fragments (approximately 0.1 and 0.3kb) were obtained from all genomic DNA extractions (Table 2). Signals were clear, even if DNA was partly degraded (frozen muscle) or not detectable (frozen fins, dried scales). Only the DNA prepared from dried scales yielded faint microsatellite signals (Table 2). Microsatellite B422 exhibited in most samples two clearly differently sized alleles representing a heterozygous genotype (Figure 3b). One sample showed only one band (lane 4 in Figure 3b), which might indicate a homozygous genotype. Microsatellite Elu64 also appeared as one band in all samples and showed different allele sizes for different DNA extractions (lanes 2-7 vs. lanes 8-10 in Figure 3b). Amplification of the complete mitochondrial control region (D-loop, approximately 1.4kb) was principally possible with DNA obtained from all extraction methods, but did not work for DNA extractions obtained from all tissue types (Table 2 and Figure 3). The best results were obtained from ethanol preserved fin and muscle tissues, but some faint signals were also detected for dried out muscle tissue. Sequence and BLAST analysis performed with some of the PCR products identified these as fish mitochondrial DNA control regions (D-loop).

DISCUSSION

The quality of a tissue intended for use in DNA analysis depends on several factors including the kind of tissue, as well as the preservation and storage method and the age of the preserved samples (De Bruyn et al. 2011). Different DNA extraction methods may cope differently with different tissue qualities concerning yield, purity, integrity and functionality (i.e. suitability for down stream analysis) of DNA. The method of choice will also depend on the specific research task. Thus an analysis requiring historical information (Hansen 2002; Larsen et al. 2005) usually provided by putatively poorer quality samples justifies more efforts than a monitoring or screening program (Beacham et al. 2010), which typically desires good quality for high throughput of a multitude of samples.

In this study different qualities of tissue of the northern pike (*Esox lucius*) were represented by different tissues (fin, muscle, scale) that have been preserved in different ways (in ethanol, frozen, dried) and stored at different temperatures (room temperature, -20°C) for different durations of time (0.5 to 10.0 years). The present work focused on some frequently used commercially available solid phase extraction methods (SPE), as these, compared to other commercially available products (e.g. the DPM method comparatively applied in this study) and commonly used lab methods (e.g. phenol-chloroform, CTAB), are the easiest and fastest methods with the additional potential for high sample throughput.

The results showed that it is possible to retrieve functional DNA with the SPE methods applied, independent of the type of tissue and the preservation method used, but the yields, especially within the methods, differed in parts substantially. Total and specific DNA yields were highest from fin tissue, whereas muscle tissue and especially scales did not perform as well. Whereas this can be expected from scales with only a marginal soft tissue share, it was a bit unexpected for muscle tissue. To improve yields mechanical pre-treatment during lysis seems appropriate, since it resulted in significant increases in specific DNA yields of aged (i.e. dried out) muscle tissue (Figure 1). Although the same treatment did not work for the 10 year old scales, it is a useful method presenting an alternative to the use of mortar and pestle in the presence of hazardous liquid nitrogen. Independent of the yields obtained from different tissue types, the genomic DNA proved to be applicable for PCR amplification (Table 2). For old dried scales the efficiency may be improved by using either an alternative extraction protocol (e.g. an Ancient-DNA-Protocol, after De Bruyn et al. 2011) or by increasing the number of PCR cycles (Lucentini et al. 2006a).

With regard to preservation and storage, fixation of fin tissue with ethanol or air drying with subsequent storage at room temperature appeared superior to storage at -20°C. Due to reduced water content especially the dried fins showed high DNA yields upon extraction with all methods. However, for muscle tissue the results were not as clear. Fin and muscle tissue stored at -20°C generally yielded low amounts of DNA with poor quality with regard to purity and integrity. Nevertheless these tissues passed the functionality test (Figure 3). A lower storage temperature (-80°C) might improve the quality of frozen tissues, but will lead to higher storage costs. Thus, as an alternative, frozen tissue may be transferred to ethanol to save costs and storage capacity. An interesting result of this study is, that significant higher specific DNA yields were obtained from tissues that are thawn in absolute ethanol at room temperature instead of 2-8°C (Figure 2). A reason for this phenomenon may be that tissue fixation occurs faster at room temperature.

Comparing all SPE methods more variability concerning yields and purity was observed within the methods (i.e. between tissue types) than between methods. The DPM method was often superior especially with regard to total yields (Table 2), which is to some extent attributable to the limited binding capacity of the silica membrane columns of the SPE methods, which should be typically 10-20μg DNA per column according to the manufacturer´s information. Although this range is sometimes also exceeded by the SPE methods (up to 30μg), the highest values were reached with the DPM method (up to 67μg).

All SPE methods were efficient enough in terms of quantity and purity to provide DNA for subsequent PCR analysis with both small nuclear (microsatellites) as well as large mtDNA fragments (D-loop). The fact that PCR with the large mtDNA fragment did not work in every case might at least to some extent be due to the fact that the primers were derived from a single pike sequence (Ishiguro et al. 2003) and did not bind to the DNA of all pike individuals tested here. Other reasons could be different tissue qualities. However, as mentioned above, dried and ethanol preserved fins appeared as good quality tissues suited for routine analysis.

The PCR results in this study seem to differ slightly from what Lucentini et al. (2006a) found, when testing fins and scales of pike and trout (*Salmo trutta*) with different methods, among them one SPE method (from a different manufacturer) and the DPM method (same as in the present study). The authors found that only extractions by the DPM and the Chelex methods provided DNA of good quality suitable for subsequent PCR-based analysis. This was all the more the case if long term storage was performed.

Despite all advantages the DPM method provides, it is not suited for high throughput sample processing. For this purpose SPE methods in 96-well formats are foremost used in monitoring and screening programs (Beacham et al. 2010; LaHood et al. 2008). In this study the SPE-96 method worked well, yielding even higher amounts of DNA compared to the single column method (SPE2) of the same manufacturer. This might, however, be founded in different lysis times specified by the manufacturer (overnight vs. three hours) rather than, e.g., differences in DNA binding capacity of the two formats. However, in order to guarantee sufficient DNA yield it is advisable to use freshly preserved samples with the most efficient preservation method available.

LaHood et al. (2008) used fins from coho salmon (*Oncorhynchus kisutch*) and vermilion rock fish (*Sebastes miniatus*) dried on chromatography paper and excised with a micro punch for subsequent extraction with 96-well format SPE (same as in the present study). They found this procedure working comparably well as with ethanol preserved fins in downstream analysis, but with the additional advantage of saving a lot of time. The present study confirms the results of LaHood et al. (2008) with fresh pike tissue samples being either preserved with ethanol or by air drying. However, the process could even be speeded up with a reverse solid phase extraction method, which is available in a 96-well format (revSPE-96) and which was used in the current study with the same kinds of tissue. Since no substantial loss in yield and functionality was observed compared to the standard SPE-96 method, this method may represent the optimal method for high throughput DNA extraction. However, before generalizing, it seems worth to test this method with tissue of fish species other than pike. Furthermore, other works (Lucentini et al. 2006b; Mirimin et al. 2011; Reid et al. 2011) concentrated on non invasive sampling of fish by using body and buccal swabs as a DNA source. It would be worthwhile to test such material with the revSPE-96 method as well. Since impurities can have a negative influence on the quality of a DNA extraction (Lucentini et al. 2006a) it is also advisable to investigate effects resulting from long-term storage.

In summary, this study has shown that the commercially available SPE methods tested here are suited for DNA extractions from extremely different qualities of pike tissues. Both, single columns as well as 96-well formats yield DNA in sufficient amounts and quality that can be used for convenient downstream genetic analysis. The reverse solid phase extraction method especially bears the potential for further accelerating routine processes requiring high sample throughput, e.g. fish screening and monitoring programs.

ACKNOWLEDGEMENTS

Funding of the current work was granted by the German Ministry of Education and Research within the project Besatzfisch (www.besatz-fisch.de) in the Program for Social-Ecological Research (Grant No. 01UU0907). I would like to thank Christian Schomaker, who helped to collect samples, Sascha Behrens and Sandro Schöning who provided brilliant laboratory assistance, Klaus Kohlmann for helpful suggestions on the manuscript and three anonymous reviewers for their constructive advices to further improve the quality of the manuscript.

REFERENCES

Aguilar, A., J.D. Banks, K.F. Levine, R.K. Wayne. 2005. Population genetics of northern pike (*Esox lucius*) introduced into Lake Davis, California. Canadian Journal of Fisheries and Aquatic Sciences 62: 1589-1599.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST, a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

Beacham, T.D., B. McIntosh, C. Wallace. 2010. A comparison of stock and individual identification for sockeye salmon (*Oncorhynchus nerka*) in British Columbia provided by microsatellites and single nucleotide polymorphisms. Canadian Journal of Fisheries and Aquatic Sciences 67: 1274-1290.

De Bruyn, M., L.R. Parenti, G.R. Carvalho. 2011. Successful extraction of DNA from archived alcohol-fixed white-eye fish specimens using an ancient DNA protocol. Journal of Fish Biology 78: 2074-2079.

Hansen, M.M. 2002. Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. Molecular Ecology 11: 1003-1015.

Hansen, M.M., D.J. Fraser, K. Meier, K.D. Mensberg. 2009. Sixty years of anthropogenic pressure: a spatio-temporal genetic analysis of brown trout populations subject to stocking and population declines. Molecular Ecology 18: 2549-2562.

Ishiguro, N.B., M. Miya, M. Nishida. 2003. Basal euteleostean relationships: a mitogenomic perspective on the phylogenetic reality of „Protacanthopterygii“. Molecular Phylogenetics and Evolution 27: 476-488.

LaHood, E.S., J.J. Miller, C. Apland, M.J. Ford. 2008. A rapid, ethanol-free fish tissue collection method for molecular genetic analyses. Transactions of the American Fisheries Society 137: 1104-1107.

Larsen, P.F., M.M. Hansen, E.E. Nielsen, L.F. Jensen, V. Loeschcke. 2005. Stocking impact and temporal stability of genetic composition in a brackish northern pike population (*Esox lucius* L.), assessed using microsatellite DNA analysis of historical and contemporary samples. Heredity 95: 136-143.

Lucentini, L., S. Caporali, A. Palomba, H. Lancioni, F. Panara. 2006a. A comparison of conservative DNA extraction methods from fins and scales of freshwater fish: A useful tool for conservation genetics. Conservation Genetics 6: 1009-1012.

Lucentini, L., A. Palomba, H. Lancioni, M. Natali, F. Panara. 2006b. A nondestructive, rapid, reliable and inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal cells. Molecular Ecology Notes 6: 257-260.

Miller, L.M., A.R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. Transactions of the American Fisheries Society 125: 971-977.

Mirimin, L., D. O´Keeffe, A. Ruggiero, M. Bolton-Warberg, S. Vartia, R. Fitzgerald. 2011. A quick, least-invasive, inexpensive and reliable method for sampling *Gadus morhua* postlarvae for genetic analysis. Journal of Fish Biology 79: 801-805.

Quinn, T.P., T.R. Seamons. 2009. Tales from scales: old DNA yields insights into contemporary evolutionary processes affecting fishes. Molecular Ecology 18: 2545-2546.

Reid, S.M., A. Kidd, C.C. Wilson. 2011. Validation of buccal swabs for noninvasive DNA sampling of small-bodied imperilled fishes. Journal of Applied Ichthyology 28: 290-292.

Rozen, S., H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology (ed. S. Krawetz, S. Misener), pp. 365-386. Humana Press, Totowa, New Jersey.