PANAMJAS
Pan-American Journal of Aquatic Sciences

Comparison of DNA extraction protocols for different marine fish tissues

LOUGAN LAGASS PEREIRA [1,*], JÚNIO DE SOUZA DAMASCENO [2,3], EDNEY LEANDRO DA VITORIA [4], MAURICIO HOSTIM SILVA [1,4] & ANA PAULA CAZERTA FARRO [1,4]

[1]*Universidade Federal do Espírito Santo, Programa de Pós-graduação em Biodiversidade Tropical, Centro Universitário Norte do Espírito Santo, Rodovia BR 101 Norte, Km 60, CEP 29932-540, Litorâneo, São Mateus, Brasil.*
[2]*PUC Minas - Pontifícia Universidade Católica de Minas Gerais - Programa de Pós-graduação em Biologia de Vertebrados, Laboratório de Genética da Conservação - Av. Dom José Gaspar 500, Coração Eucarístico, CEP: 30535-901, Belo Horizonte, MG, Brasil.*
[3]*UniBH - Centro Universitário de Belo Horizonte, Instituto de Engenharia e Tecnologia (IET),. Av. Prof. Mário Werneck, 1685, Buritis, CEP: 30455-610, Belo Horizonte, MG, Brasil.*
[4]*Universidade Federal do Espírito Santo, Departamento de Ciências Agrárias e Biológicas, Centro Universitário Norte do Espírito Santo, Rodovia BR 101 Norte, Km 60, CEP 29932-540, Litorâneo, São Mateus, Brasil.*

* Corresponding author: *lougan.lagass@gmail.com*

Abstract. The quality of extracted DNA for genetic study depends on the extraction method used and the type of tissue sampled. For studies that address endangered species, it often becomes necessary to choose a tissue that involves a minimally invasive and non-lethal methodology rather than one that results in the greatest DNA quantity and quality. In this study, three DNA extraction protocols (phenol/chloroform, saline extraction, and Chelex) were tested using three tissues (muscle, liver and caudal fin) from three species of rayed fin bony fish: *Epinephelus itajara* (Atlantic goliath grouper), *Lutjanus jocu* (dog snapper), and *Centropomus parallelus* (fat snook). A sufficient amount of DNA was extracted from the caudal fin samples from all species regardless of the protocol used. Thus, we suggest extracting DNA from the caudal fin of these and other threatened species using the saline solution method, which does not sacrifice the specimens evaluated. This method presents the best cost-benefit regarding the amount of DNA obtained, toxicity and cost.

Keywords: Conservation, molecular analysis, non-lethal sampling.

Resumo. Comparação entre protocolos de extração de DNA para diferentes tipos de tecidos de peixes marinhos. A qualidade do DNA extraído para estudo genético depende do método de extração utilizado e do tipo de tecido. Para estudos que abordam espécies ameaçadas de extinção, muitas vezes torna-se necessário escolher um tecido que envolve uma metodologia minimamente invasiva e não-letal, em vez de um que resulta numa grande quantidade e qualidade de DNA. Neste estudo, três protocolos de extração de DNA (fenol / clorofórmio, extração salina e Chelex) foram testados utilizando três tecidos (músculo, fígado e nadadeira caudal) de três espécies de peixes ósseos de nadadeira raiada: *Epinephelus itajara* (Mero), *Lutjanus jocu* (Vermelho) e *Centropomus parallelus* (robalo peba). Uma quantidade suficiente de DNA foi extraído a partir de amostras de nadadeira caudal, para as espécies avaliadas, independentemente do protocolo utilizado. Assim, sugerimos a extração do DNA utilizando nadadeira caudal para estas e outras espécies ameaçadas, utilizando o método de solução salina,

o que não necessita sacrificar os espécimes estudados. Este método apresenta o melhor custo-benefício em relação à quantidade de DNA obtido, toxicidade e custo.

Palavras-chave: Amostragem não letal, análise molecular, conservação.

Introduction

For use in genetic studies, DNA can be obtained from different tissue sources; however, most studies developed for fish DNA extraction use invasive methods to remove liver or muscle samples from individuals (Artoni & Matiello 2003, Marengoni *et al.* 2006, Gomes *et al.* 2008). Fewer studies use fins (Carreiro *et al.* 2003, Povh *et al.* 2003, Wasko *et al.* 2003, Marengoni *et al.* 2006, Gold *et al.* 2011), and some use more unusual tissues such as the kidney, heart, and gills (Marengoni *et al.* 2006). Although there are many genetic studies on fish, there has been little emphasis on comparing DNA extraction protocols to determine the efficiency of DNA extraction protocol in different tissues in conservation research of marine fish.

Extracting a proper amount of DNA that is of good quality is of great importance for molecular genetic studies. Thus, the tissue and protocol adequate for the species must be chosen very carefully. It is necessary to apply non-lethal techniques for tissue sampling endangered species. It is important to maximize the DNA extraction results by removing the smallest piece of tissue with the least injury to specimen possible, thereby enabling quick and safe regeneration of the tissue sample collection site (Wasko *et al.* 2003).

Non-invasive techniques are important because we can assess the individual without the need to capture the specimen. These techniques involve the use of trace material such as hair, faeces, feathers, or loose skin in the environment, among others (Taberlet *et al.* 1999).

Wasko *et al.* (2003) evaluated DNA extraction from the caudal and anal fins and the scales of species *Brycon cephalus*, *B. orbignyanus*, *Leporinus elongatus*, *Astyanax scabripinnis*, *Hypostomus* sp., and *Geophagus brasiliensis* using phenol/chloroform protocols. The authors obtained an amount of DNA sufficient to properly amplify RAPD, D-loop, and 5S rDNA. Thus, this low invasive methodology can be used for some studies. However, other procedures less toxic than phenol/chloroform-based can be applied that generate similar efficiency.

Many protocols of DNA extraction are cited in the literature (Han & McPheron 1997, Sambrook *et al*. 1998, Ward *et al*. 2005, Weber *et al*. 2010), furthermore, for different organisms, specific protocols are used. Regarding the protocols used in this study: Costa (2015) constructed a chromosomal mapping of repetitive DNAs in marine fish of commercial interest, and for obtaining genomic DNA was used the phenol-chloroform protocol; Hollenbeck *et al*. (2015) for obtaining *Lutjanus campechanus* DNA used the Chelex resin protocol; Damasceno *et al.* (2015) moreover, working with *Epinephelus itajara* used the salt extraction protocol for obtaining genomic DNA. We can separate in a simple, two groups of extraction protocols, where the researcher uses chemical reagents purchased separately or kits, which comes with specific reagents and certain concentrations in a closed package sold by the manufacturer.

In molecular analysis, is necessary to consider three main questions to choose the more adequate DNA extraction protocol to be followed: - the available animal tissue, mainly if it was obtained by low or non-invasive method that normally have less DNA amount; - the cost of the total procedure: some kits are expensive; - the risky for the researcher's health: protection equipment and careful procedures are necessary.

In addition, low cost in DNA extraction methods and non-lethal sampling collection has been increasingly used, due the restrictions on access to data of threatened species and high cost of using commercial kits.

In this study we compared three DNA extraction protocols on different tissues obtained from marine fish species, to identify enough quality and DNA concentration for each method and tissue, looking for an efficient, cheap and with low health risky.

Materials and Methods

Experimental Setup: Specimens from three fish species, *Epinephelus itajara* (Lichtenstein, 1822), *Lutjanus jocu* (Bloch & Schneider, 1801) and *Centropomus parallelus* Poey, 1860, were collected from the northern end of the Espírito Santo State coast, Brazil, from the estuary of EPA (Environmental Protection Area) of Conceiçao da Barra in the municipality of Conceiçao da Barra and from the Barra Nova Ecological Station in the

municipality of Sao Mateus. The samples were collected from 2010-2012. These species were chosen because of their biological and economical importance and the scarce genetic information about that. The species occur in almost all Brazilian cost, and at least in their juvenile life phase can be found in the same habitats, as mangroves. *Lutjanus jocu* and *C. parallelus* has commercial importance and may have been suffer with overfishing, and *E. itajara* already is considered an endangered species.

The specimens were identified for an expert using the keys developed by the FAO (United Nations Food and Agriculture Organization) (Allen 1985, Heemstra & Randall 1993, Carpenter 2002). A piece of the caudal fin of each specimen was collected in the field and other tissues were collected in the laboratory. After the sampling, the tissues were preserved in alcohol 70% and stored at a -20ºC.

Three DNA extraction protocols were tested using the three following types of tissues removed from the species: muscle, liver, and caudal fin. Eight individuals of each species were sampled for a total of 216 samples. The extraction protocols used were as follows: 1) a protocol established by Han & McPheron (1997) and adapted by Siquara (2010) with phenol and chloroform, proteinase K digestion and SDS detergent. This method is called phenol/chloroform in this study; 2) extractions using saline solution (Bruford *et al.* 1992); 3) DNA extraction using Chelex resin (SIGMA). These protocols were chose because they are being used in our laboratory for different organisms and because they are widely cited in the literature (Costa 2015, Damasceno *et al.* 2015, Hollenbeck *et al.* 2015), we tested these protocols because phenol / chloroform is one of the most widely used, but toxic, laborious and time-consuming with respect to the other; saline protocol is more rapid and less toxic with respect to phenol-chloroform; and the Chelex resin that is easily manipulated and less toxic, and although been more expensive than the other two protocols, is cheaper than sophisticated kits.

DNA quantification was performed using a spectrophotometer (Nanodrop). The amount of DNA extracted was measured in ng/µl, and the 260/280 ratios were also assessed using the 260nm and 280nm lengths, which are absorbed by nucleic acids and proteins, respectively. The ideal value should range between 1.8 and 2.0 or 2.2 depending on the author (Ferreira & Grattapaglia 1998, Sambrook *et al.* 1998, Parpinelli & Ribeiro 2009).

Amplification tests were performed for all treatments for some of the samples extracted. To this end, primers specific for cytochrome oxidase subunit I (COI) gene of the mitochondrial DNA were used, as described by Ward *et al.* (2005). The concentrations were modified as follows in the 12.5µl reaction: buffer 10X, 10 ng of DNA, 2.0 mM of $MgCl_2$, 0.12 µM of each primer, 0.06 U of Taq DNA polymerase, 0.2 mM of dNTP, and ddH_2O (ultrapure water) to complete. The amplification conditions consisted of a denaturation step at 95°C for two min followed by 35 amplification cycles (denaturation at 94°C for 30 s, annealing at 52.5°C for 30 s, and extension at 72°C for one min), and final extension cycle at 72°C for 10 minutes.

The PCR fragments were separated on 1% agarose gel, and visualized under UV light after staining the DNA with Gel Red. For verifying the appropriate size of the amplified band a 100bp ladder was run on the same gel. The amplification products were sequenced to confirm that the appropriate gene fragment was amplified.

Statistical analyses: Statistical analyses were performed to assess differences in the efficiency between DNA extraction methods. The data for the combinations of methods and tissues were divided into nine groups for each fish species (i.e., three tissues for three extraction protocols). These groupings were analyzed using the Kolmogorov-Smirnov test to verify normal distribution of the DNA data. The experiment was a randomized block design. Thus, the species were divided into three blocks: the *E. itajara*, *L. jocu*, and *C. parallelus* groups. Treatments were divided into nine groups: muscle - phenol (MP); liver - phenol (LP); fin - phenol (FP); muscle - saline (MS); liver - saline (LS); fin - saline (FS); muscle - chelex (MC); liver - chelex (LC); and fin - chelex (FC). The effects of treatments and blocks on DNA concentration were interpreted using analysis of variance (ANOVA). The differences between the means of treatments and blocks were evaluated using Tukey's test. A p-value of < 0.05 was considered to be significant.

Results

For the phenol/chloroform protocol, the lowest DNA concentration (52.9 ng/µl) was obtained from liver of *E. itajara*, and the highest concentration 8301.5 ng/µl, which was also extracted from the liver of *E. itajara* (Figs 1, A, B and C). For the saline extraction method, the lowest concentration obtained was 45.4 ng/µl from fin of *L. jocu*, and the highest concentration was 10245.2 ng/µl from the fin of *E. itajara*. For the Chelex resin

extraction method, the concentrations ranged from 55.0 ng/µl from the fin of *C. parallelus* to 1639.8 ng/µl from the liver of the dog snapper (Figs 1a-1c).

The 260/280 ratio ranged from 1.9 to 2.0 for DNA extracted using phenol/chloroform, between 1.9 and 2.1 using the saline extraction with the exception of *E. itajara* liver and the *L. jocu* muscle samples (ratio of 1.1 to 1.2, respectively), and a wide range from Chelex extracted DNA, which had a range from 0.9 to 2.1 (Table I).

Based on our statistical analyses, the data had a normal curve and the treatments (extraction methods) were significantly different. The results were divided into three groups (a, b, c). The treatments with the highest mean concentrations were (LP), (MC), (MP), and (LC), which were significantly different from the others, noted by the letter "a" (Table II). The treatments for the *E. itajara* demonstrated no significant difference, whereas the treatments for the *L. jocu*, that led to the largest amount of DNA extracted were as follows: LP, MP, MC, and FC ($p < 0.05$: group "a"). For the *C. parallelus*, the highest concentrations of DNA extracted were in the LP, LC, MC, MP, and FP treatments ($p < 0.05$: group "a"). For *E. itajara*, the treatments did not statistically differ, thus demonstrating that there is no significant difference between treatments.

In amplification tests, a fragment of approximately 600bp was observed, which was expected for the COI marker (Fig 2).

The mean DNA concentration isolated from the caudal fin was 554.4 ng/µl, when using the phenol/chloroform extraction method. The mean DNA concentration isolated from the caudal fin was 433.8 ng/µl when using the saline extraction method.

Discussion

The phenol/chloroform and saline solution protocols did not differ in band intensities, which may represent differences in the integrity of the DNA extracted. This may suggest that both methods functions adequately for molecular markers amplifications from caudal fin sampling (Fig 2).

When assessing the quality and purity of the DNA from the extraction protocols using the 260/280 ratio, it was determined that the treatments using phenol/chloroform and saline solution ranged from 1.8 to 2.2, which is considered to be "ideal" based on the literature (Ferreira & Grattapaglia 1998, Sambrook *et al.* 1998, Parpinelli & Ribeiro 2009) (Table I).

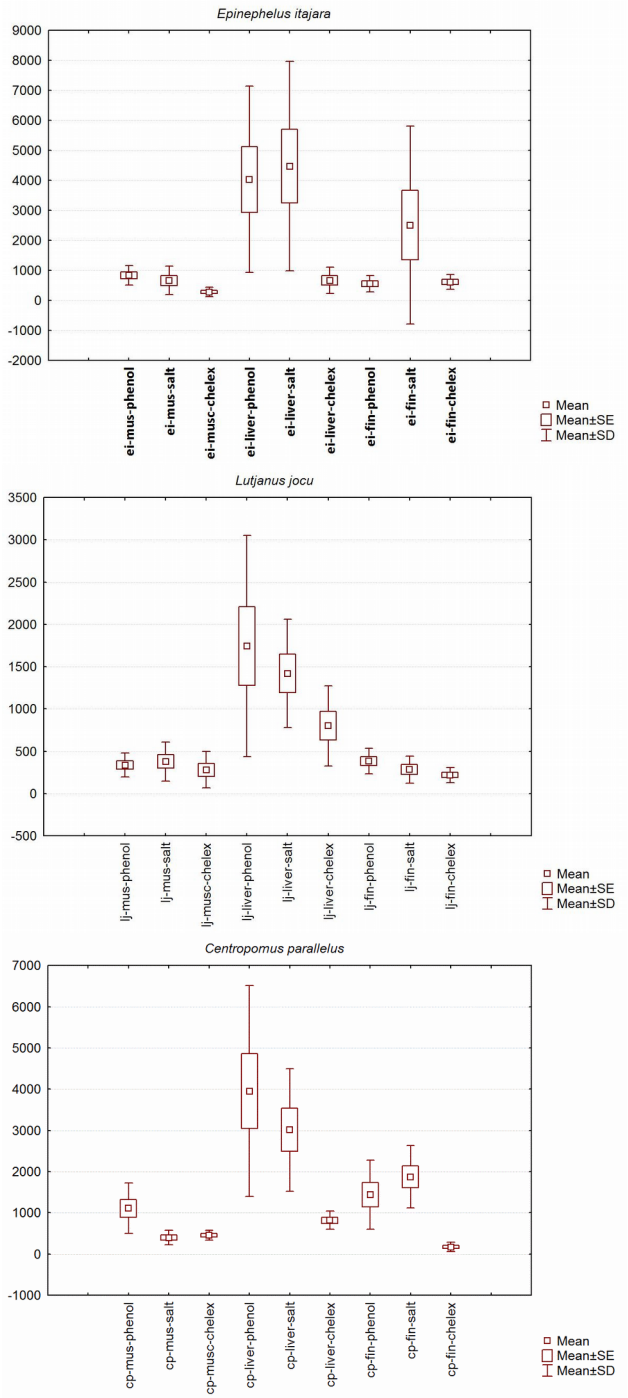


Figure 1. Minimum and maximum concentrations of DNA (ng/µl) obtained from phenol, saline, and chelex extraction protocols in different tissues (liver, muscle, and caudal fin) from marine fishes. a = *Epinephelus itajara*; b = *Lutjanus jocu*; c = *Centropomus parallelus*. Values in parentheses represent the mean and standard deviation, respectively ($N = 8$).

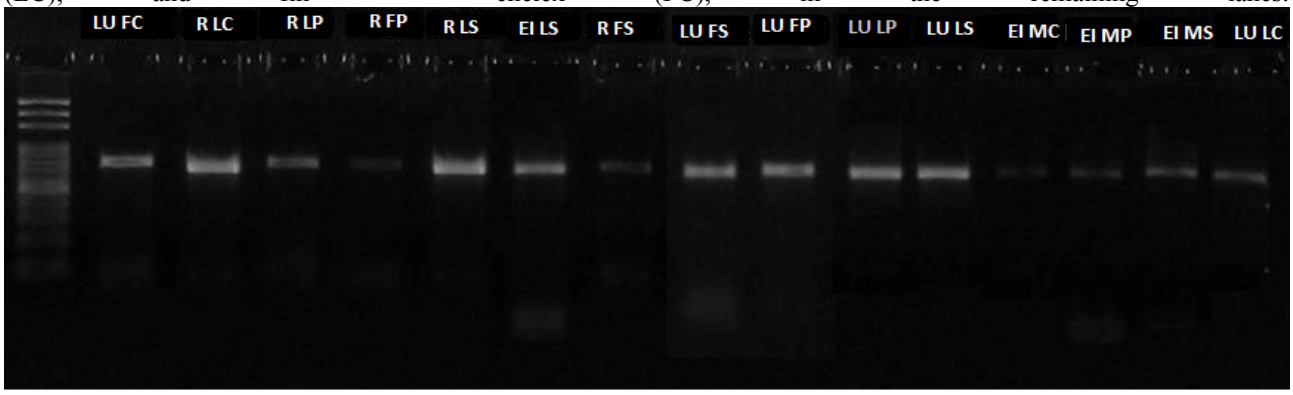
Table I. Mean and and standard deviation of the 260nm/280nm absorbance ratios (N = 8). P/ C= phenol/chloroform

	E. itajara			*L. jocu*			*C. parallelus*		
Tissue	P/ C	Saline	Chelex	P/ C	Saline	Chelex	P/ C	Saline	Chelex
Muscle	2.01+-0.02	1,99+-0.07	1,71+-0.23	2.02+-0.03	1.94+-0.29	1.23+-0.15	2.03+-0.04	1.98+-0.02	1.36+-0.11
Liver	1.93+-0.05	1,85+-0.30	1.67+-0.13	2.08+-0.03	2.02+-0.13	1.60+-0.25	2.04+-0.02	2.03+-0.01	1.65+-0.12
Fin	2.01+-0.03	1.97+-0.05	1.75+-0.09	2.05+-0.05	2.06+-0.11	1.80+-.08	2.01+-0.04	1.97+-0,02	1.59+-0.12

Table II. Analysis of Variance test (ANOVA) of the mean DNA concentrations of the treatments and of the interactions in blocks. Tukey's test (N = 8). Legend: Means followed by the same letter do not statistically differ ($p < 0.05$). Lower case letters correlate to the columns and uppercase letters correlate to the rows. Means of the treatments and of treatments X blocks are not correlated in this table. The treatments are as follows: muscle phenol (MP); liver phenol (LP); fin phenol (FP); muscle saline (MS); liver saline (LS); fin saline (FS); muscle chelex (MC); liver chelex (LC); fin chelex (FC).

Treatment	Mean of treatment	Treatments X blocks		
		B1 (*E. itajara*)	B2 (*L. jocu*)	B3 (*C. parallelus*)
MP	1809.5 ab	840.9 aB	4033.0 aA	5554.6 abB
LP	2547.6 a	660.9 aC	4471.3aA	22510.5 aB
FP	521.4 c	281.2 aA	669.7 cA	6613.2 abA
MS	821.4 bc	337.4 aA	1743.4 bcA	3383.3 bA
LS	694.3 bc	379.4 aA	1419.4 bcA	2283.9 bA
FS	433.8 c	281.5 aA	800.6 cA	2219.2 bA
MC	2165.7 a	1108.2 aB	3952.5 aA	11436.4 abB
LC	1760.5 ab	399.3 aB	3009.4 abA	11872.7 abAB
FC	482.5 c	456.1 aA	818.5 cA	1173.0 bA

Figure 2: 1% agarose gel with ladder (lane 1) and the 590 bp fragments *COI* amplified marker from *Lutjanus jocu* (LU), *Centropomus parallelus* (R) and *Epinephelus itajara* (EI), to all treatments muscle - phenol (MP); liver - phenol (LP); fin - phenol (FP); muscle - saline (MS); liver - saline (LS); fin - saline (FS); muscle - chelex (MC); liver - chelex (LC); and fin - chelex (FC), in the remaining lanes.



The phenol/chloroform procedure requires an average of seven hours of work for 12 samples. It is a traditional protocol that has historically generated good results; however, it is highly toxic to the user, this protocol has greater health risks and requires the use of laminar flow and PPE (personal protective equipment) mainly due to the reactants, phenol and chloroform (Weber *et al.* 2010).

The saline extraction method protocol takes an average of five hours of work, has low toxicity, inexpensive, and satisfactory results (Weber *et al.* 2010). Extractions using the saline protocol

exhibited higher concentrations than those obtained from the phenol/chloroform isolations performed on some samples (*E. itajara* and *C. parallelus*) and this method can to extract DNA quality with very small samples (Aljanabi & Martinez 1997). This highlights that this is a viable protocol and has several positive characteristics. Fragments amplified using the DNA extracted from the saline extraction protocol were sequenced for Cyt b, COI, and D-Loop markers. The sequencing results were satisfactory, as has been reported in other studies on fish muscle, fin, and barbell tissues (Teixeira 2011, Orrego 2012).

The Chelex resin protocol has a relatively higher cost because it is a more advanced technology, and it is more practical compared to the other two protocols. Despite 16 hours in the water bath, the time spent working on the procedures is relatively short. Indeed, it requires less than an hour for every 10 samples, and there is lower toxicity compared to the other protocols. Therefore, the cost-benefit, combined with this method's flexibility, makes the Chelex resin protocol very attractive. An important aspect to highlight is the variation in DNA concentrations extracted using Chelex, which had lower standard deviation values (Table I). Even with some discussions about the quality of DNA extracted with Chelex resin, some studies confirm that this procedure can be very useful, as Ward *et al.* (2005) that conducted a barcoding study on Australian fish with a total of 207 amplified and sequenced species using Chelex extractions, demonstrating that this protocol can be successful in a large number of fish species.

The liver and muscle samples are among the treatments that had the highest amounts of total DNA extracted; however, the specimen is sacrificed for this type of sampling. The treatments with the lowest concentrations of genetic material were those from the caudal fin (FP, FS, and FC), which are significantly different from the others (Table I). However, it was determined that despite the low amount of DNA obtained with the amplification tests, it is sufficient for performing molecular analyses using different types of markers, and it are very indicated to work with overfishing and endangered species (Taberlet *et al.* 1999, Wasko *et al.* 2003).

Based on Table II, it is possible to compare the treatments for each species and to verify that one is more effective. For *L. jocu* and *C. parallelus*, treatments that have the highest quantities of extracted DNA are the same, indicating that the most successful protocols are phenol / chloroform and Chelex for muscle and liver tissues of these species. For *L. jocu* and *C. parallelus* the treatments that showed the highest efficiency were respectively: FF, MF, MC, FC, but no significant statistic difference. And the same can be considered for *E. itajara* that had no significant statistic difference among the treatments, thus demonstrating that all tissues and protocols can be used.

Although the difference between the quantity of DNA extraction, the smallest amount reported was 49.7 ng/µl for the MS treatment of *E. itajara*, which falls within the concentration range often used to perform PCR (10-100 ng/µl), thus, it can be concluded that all of the protocols, for all tissues tested, presented satisfactory results because all samples exhibited sufficient DNA concentrations, it was confirmed in PCR test (Fig 2).

Adequate sequencing of the fragments indicated that the DNA was of good quality for downstream genetic studies, further reinforcing that the samples extracted using the saline extraction exhibited acceptable sequencing results. Thus, the saline method is a suitable extraction method because it is viable, inexpensive, and has low toxicity. Considering that the phenol/chloroform method represents a toxicity risk to the user, that using Chelex leads to lower DNA quality, and that to obtain muscle and liver we need to sacrifice the specimen, we suggest that fish DNA samples should be isolated from caudal fin tissue using the saline method, especially if dealing with endangered species.

The importance of studies with fish population genetics is increasing due to the impacts of overfishing and because endangered species, we need understand the genetic of this species, to better conservation, and for this, we need obtain a good quality DNA without sacrifice specimens. Thus, the genetic techniques of low cost and high efficiency are important to expanding the lines of research, mainly in laboratories with limited financial support for high- efficiency extraction kits that despite the quality are expensive. This work shows a cheap, safe and efficient protocol of DNA extraction, using a non-lethal sampling method.

Acknowledgments

We wish to thank the Projeto Meros do Brasil, sponsored by Petrobras-Programa Petrobras Ambiental for financial suport, Dr. Luiz Fernando Duboc for graphics and João Victor Silva and Rayane Moraes Batalha for biological material.

References

Aljanabi, S. M. & Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. **Nucleic Acids Res.** 25: 4692–4693.

Allen, G. R. 1985. FAO species catalogue. Snappers of the world. An annotated and illustrated catalogue of Lutjanid species known to date. **FAO**, Rome, Italy, 208 p.

Artoni, R. F. & Matiello, M. C. A. 2003. Neotropical fish genetics. I. Aspects of genetic conservation of fish in the State Park of Vila Velha. **Publicações da UEPG em Ciências Biológicas e da Saúde**, 9: 7-15.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1992. Single-locus and multilocus DNA fingerprinting. pp. 225–269. *In:* HOELZEL, A. R. 1 Ed. **Molecular genetic analyses of populations: A Pratical Approach**. Oxford: IRL Press, 315 p.

Carpenter, K. E. 2002. The living marine resources of the Western Central Atlantic Volume 2. Bony fishes part 1 (Acipenseridae to Grammatidae). **FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologistsand Herpetologists Special Publication**. Fao: Rome, Italy, 601-1374.

Carreiro, C. R. P., Mesquita, P. E. C., Teixeira, E. G., Vieira, C. M. T.; Lima, A. D. F. & Furtado-neto, M. A. A. 2003. Isolamento de DNA genômico de Tilápia (Oreochromis niloticus) var. Chitralada, como base para monitoramento genético. **Congresso Brasileiro de Engenharia de Pesca**, 13. Porto Seguro, p. 322.

Costa, G. W. W. F. da. 2015. Mapeamento cromossômico de DNAs repetitivos com fins biotecnológicos em peixes marinhos de interesse comercial. **Doctor thesis**, Universidade Federal do Rio Grande do Norte, Natal, Brazil, 141 p.

Damasceno, J. S., Siccha-Ramirez, R., Morales, M. J. A., Oliveira, C., Torres, R. A., 5, Costa, E. N., Silva-Oliveira, G. C., Vallinoto, M., Machado, L. F., Tosta, V. C., Farro, A. P. C., Hostim-Silva, M. 2015. Mitochondrial DNA evidences reflect an incipient population structure in Atlantic goliath grouper (Epinephelus itajara, Epinephelidae) in Brazil. **Scientia Marina**, 79:419-429.

Ferreira, M. E. & Grattapaglia, D. 1998. Introdução ao uso de marcadores moleculares em análise genética. **Embrapa Recursos Genéticos e Biotecnologia**. Brasília, Brazil, 220 p.

Gold, J. R., Voelker, G. & Renshaw, M. A. 2011. Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. **Biological Journal of Linnean Society**, 102: 915–929.

Gomes, G., Schneider, H., Vallinoto, M., Santos, S., Orti, G. & Sampaio, L. 2008. Can Lutjanus purpureus (South red snapper) be "legally" considered a red snapper (Lutjanus campechanus)? **Genetic and Molecular Biology**, 31: 372-376.

Han, H. Y. & McPheron, B. A. 1997. Molecular phylogenetic study of Tephritidae (Insecta: Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. **Molecular Phylogenetic and Evolution**, 7: 17-32.

Heemstra, P. C. & Randall, J. E. 1993. FAO species catalogue. Groupers of the world (Family Serranidae, Subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coralgrouper and lyretail species known to date. **FAO Fisheries Synopsis**, Rome, Italy, 382 p.

Hollenbeck, C. M., Portnoy, D. S., Saillant, E., Gold, J. R. 2015. Population structure of red snapper (Lutjanus campechanus) in U.S.waters of the western Atlantic Ocean and the northeastern Gulf of Mexico. **Fisheries Research**, 172: 17-25.

Orrego, L. E. O. 2012. Análise filogeográfica de Brachyplatystoma platynemum (Siluriformes: Pimelodidae). **Master thesis**, Universidade estadual Paulista, Botucatu, Brazil, 89 p.

Marengoni, N. G., Machado, M. R. F. & Gasparino, E. 2006. Extração de DNA genômico em tecidos sólidos de peixes teleósteos. **Semina: Ciências Agrárias**, 27: 99-106.

Parpinelli, R. S. & Ribeiro, R. P. 2009. Estudo comparativo de protocolos de extração de DNA em diferentes tecidos de tilápia do nilo (Oreochromis niloticus). **Global Science and Technology**, 02: 22-33.

Povh, J. A., Blanck, D. V., Petronilo V., Moreira, H. L. M., Ribeiro, R. P., Vargas, L., Cavallieri, R. F. D. & Benites, C. 2003. Comparação de duas linhagens de tilápia do Nilo (Oreochromis niloticus). **Congresso internacional de zootecnia**, 5, congresso nacional de zootecnia, 12, Uberaba, Brazil,

417-421.

Sambrook, J., Fritsh, E. F. & Matias, T. 1998. **Molecular cloning: a laboratory manual**, 2nd ed.; Cold Sring Harbor Lab Press, Cold Sring Harbor, New York.

Siquara, L. C. 2010. Padronização de protocolos para análise de diversidade genética em cavalos-marinhos (syngnathiformes: syngnathidae: hippocampus). **Degree Thesis**, Universidade Federal do Espírito, São Mateus, Brazil, 47 p.

Taberlet, P., Waits, L. P. & Luikart, G. 1999. Noninvasive genetic sampling: look before you leap. **Trends Ecologic Evolution**, 14: 323–327.

Teixeira, A. F. 2011. Análise da variabilidade e estruturação genética do tubarão azul, Prionace glauca (Chondrichthyes, Carcharhinidae) no Oceano Atlântico Sul Ocidental utilizando marcador molecular do DNA mitocondrial. **Master thesis**, Universidade Estadual Paulista, Botucatu, Brazil, 63 p.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert P. D. N. 2005. DNA barcoding Australia's fish species. **Philosophical Transaction of the Royal Society of London. Series B: Biological Sciences**, 360(1462): 1847–1857.

Wasko, A. P., Martins, C., Oliveira, C. & Foresti, F. 2003. Non-destructive genetic sampling in fish. An improved method for DNA extraction from fish fins and scales. **Hereditas**, 138: 161–165.

Weber, G. R., Disner, G. R., Nicolau, L. S., Giovanaz, G., Trott, A. & Miranda, G. B. 2010. Use of saline solution (NaCl) in DNA extraction from a hair bulb. **Evidência**, 1-2: 115-120.

Received: November 2015
Accepted: May 2016
Published: August 2016