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Comparising DNA extraction from environmental DNA samples to reveal the diversity of freshwater metazoans

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ABSTRACT. Environmental DNA (eDNA) monitoring has gained popularity in the last decade as one of the most sensitive and cost-effective monitoring methods. However, information regarding the type of DNA extraction used still needs to be studied, especially for metazoan in fresh water samples. This parameter is also critical for a project's experimental design. This study aims to compare the effectiveness of two extraction kits between DNeasy® Blood & Tissue Kit (Qiagen) silica column-based and ZymoBIOMICS 96 MagBead DNA Kit (Zymo Research) magnetic bead-based. The quantity of DNA extracts was measured using a spectrophotometer at 260/280 nm. Following that, we continued the metazoa PCR procedure. Qiagen has higher mean value of DNA concentration (88.48 ng/µl) than Zymo (20.89 ng/µl). For DNA purity, Zymo has higher mean value of DNA purity (1.84) than the Qiagen (1.59). However, both kits were equally successful in amplifying universal metazoan primers. We recommend that the use of these types of kits appears to be the least important consideration. Other important factors that may have a major impact on DNA extraction such as water volume, membrane type, sampling strategy need to be investigated in freshwater samples.

Keywords: Code River; magnetic bead-based method; metazoan biodiversity; silica column-based method; universal metazoan primers

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INTRODUCTION

The fauna of Indonesia is diverse due to its vast size as a tropical island with a variety of microhabitats in river ecosystems, notably the Code River that divides D.I.Yogyakarta into western and eastern regions. The Laboratory of Animal Systematics, Faculty of Biology, Universitas Gadjah Mada has been conducted an inventory and research on the diversity of herpetofauna and ichthyofauna along Boyong-Code River in 2012 and 2017 (Yudha *et al*., 2016; Yudha *et al*., 2019). Due to the fact that data from both research years were collected in the same month, between April and October, it is probable that certain faunal species were missed, particularly outside the sample month. Data obtained so far was based on direct faunal sampling and direct morphological determination on fauna. The direct faunal sample used the visual encounter survey and purposive sampling methods.

Animal inventory methods often face challenges due to several factors, such as extreme environments, habitat quality, distribution areas that are difficult to access and can only be detected during certain periods or weather conditions (Grattepanche *et al*., 2011; Bang *et al*., 2018; Zeppilli et *al*., 2018). However, in recent decades, many studies have shown that DNA of organisms can be extracted from environmental samples, such as water, soil, feces, mucus, and ancient sediments (Olson *et al*., 2012; Walker *et al*., 2017; Staley *et al*., 2018). DNA taken from the environmental samples is called environmental DNA (eDNA) and can be used to obtain important information on the presence or absence of target species in a specific environment. The growing use of eDNA has revolutionized researchers understanding of biodiversity studies, as it can increase the ability and resolution to detect rare and elusive

species (Bylemans *et al*., 2019; Beng & Corlett, 2020), and also opens up new avenues of research, including to obtain information that was almost inaccessible in the past few decades (Valentini *et al*., 2016; Ficetola *et al*., 2019).

eDNA has the advantage of being less invasive, the lower cost and effort, also faster than traditional species collection approaches while yet recognizing numerous species owing to metabarcoding (Huver *et al*., 2015; Pawlowski *et al*., 2018). Research on the diversity of vertebrate fauna living in and along rivers using the eDNA approach is needed to offer complete information and improved resolution for fauna detection in the Boyong-Code River. Several DNA extraction kits are commercially accessible, however it is vital to examine which kits can be used properly for DNA extraction from freshwater, especially from the river. We evaluate two commercial extraction kits, notably DNeasy® Blood & Tissue Kit (Qiagen) and the ZymoBIOMICS 96 MagBead DNA Kit (Zymo Research) to establish the two kits' efficacy in amplifying metazoan primers. Although next-generation sequencing, particularly for environmental DNA, is becoming more popular, experimental designs for revealing biodiversity, particularly DNA extraction methods, are still under investigation. This study is the first to compare two commercial extraction kits using freshwater metazoan primers. These discoveries will constitute an important basis for further studies of eDNA in freshwater.

MATERIALS AND METHODS

Samples collection. The sampling location was upstream of the Code River (Co. Hu. TS4B; 7°43'26.6"S, 110°23'21.5"E). Environmental DNA samples were collected directly in the water column from the river (Fig 1). eDNA samples were collected directly in the water column from the river. The water samples were taken as much as four liters each. The sample was filtered with 0.4 µm PCTE polycarbonate filter membrane (Sterlitech™, USA) to remove any large organisms or debris (Bruland *et al*., 2001). Subsequently, the material was filtered with 0.4 µm filter paper using a peristaltic pump. The filter paper, which is presumed to contain genetic material from the river sample, was placed in a 2 ml cryotube containing 1 ml of DNA shield.

Fig. 1. The landscape condition of eDNA sampling in Code River, D.I.Yogyakarta, Indonesia.

DNA amplification. DNeasy® Blood & Tissue Kit (Qiagen) (referred as Qiagen) and the ZymoBIOMICS 96 MagBead DNA Kit (Zymo Research) (referred as Zymo) were used to extract the eDNA from the filter papers, according to the manufacturer's instructions. We compared these two kits by extracting DNA samples 15 times each. We compared the quality and quantity of DNA extracts using spectrophotometer NANODROP 2000c (Thermo Fisher Scientific). The parameters compared in the DNA extracts were the yield

DNA concentration and the DNA purity level at the 260/280 nm. Then, we continued on the metazoa PCR process. We used the degenerate metazoan primers miCOIintF: 5′–GGWACWGGWTGAACWGTWTAYCCYCC and jgHCO2198: 5′–TAIACYTCIGGRTGICCRAARAAYCA (Deiner *et al*., 2017). The primers target a COI gene (313 bp), including enough information to identify fish to a taxonomic family, genus, and species. The first PCR reaction contained 12.5 µl of KAPA Hifi Hotstart Readymix, µl each of 1 nM primers (F and R), 4 µl ddH_2O, and 7 µl DNA template. The phases of the DNA amplification PCR profile including pre-denaturation of the template DNA at 95°C for 5 min, denaturation of the template DNA at 98°C for 10 s, annealing at 65°C for 10 s, primary extension at 72°C for 10 s, and final extension (post extension) at 72°C for 5 min with 35 cycles (Deiner *et al*., 2017). The 96 Universal peqStAR PCR machine (Peqlab Ltd, USA) was used with negative controls (blank template) to check for contamination.

PCR product quality was visualized using electrophoresis on 2% agarose gel (100 ml TAE buffer and 2 g agarose). A total of 3 µl aliquot of PCR product was then inserted into each agarose well with 100 bp DNA ladder in one of the wells. The electrophoresis machine was run at 50 V for 60 min, and the results were visualized using an UV fluorescent via an Alphaimager mini gel documentation system (Protein Simple Ltd, USA). All PCR products which passed the electrophoresis quality control underwent a second PCR for indexing purposes. The IDT double index and Illumina sequencing adapter for Illumina-Nextera DNA Unique Dual Index, Set A (Illumina, 20027213, USA) were added to the target amplicon in the second PCR, using 12.5 µl of Kapa HotStart HiFi 2 × ReadyMix DNA polymerase (Kapa Biosystems Ltd., UK) and 2 µl of PCR product. The PCR cycle comprised an initial denaturation at 95°C (3 min), then 9 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. PCR purification was conducted on the first and second PCR products using AMPure XP (Beckman Coulter Inc., US) before proceeding to the next step.

RESULTS AND DISCUSSION

To measure the efficiency of DNA isolation, the DNA purity and concentration of DNA extracted were determined. We discovered that the Qiagen kit can generate larger amounts of DNA. The mean concentration of DNA in this kit was 88.48 ng/l, but the mean concentration in the Zymo kit was lower (20.89 ng/l) (Fig. 2). The silica column is used to extract DNA from the Qiagen. The stages of the technique are lysed, bind, wash, and elute (Katevatis *et al*., 2017). This process comprises lysing target cells to liberate nucleic acids, binding the nucleic acid selectively to a silica membrane, washing away non-bound particulates and inhibitors, and elution of the nucleic acid, resulting in purified nucleic acid in an aqueous solution (Diefenbach *et al*., 2018). This principle has been demonstrated to be effective at increasing the DNA concentration in milk and stool samples during the DNA extraction process (Husakova *et al*., 2020).

The mean value of the DNA purity level (A260/280) indicates that the Zymo kit is capable of producing a better DNA purity level (1.84) than the Qiagen kit (1.59) (Fig. 2). The Zymo is a commercial product that purifies DNA using the magnetic bead principle. Magnetic beads are an efficient way to concentrate biomarkers and remove background biomolecules (Bordelon *et al*., 2013; Bitting *et al*., 2015). The magnetic beads are then collected magnetically, and the biomarkers captured on their surface are released into a more amenable reaction buffer (Sasso *et al*., 2012; Shields *et al*., 2012; Bordelon *et al*., 2013). In comparison to the spin column approach, the extraction of DNA using magnetic beads has been widely reported to be successful (Husakova *et al*., 2020), including eDNA studies (Shahraki *et al*., 2019; Wood *et al*., 2019).

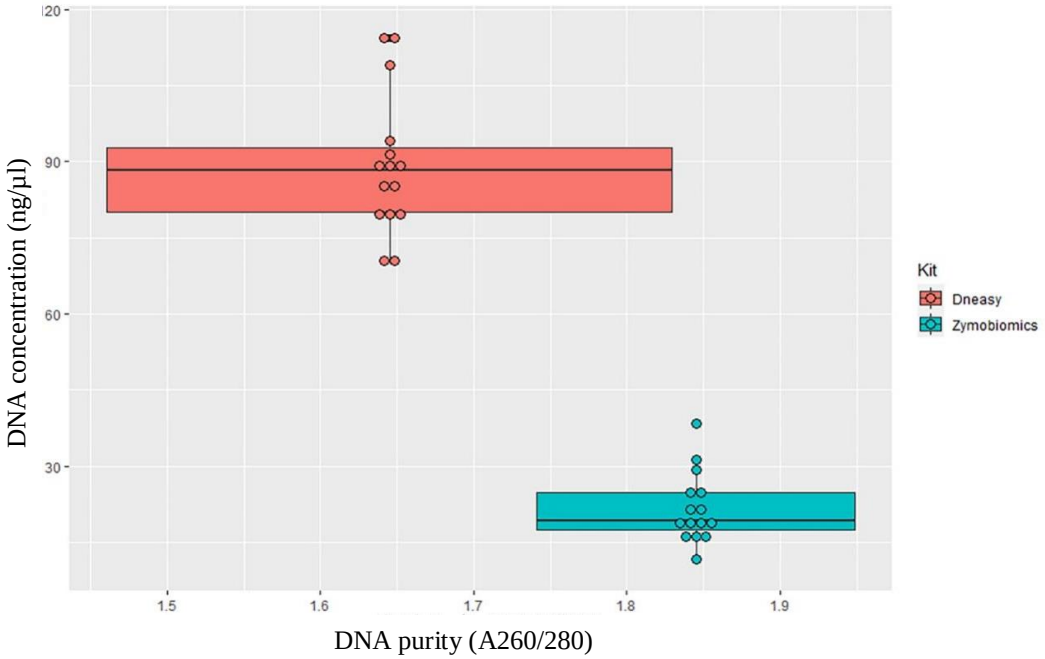

Fig. 2. Comparison of DNA extraction result based on DNA concentration and purity.

The findings of PCR amplification for metazoan primers indicated that no sample was not amplified successfully (Fig. 3). The data indicate that both kits had a very high success rate for amplification (100%). This finding demonstrated that, despite the kits' discrepancies in DNA purity and concentration, both kits successfully amplified metazoan primer. Additionally, based on this finding, when it comes to selecting eDNA capture sample methodologies, the extraction kit appears to be the least significant factor to consider.

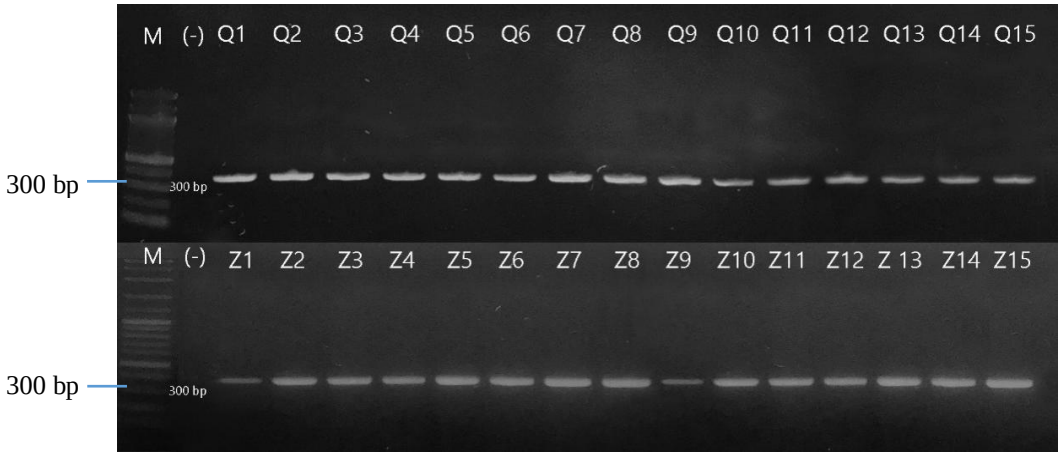

Fig. 3. PCR amplification results using miCOIintF primer for metazoa with target length of 313 bp (M= DNA ladder; (-) = Negative control; Q1-15= Sample with DNeasy® Blood & Tissue Kit (Qiagen) protocol; Z1-15= sample with ZymoBIOMICS 96 MagBead DNA Kit (Zymo Research) protocol).

On other cases, extraction processes utilizing commercial kits produced identical results (Djurhuus *et al*., 2017; Muha *et al*., 2019). Metazoan primer having a 313-bp target sequence that has been demonstrated to be effective in amplifying a variety of metazoa in eDNA studies (Elbrecht & Leese, 2017; Pawlowski *et al*., 2018). The use of eDNA metabarcoding has been pushed as an useful technique for gathering inventories of aquatic organisms. It has already been established that employing universal primers, a combination of different capture and extraction procedures can result in significantly varying success rates for

eDNA metabarcoding for different target groups (Djurhuus *et al*., 2017; Stat *et al*., 2017). Several critical aspects affecting the performance of river eDNA studies include the number of water samples used (Piaggio *et al*., 2014; Cantera *et al*., 2019), the sampling strategy (Carraro *et al*., 2021), the preservation (Williams *et al*., 2016), and the type of filtration (Lacoursière-Roussel *et al*., 2016; Muha *et al*., 2019). We found interesting results that each extraction kit has its own advantages in DNA concentration and purity, but no difference when amplifying metazoan primers. This result is certainly an important basis for the eDNA study which is still wide open in revealing Indonesia's biodiversity.

CONCLUSION

Qiagen kit which uses the silica column principle has a higher DNA concentration level (88.48 ng/µl) than Zymo kit (20.89 ng/µl). For DNA purity, Zymo kit which uses the principle of magnetic beads, has a higher level of purity (1.84) compared to the Qiagen kit (1.59). However, both kits were equally successful in amplifying universal metazoan primers. We recommend that the use of these types of kits appears to be the least important consideration. Other important factors that may have a major impact on DNA extraction such as water volume, membrane type, sampling strategy need to be investigated in freshwater samples.

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REFERENCES

Bang C, Dagan T, Deines P, Dubilier N, Duschl WJ, Fraune S, Hentschel U, Hirt H, Hülter N, Lachnit T, Picazo D, Pita L, Pogoreutz C, Rädecker N, Saad MM, Schmitz RA, Schulenburg H, Voolstra CR, Bosch TCG. 2018. Metaorganisms in extreme environments: do microbes play a role in organismal adaptation?. *Zoology*. vol 127: 1–9. doi: https://doi.org/10.1016/j.zool.2018.02.004.

Beng KC, Corlett RT. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodiversity and Conservation*. vol 29(7): 2089–2121. doi: https://doi.org/10.1007/s10531-020-01980-0.

Bitting AL, Bordelon H, Baglia ML, Davis KM, Creecy AE, Short PA, Albert LE, Karhade AV, Wright DW, Haselton FR, Adams NM. 2016. Automated device for asynchronous extraction of RNA, DNA, or protein biomarkers from surrogate patient samples. *Journal of Laboratory Automation*. vol 21(6): 732–742. doi: https://doi.org/10.1177%2F2211068215596139.

Bordelon H, Russ PK, Wright DW, Haselton FR. 2013. A magnetic bead-based method for concentrating DNA from human urine for downstream detection. *PloS One*. vol 8(7): 1–9. doi: https://doi.org/10.1371/journal.pone.0068369.

Bruland KW, Rue EL, Smith GJ. 2001. Iron and macronutrients in California coastal upwelling regimes: Implications for diatom blooms. *Limnology and Oceanography*. vol 46(7): 1661–1674. doi: https://doi.org/10.4319/lo.2001.46.7.1661.

Bylemans J, Gleeson DM, Duncan RP, Hardy CM, Furlan EM. 2019. A performance evaluation of targeted eDNA and eDNA metabarcoding analyses for freshwater fishes. *Environmental DNA*. vol 1(4): 402–414. doi: https://doi.org/10.1002/edn3.41.

Cantera I, Cilleros K, Valentini A, Cerdan A, Dejean T, Iribar A, Taberlet P, Vigouroux R, Brosse S. 2019. Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. *Scientific Reports*. vol 9(1): 1–11. doi: https://doi.org/10.1038/s41598-019-39399-5.

Carraro L, Stauffer JB, Altermatt F. 2021. How to design optimal eDNA sampling strategies for biomonitoring in river networks. *Environmental DNA*. vol 3(1): 157–172. doi: https://doi.org/10.1002/edn3.137.

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, De Vere N, Pfrender ME, Bernatchez L. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*. vol 26(21): 5872–5895. doi: https://doi.org/10.1111/mec.14350.

Diefenbach RJ, Lee JH, Kefford RF, Rizos H. 2018. Evaluation of commercial kits for purification of circulating free DNA. *Cancer Genetics*. vol 228–229: 21–27. doi: https://doi.org/10.1016/j.cancergen.2018.08.005.

Djurhuus A, Port J, Closek CJ, Yamahara KM, Romero-Maraccini O, Walz KR, Goldsmith DB, Michisaki R, Breitbart M, Boehm AB, Chavez FP. 2017. Evaluation of filtration and DNA extraction methods for environmental DNA biodiversity assessments across multiple trophic levels. *Frontiers in Marine Science*. vol 4: 1–11. doi: https://doi.org/10.3389/fmars.2017.00314.

Elbrecht V, Leese F. 2017. Validation and development of COI metabarcoding primers for freshwater

macroinvertebrate bioassessment. *Frontiers in Environmental Science*. vol 5: 1–11. doi: https://doi.org/10.3389/fenvs.2017.00011.

Ficetola GF, Manenti R, Taberlet P. 2019. Environmental DNA and metabarcoding for the study of amphibians and reptiles: species distribution, the microbiome, and much more. *Amphibia Reptilia*. vol 40(2): 129–148. doi: https://doi.org/10.1163/15685381-20191194.

Grattepanche JD, Breton E, Brylinski JM, Lecuyer E, Christaki U. 2011. Succession of primary producers and micrograzers in a coastal ecosystem dominated by *Phaeocystis globosa* blooms. *Journal of Plankton Research*. vol 33(1): 37–50. doi: https://doi.org/10.1093/plankt/fbq097.

Husakova M, Kralik P, Babak V, Slana I. 2020. Efficiency of DNA isolation methods based on silica columns and magnetic separation tested for the detection of *Mycobacterium avium* subsp. *Paratuberculosis* in milk and faeces. *Materials.* vol 13(22): 1–11. doi: https://doi.org/10.3390/ma13225112.

Huver JR, Koprivnikar J, Johnson PT, Whyard S. 2015. Development and application of an eDNA method to detect and quantify a pathogenic parasite in aquatic ecosystems. *Ecological Applications*. vol 25(4): 991–1002. doi: https://doi.org/10.1890/14-1530.1.

Katevatis C, Fan A, Klapperich CM. 2017. Low concentration DNA extraction and recovery using a silica solid phase. *PLoS One*. vol 12(5): 1–14. doi: https://doi.org/10.1371/journal.pone.0176848.

Lacoursière-Roussel A, Rosabal M, Bernatchez L. 2016. Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Molecular Ecology Resources*. vol 16(6): 1401–1414. doi: https://doi.org/10.1111/1755-0998.12522.

Muha TP, Robinson CV, de Leaniz CG, Consuegra S. 2019. An optimised eDNA protocol for detecting fish in lentic and lotic freshwaters using a small water volume. *PloS One*. vol 14(7): 1–20. doi: https://doi.org/10.1371/journal.pone.0219218.

Olson ZH, Briggler JT, Williams RN. 2012. An eDNA approach to detect eastern hellbenders (Cryptobranchus a. alleganiensis) using samples of water. *Wildlife Research*. vol 39(7): 629–636. doi: https://doi.org/10.1071/WR12114.

Pawlowski J, Kelly-Quinn M, Altermatt F, Apothéloz-Perret-Gentil L, Beja P, Boggero A, Borja A, Bouchez A, Cordier T, Domaizon I, Feioh MJ, Filipe AF, Fornaroli R, Graf W, Herderk J, der Hoorn B, Jones JI, Sagova-Mareckovan M, Moritz C, Barquín J, Piggott JJ, Pinna M, Rimet F, Rinkevichs B, Sousa-Santos C, Specchia V, Trobajo R, Vasselon V, Vitecek S, Zimmerman J, Weigand A, Leese F, Kahlert M. 2018. The future of biotic indices in the ecogenomic era: Integrating (e) DNA metabarcoding in biological assessment of aquatic ecosystems. *Science of the Total Environment.* vol 637–638: 1295–1310. doi: https://doi.org/10.1016/j.scitotenv.2018.05.002.

Piaggio AJ, Engeman RM, Hopken MW, Humphrey JS, Keacher KL, Bruce WE, Avery ML. 2014. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. *Molecular Ecology Resources*. vol 14(2): 374–380. doi: https://doi.org/10.1111/1755-0998.12180.

Sasso LA, Johnston IH, Zheng M, Gupte RK, Ündar A, Zahn JD. 2012. Automated microfluidic processing platform for multiplexed magnetic bead immunoassays. *Microfluidics and Nanofluidics*. vol 13: 603–612. doi: https://doi.org/10.1007/s10404-012-0980-0.

Shahraki AH, Chaganti SR, Heath D. 2019. Assessing high-throughput environmental DNA extraction methods for meta-barcode characterization of aquatic microbial communities. *Journal of Water and Health*. vol 17(1): 37–49. doi: https://doi.org/10.2166/wh.2018.108.

Shields MJ, Hahn KR, Janzen TW, Goji N, Thomas MC. Bin Kingombe CI, Paquet C, Kell AJ, Amoako KK. 2012. Immunomagnetic capture of *Bacillus anthracis* spores from food. *Journal of Food Protection*. vol 75: 1243–1248. doi: https://doi.org/10.4315/0362-028X.JFP-12-048.

Staley ZR, Chuong JD, Hill SJ, Grabuski J, Shokralla S, Hajibabaei M, Edge TA. 2018. Fecal source tracking and eDNA profiling in an urban creek following an extreme rain event. *Scientific Reports*. vol 8(1): 1–12. doi: https://doi.org/10.1038/s41598-018-32680-z.

Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, Newman SJ, Harvey ES, Bunce M. 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports.* vol 7(1): 1–11. doi: https://doi.org/10.1038/s41598-017-12501-5.

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, Gaboriaud C, Jean P, Poulet N, Roset N, Copp GH, Geniez P, Pont D, Argillier C, Baudoin JM, Peroux T, Crivelli AJ, Olivier A, Acqueberge M, Le Brun M, Møller PR, Willerslev E, Dejean T. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*. vol 25(4): 929–942. doi: https://doi.org/10.1111/mec.13428.

Walker DM, Leys JE, Dunham KE, Oliver JC, Schiller EE, Stephenson KS, Kimrey JT, Wooten J, Rogers MW. 2017. Methodological considerations for detection of terrestrial small-body salamander eDNA and implications for biodiversity conservation. *Molecular Ecology Resources*. vol 17(6): 1223–1230. doi: https://doi.org/10.1111/1755-0998.12667.

Williams KE, Huyvaert KP, Piaggio AJ. 2016. No filters,

no fridges: a method for preservation of water samples for eDNA analysis. *BMC Research Notes*. vol 9(1): 1–5. doi: https://doi.org/10.1186/s13104-016-2104-5.

Wood SA, Pochon X, Laroche O, von Ammon U, Adamson J, Zaiko A. 2019. A comparison of droplet digital polymerase chain reaction (PCR), quantitative PCR and metabarcoding for species-specific detection in environmental DNA. *Molecular Ecology Resources*. vol 19(6): 1407–1419. doi: https://doi.org/10.1111/1755-0998.13055.

Yudha, DS, Eprilurahman R, Jayanto H, Wiryawan IF. 2016. Keanekaragaman jenis kadal dan ular (Squamata: Reptilia) di sepanjang Sungai Code Daerah Istimewa Yogyakarta. *Biota: Jurnal Ilmiah Ilmu-ilmu Hayati*. vol 1(1): 31–38. doi: https://dx.doi.org/10.24002/biota.v1i1.710.

Yudha DS, Akmal WR, Eprilurahman R. 2019. Monitoring anurans diversity along Code River, Province of Daerah Istimewa Yogyakarta, Indonesia. *Biogenesis: Jurnal Ilmiah Biologi*. vol 7(2): 132–138. doi: https://doi.org/10.24252/bio.v7i2.10174.

Zeppilli D, Leduc D, Fontanier C, Fontaneto D, Fuchs S, Gooday AJ, Goineau A, Ingels J, Ivanenko VN, Kristensen RM, Neves RC, Sanchez N, Sandulli R, Sarrazin J, Sørensen MV, Tasiemski A, Vanreusel A, Autret M, Bourdonnay L, Claireaux M, Coquillé V, De Wever L, Rachel D, Marchant J, Toomey L, Fernandes D. 2018. Characteristics of meiofauna in extreme marine ecosystems: a review. *Marine Biodiversity*. vol 48(1): 35–71. doi: https://doi.org/10.1007/s12526-017-0815-z.