Avens Publishing Group
J Analyt Molecul Tech
January 2025 Volume:4, Issue:1

Parboiled Preservation of Odonata Nymphs for DNA Related Research

Open Access Research Article

Journal of Analytical & Molecular Techniques

Sutherland LN[1]*, Fomekong-Lontchi J[1,5]*, Lupiyaningdyah P[1,4], Tennessen KJ[3], Carter P[1] and Bybee SM[1,2]

[1]Department of Biology, Brigham Young University, 4102 LSB, Provo, UT 84602, USA
[2]Bean Life Science Museum, Brigham Young University, Provo, UT 84602, USA
[3]Florida State Collection of Arthropods, Gainesville, Florida, USA
[4]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia
[5]Institute of Medical Research and Medicinal Plants Studies (IMPM), Centre of Medical Research, P.O Box: 6163 Yaoundé, Cameroon

***Address for Correspondence:**
Sutherland LN, Department of Biology, Brigham Young University, 4102 LSB, Provo, UT 84602, USA E-mail Id: lns25@byu.edu

Submission: 09 December, 2024
Accepted: 15 January, 2025
Published: 20 January, 2025

Keywords

Dragonfly; Damselfly; Nymphs; DNA; Parboil

Abstract

Odonate nymph preservation is less standardized and more prone to long-term preservation issues than adults.Two preemptive measures that can be taken to help prevent the high level of decay often seen in older nymph specimens are 1. Ethanol injection 2. Parboiling. Parboiling offers the long-term advantage of better retaining the morphology, which is a great advantage for future taxonomic projects. Here we found that parboiling is as good for preserving DNA as ethanol injection.

Introduction

Natural history collections have been the backbone of systematic and taxonomic research by housing well-preserved specimens that offer repeatability and comparability in phenotypic analyses [1]. The importance of natural history collections has received a growing amount of attention [1-6] in the face of the biodiversity crisis and continual underfunding. This resurgence in museum interest is due in part to the increase in digitization efforts and advances in technology that allows for the sharing of data at a global scale [7]. Museum specimens have long been used in large-scale evolutionary, biodiversity, and ecological research, but the increase in publicly available data has created opportunities for broader and more collaborative research to take place [8]. Recently, there have been calls to address issues (*e.g.,* lack of administrative support, understaffing, declines in specimens being deposited, specimen degradation) in natural history collections to set them up for success in this next phase of broader museum-based research [4,1]. One way to ensure museum-based research will continue long into the future is to consistently use best practices for long-term preservation and storage methods, which will vary by group.

In general, insect collections are extensive, and odonates (dragonflies and damselflies) collections are no exception. For instance, a recent survey was conducted at 13 institutions that possess at least 100 odonate-type specimens [9]. Among these are the Florida State Collection of Arthropods (~1.1 million specimens), the Naturalis Biodiversity Center (~200,000 specimens), and the Natural History Museum at London (~110,000 specimens) demonstrating the sheer size of odonate collections. Due to the scale of these collections and limited expertise, there is often a backlog of processing and taxonomic work where specimens potentially sit for years, so morphological and genetic preservation is critical.

Odonata is a medium-sized hemimetabolous order that has become highly studied and collected due to brilliant coloration, charismatic behavior, and aquatic lifestyle. Historically, odonates have been used as biological indicators of freshwater health [10-13] and often collected as nymphs in large biodiversity projects and deposited in museums for later identification. While adult taxonomy is relatively well known, nymph taxonomy is greatly understudied, with less than half of the recognized species having a documented nymph, as more effort has traditionally been spent on rearing nymphs to adults for identification [14]. While nymph taxonomy is greatly lagging behind adult taxonomy, there has been an effort to close this gap in recent decades. For example, the majority of species in *Ischnura* are known compared to approximately 20% of *Telebasis*. Historically, more work has been done on Anisoptera than Zygoptera, but early nymph descriptions are often brief, lacking detailed illustrations, and in need of reexamination, highlighting the need for well-preserved nymph specimens in collections.

Adult odonates preservation has become standardized with three methods. First, specimens are air-dried and stored in glassine envelopes without any additional preservation measures, which allows for the coloration to fade over time and provides inconsistent results in genetic studies. Second, the specimens are adjusted (*i.e.*, wings folded above the abdomen and legs stretched) and then soaked in acetone for 12-14 hours after which they are dried and again stored in glassine envelopes. Acetone is excellent for preserving coloration which is an important character in adult odonate taxonomy and evolutionary research. Lastly, adults are stored in 70%-95% ethanol. Ethanol preservation is ideal for genetic studies but can cause significant color fading if left in the light.

Nymph preservation is less standardized and more prone to long-term preservation issues than adults. Nymphs generally are preserved straight into 70-95% ethanol, but ethanol does not readily penetrate the cuticle, and often results in the specimen decaying to varying degrees [15,16]. Perforations are often made in the abdomen of large

ISSN: 2474-1914

specimens in an attempt to help the ethanol penetrate the specimen, but this compromises morphological features that could be needed for identification in the future. Also, neglecting to refresh the ethanol will affect the long-term preservation of DNA. There are two preemptive measures (ethanol injection and parboiling) that can be taken to help prevent this high level of decay. Ethanol injection, a fairly common preemptive measure, is when 95% ethanol is directly injected into the specimens upon capture. Parboiling, a technique not often utilized in odonates, consists of dispatching the specimen by placing it briefly in boiling water [14]. Boiling insects fixes the proteins in the body and prevents them from decaying over time [17].

Parboiling offers the long-term advantage of retaining the morphological dimensions and patterns of the specimens, closer to what they are under living conditions, which is a great advantage for future taxonomic projects. The benefits of parboiling have been demonstrated in other groups. For example, caterpillars, if not boiled, will decompose and turn black [18,19]. Parboiling has additionally been shown to be effective in forensic research when estimating post-mortem intervals as it keeps the size, coloration, and internal organs of larvae closer to their natural states [20]. Although parboiling provides long-term stability of morphological features in odonate nymphs (KTJ, personal comment) it is still unclear how efficiently it preserves genetic material. Here we aim to

1. Test if there is a difference between the DNA quantity captured based on the initial preservation method (parboiled vs. ethanol injected) in odonate nymphs and
2. Test if there is a difference in DNA degradation (fragment length) between the initial preservation methods.

Materials and Methods

Specimen preservation and sampling

Odonate nymphs were collected from multiple locations in Wisconsin, and one location in New York. Freshly collected nymphs were initially preserved in one of two ways: ethanol injection or parboiling. Parboiling consisted of submerging the nymph in boiling water for 30–60 seconds, depending on size. All specimens, regardless of initial preservation method, were stored in 80% ethanol in a -20°C freezer. Thirty-eight specimens were selected for extraction which represent nine species (6 Anisoptera, 3 Zygoptera) (Table 1).

Table 1: Number of specimens for extraction by preservation method for each species.

Suborder	Family	Species	# EtOH injected specimens	# Parboiled specimens
Anisoptera	Aeshnidae	*Anax junius*	2	4
	Cordulegasteridae	*Cordulegaster maculata*	2	2
	Corduliidae	*Epitheca spinigera*	2	2
	Gomphidae	*Phanogomphus spicatus*	2	2
	Libellulidae	*Ladona julia*	2	4
		Leucorrhinia intacta	2	2
Zygoptera	Calopterygidae	*Calopteryx maculata*	0	2
	Coenagrionidae	*Enallagma boreale*	2	2
	Lestidae	*Lestes eurinus*	2	2

DNA Extraction and Quantification

For each species at least four specimens (two per initial preservation method) were extracted, except for *Calopteryx maculata* where no ethanol injected specimen was available (Table 1). Each specimen was extracted three times (pro-, meso- and metathoracic legs) and the variation in concentration between species is depicted (Figure 1). DNA extractions were performed using a Qiagen DNeasy Blood & Tissue kit (Valenica, CA) following manufacturer protocols, with two exceptions. The sample was incubated at 56°C for 72 hours during tissue lysis and the final elution volume was changed to 25 µL which resulted in a final volume of ~45 µL. A Qubit 4 fluorometer was used to quantify the DNA concentration with the dsDNA High sensitivity procedure.

DNA Degradation

To test for DNA degradation over time, specimens were re-extracted in 2024, three years after the initial extractions. In total 32 extractions were selected, 16 extractions (8 ethanol injected, 8 parboiled) from 2021 and 16 new extractions performed in 2024. All but *Lestes eurinus* was re-extracted. As an estimate for DNA degradation, a fragment analysis was performed using the Agilent Genomic 55 kb BAC Kit Quick Guide for Femto Pulse Systems at the BYU DNA Sequencing Center. Two parboiled extractions from 2024 (*Calopteryx maculata* and *Leucorrhinia intacta*) were removed from further analysis as no fragment length was recovered.

Statistical analysis

To test for a difference in DNA concentration between the initial preservation methods (ethanol injected and parboiled), a Mann-Whitney U Test was run in Rstudio v. 4.4.1 using the wilcox.test function in the *dplyr* package [21]. To test the stability of the genetic material by initial preservation two Mann-Whitney U Tests were run, one for each year (2021 and 2024).

Results

While there is variation in the DNA concentration between species (Figure 1), in general, the average DNA concentration (ng/µl) was higher for species in Anisoptera (24.3) than Zygoptera (11.5). The average DNA concentration was 20.7 and 21.0 for ethanol injected and parboiled specimens, respectively. The results of the Mann-Whitney U Test showed that there is no significant difference (W = 1590.5, p-value = 0.97) in DNA concentration recovered between parboiled and ethanol-injected specimens (Figure 2).

For the extractions completed in 2021, the average fragment length (bp) for ethanol injected specimens was 6,620 compared to 7,529 for the parboiled specimens. However, based on the results of the Mann-Whitney U Test, there is no difference (W = 25, p-value = 0.49) in fragment length between the methods. For the extractions completed in 2024, the average fragment length (bp) for ethanol injected specimens was 8,113 compared to 6,754 for the parboiled specimens. The results of the Mann-Whitney U Test for the 2024 extractions indicate that there is no difference (W = 33, p-value = 0.27) in fragment length between the methods (Figure 3).

ISSN: 2474-1914

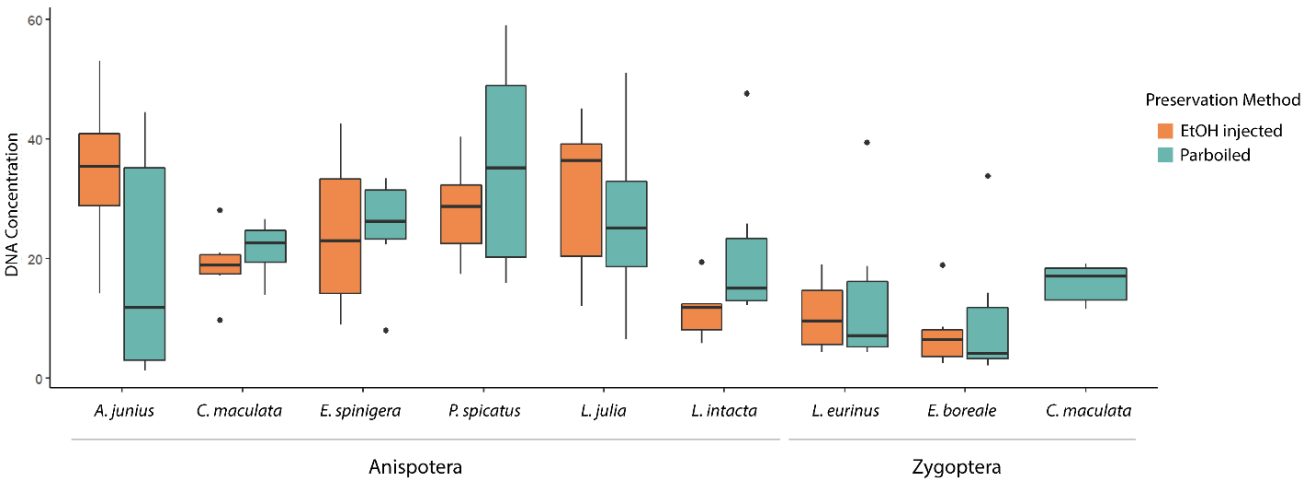

Figure 1: Variation within DNA concentration (ng/µl) by species and preservation method.

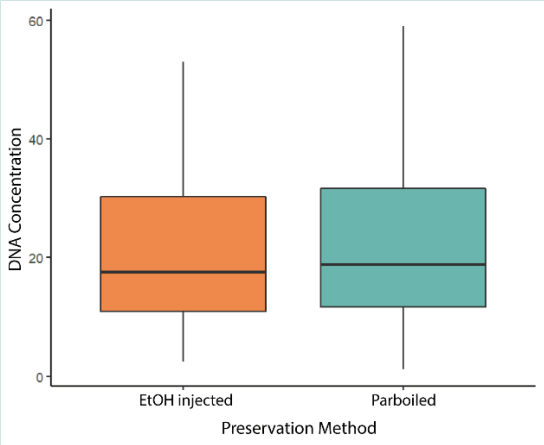

Figure 2: Variation in recovered DNA concentration (ng/µl) based on initial preservation method.

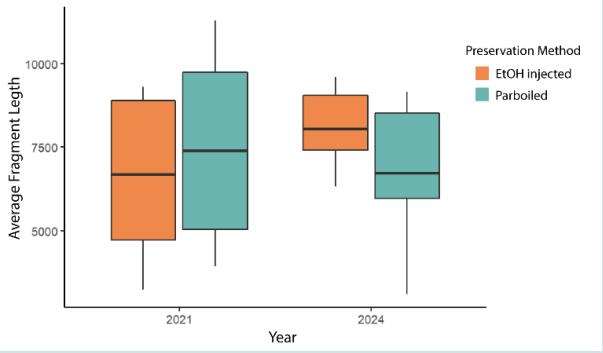

Figure 3: Variation in average DNA fragment length (bp) by initial preservation method over time.

ISSN: 2474-1914

Discussion

The results above demonstrate that there is no difference in DNA quantity or quality between initial preservation methods (ethanol injected and parboiling) over time. Therefore, to better preserve nymph morphology, parboiling should be the standard practice. Parboiling has the benefit of slowing down degradation caused by enzymes, which helps preserve the morphological integrity of the nymph [20]. For long-term storage, nymphs should be kept in either high concentration ethanol (95-100) or -80°C freezers with a lower ethanol concentration (70-100). While both have issues, such as brittle specimens leading to a high probability of breakage for high concentration ethanol specimens, and DNA degradation due to the rapid freezing and thawing when working with -80°C freezers, they are still the current best practices [22,23].

Nymph taxonomy and identification are based almost solely on the final instar. However, with the high level of adult sequences that are publicly available, it is possible to associate unknown nymphs at various stages with relative ease and accuracy. This approach will allow researchers to get better insight into the different instars, there by expanding our understanding of nymph development, morphology, and taxonomy. Additionally, it will allow for increased use of museum s pecimens, as more nymphs will be utilized which are not at final instar. The benefits will be increased if parboiled specimens are utilized, as a more detailed description can be provided at the end.

Relatively little is known about which morphological nymph characters demonstrate phylogenetic synapomorphies across the order, although some integrative studies have appeared [24-27]. Nymph characters have been found to help resolve generic limits in the past [28-30]. Questions remain regarding whether certain mouthparts (*i.e.,*premental and mandibular structure), thoracic morphology, and lateral abdominal and anal gills show relationships within or between groups [29,14]. Therefore, nymph preservation is critical to all stages of systematic, phylogenetic, and evolutionary research.

Acknowledgments

We would like to thank the BYU undergraduates who helped with DNA extractions and the BYU sequencing center for performing the fragment analysis. We also thank Dr. Dennis Paulson for information on the importance of insect collections for many fields of biology.

References

1. Hilton EJ, Watkins-Colwell GJ, Huber SK (2021)The expanding role of natural history collections. Ichthyology & Herpetology 109: 379-391.
2. Bradley RD, Bradley LC, Garner HJ, Baker RJ (2014) Assessing the value of natural history collections and addressing issues regarding long-term growth and care. BioScience64: 1150-1158.
3. Gropp RE (2018) Specimens, collections, and tools for future biodiversity-related research. BioScience68: 3-4.
4. Miller SE, Barrow LN, Ehlman SM, Goodheart JA, Greiman SE, et al. (2020) Building natural history collections for the twenty-first century and beyond. BioScience70: 674-687.
5. Suarez AV, Tsutsui ND (2004) The value of museum collections for research and society. BioScience 54: 66-74.
6. Pyke GH, Ehrlich PR (2010) Biological collections and ecological/ environmental research: a review, some observations and a look to the future. Biological reviews 85: 247-266.
7. Rocha LA, Aleixo A, Allen G, Almeda F, Baldwin CC, et al. (2014) Specimen collection: An essential tool. Science 344: 814-815.
8. Meineke EK, Davies TJ, Daru BH, DavisCC (2019)Biological collections for understanding biodiversity in the Anthropocene. Philosophical Transactions of the Royal Society B374: 20170386.
9. AbbottJC, SandallEL (2022)Odonata collections and databases. Dragonflies and Damselflies Pp: 327-340.
10. Heino J, Soininen J, Lappalainen J, Virtanen R (2005) The relationship between species richness and taxonomic distinctness in freshwater organisms. Limnology and Oceanography 50: 978-986.
11. O'Malley ZG, Compson ZG, Orlofske JM, Baird DJ, Curry RA, et al. (2020). Riparian and in-channel habitat properties linked to dragonfly emergence. Scientific reports10: 1-12.
12. Polhemus DA (1993) Damsels in distress: a review of the conservation status of Hawaiian Megalagrion damselflies (Odonata: Coenagrionidae). Aquatic Conservation: Marine and Freshwater Ecosystems 3: 343-349.
13. Vorster C, Samways MJ, Simaika JP, Kipping J, Clausnitzer V, et al. (2020) Development of a new continental-scale index for freshwater assessment based on dragonfly assemblages. Ecological Indicators 109: 105819.
14. Tennessen KJ (2019) Dragonfly nymphs of North America: an identification guide. Springer.
15. Dijkstra K.-DB, Clausnitzer V (2014) The dragonflies and damselflies of eastern Africa: Handbook for all Odonata from Sudan to Zimbabwe.
16. Suhling F, Sahlén G, Gorb SN, KalkmanVJ, DijkstraK.-DB, et al. (2015)Order Odonata.Thorp and Covich's Freshwater Invertebrates 1: 893-932.
17. Adams ZJ, Hall MJ. (2003). Methods used for the killing and preservation of blowfly larvae, and their effect on post-mortem larval length. Forensic Science International 138:50-61.
18. Solis MA, Harms NE, Phillips-Rodríguez E, Scheffer SJ, Lewis LM, Janzen, et al. (2018)Aquatic Larvae of Two Acentropines, Usingeriessaonyxalis (Hampson) and Oxyelophilacallista (Forbes) (Lepidoptera: Crambidae). Proceedings of the Entomological Society of Washington 120: 180-195.
19. Winter WD (2000) Basic techniques for Observing and studying Moths and butterflies. Memoirs of the Lepideptorists' Society 5: 1-444.
20. Brown K., Thorne A, Harvey M (2012) Preservation of Calliphoravicina (Diptera: Calliphoridae) pupae for use in post-mortem interval estimation. Forensic Science International 223: 176-183.
21. Wickham H, François R, Henry L, Müller K, Vaughan D (2023) dplyr: A Grammar of Data Manipulation. R package version 1.1.4.
22. King JR, Porter SD (2004)Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae). Insectes Sociaux 51 : 197-202.
23. Marquina D, Buczek M, Ronquist F, Łukasik P (2021) The effect of ethanol concentration on the morphological and molecular preservation of insects for biodiversity studies. PeerJ 9: e10799.
24. BlankeAC, GreveR, MoskoF, BeckmanB, Misof(2013)An updated phylogeny of Anisoptera including formal convergence analysis of morphological characters. Systematic Entomology 38:474–490.
25. CarleF,Kjer K,May M(2015) A molecular phylogeny and classification of Anisoptera (Odonata). Arthropod Systematics & Phylogeny 73:281-301.
26. KohliMK,Ware JL,Bechly G (2016) How to date a dragonfly: Fossil calibrations for odonates. Paleontologia Electronica 19:1-14.
27. Letsch H, Gottsberger B, Ware JL (2016) Not going with the flow: a comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. Molecular Ecology 25: 1340-1353.
28. WestfallMJ (1988)Elasmothemis gen. nov., a new genus related to Dythemis

ISSN: 2474-1914

(Anisoptera: Libellulidae). Odonatologica17:419-428.

29. RamirezA, Novelo-Gutiérrez R(1999). The Neotropical dragonfly genus Macrothemis: new larval descriptions and an evaluation of its generic status based on larval stages (Odonata: Libellulidae). Journal of the North American Benthological. Society 18:67-73.

30. FleckG, Brenk M, Misof B (2008)Larval and molecular characters help to solve phylogenetic puzzles in the highly diverse dragonfly family Libellulidae (Insecta: Odonata: Anisoptera): The Tetrathemistinae are a polyphyletic group. Organisms Diversity & Evolution 8:1-16.

31. Büsse S, Hörnschemeyer T, Gorb SN (2017)The head morphology of Pyrrhosomanymphula larvae (Odonata: Zygoptera) focusing on functional aspects of the mouthparts. Frontiers in Zoology14:25.

32. ArnoldPR, LordNP, SmithAN, BybeeSM (2016)The effects of non-ideal temperature regimes on RNA quality from samples stored in RNAlater-like buffer: an attempt to replicate field conditions. Journal of Analytical & Molecular Techniques2: 1-8.

33. CorbetPS(1999)Dragonflies: behaviour and ecology of Odonata. Harley books.

34. KutcherTE, BriedJT(2014)Adult Odonata conservatism as an indicator of freshwater wetland condition. Ecological Indicators38: 31-39.