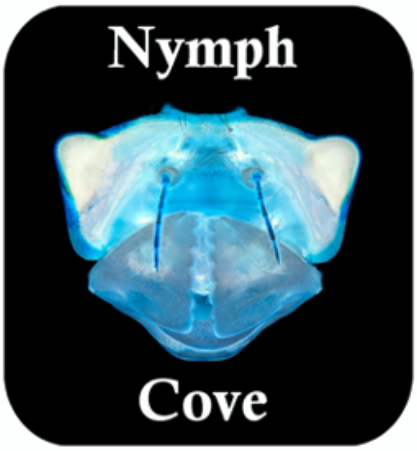
# Nymph Cove: Preserving and Storing Odonate Nymphs and Exuviae




**By Marla C Garrison and Ken J Tennessen**

In the previous Nymph Cove installment, we discussed how and where to collect nymphs. Of course, a major goal of collecting nymphs is to learn how to identify them. Once proficient in identifying local species, final and penultimate instars can often be diagnosed in hand and released on site after data is recorded (i.e. common or uncommon, size classes present, near emergence? etc.). However, if the nymphs cannot be identified in hand, and/or they are purposefully collected for a specific scientific study, it is necessary to retain and preserve them. Sampling nymphs is an important means of documenting habitat, maintaining natural history databases, identifying species assemblages, assessing population trends, and obtaining other valuable life history information. As with all aquatic insects, identifying many species of Odonata nymphs requires examining small details, taking precise measurements, and counting minute setae, all exercises best conducted under a dissecting microscope. This usually means collecting and preserving nymphs or exuviae; we discuss here the most proven methods (in our experience) to ensure their proper preservation and storage.

Here in Nymph Cove, we encourage the collection of exuviae whenever possible (no, we're not open to changing the title to Exuviae Cove). Exuviae are, after all, the best proof of successful breeding and development in a particular aquatic habitat, and most can be specifically identified. Adding to that, an exuvia is the exoskeleton (nonliving integument) left behind by the metamorphosing insect, so no loss of insect life is inflicted when exuviae are collected. However, there are occasions when exuviae are either damaged and unidentifiable, not available seasonally or are in habitats such as peatlands or heavily vegetated seeps where they are difficult to find. So, we realize that, under certain circumstances, nymph collecting is warranted. Therefore, in this installment we describe techniques for preserving and storing both exuviae and nymphs. Proper curation of specimens is essential for them to be of long-term significance for evaluating a species' past, present, and future and for using them as a source of viable DNA for future molecular research. Whether netting and releasing or actually collecting nymphs and exuviae, researchers and citizen scientists should always abide by the collection guidelines set out by the DSA.

In our experience, exuviae are best preserved in alcohol for ease of identification. Keeping the exuviae wet minimizes breakage, facilitates brushing off sand, silt and debris, and it renders them pliable so that certain key structures, such as the labium, can be manipulated and their parts examined (many species have palpal and premental setae that must be counted). Manipulating dry exuviae can result in substantial damage and sometimes loss of a key structure. Either ethyl or isopropyl alcohol can be used; we recommend a 70–75% solution.

Preservation of Odonata nymphs is slightly more involved. The best methods maintain near-natural shape, posture and color pattern (although color itself usually changes) but differ for Anisoptera (dragonfly) versus Zygoptera (damselfly) nymphs. Zygoptera nymphs are best preserved by placing them directly into 80% ethanol. It is advised to put each Zygoptera nymph in a separate container of alcohol, as they tend to shed their caudal gills, and if nymphs are put together, matching gills and nymphs can become a difficult to impossible task.

Most researchers, however, have found that live Anisoptera nymphs that have been dropped directly into alcohol deteriorate in a matter of weeks, usually decomposing internally and losing their shape and color. Instead, immersing Anisoptera nymphs in near-boiling water for up to one minute, depending on size, kills most microbes and fixes proteins and fats preventing internal decomposition. Specimens are removed from the hot water, cooled and placed on paper toweling to remove excess water, and immediately put in 80% ethanol (isopropanol will suffice but may not yield as high quality specimens as ethanol).

Following this treatment, nymphs should be placed in glass vials or bottles that can be sealed to prevent evaporation of the alcohol. We recommend using screw-cap vials with poly-seal plastic caps (Fig. 1); the caps have a pliable plastic insert that effectively seals the rim of the vial. These vials have been utilized for 50 years now with minimal to no visible

loss of alcohol. However, collections of vials still should be examined annually to check the alcohol level in each vial. Other vials that prevent evaporation are the patent lip vials with neoprene stoppers, although some types of stoppers swell with time making them difficult to remove from the vial. A source for vial purchase has been the BioQuip company, but we have just learned that the company is going out of business on 11 March 2022. An alternate source to check for vials is www.discountvials.com. The vial size depends on the size and number of nymphs placed in them. Most nymphs will fit in the 4-dram size, but final instars of very large species, such as *Hagenius* and most *Macromia*, require larger vials. We place nymphs in the vials posterior end first. This makes it easier to remove them for study as the legs do not hook on the constricted vial neck.

Vial labels should be printed on high quality, acid-free paper using a laser printer with permanent ink. They should include the species identification, the name of the person who identified the specimen, the locality with lat/long coordinates, the date, and the name of the collector. Also useful is the habitat in which the specimen was found, which can be printed on the back of the label if space is lacking. The example below is Arial font sizes 9 and 7 and fits well in a 4-dram vial.

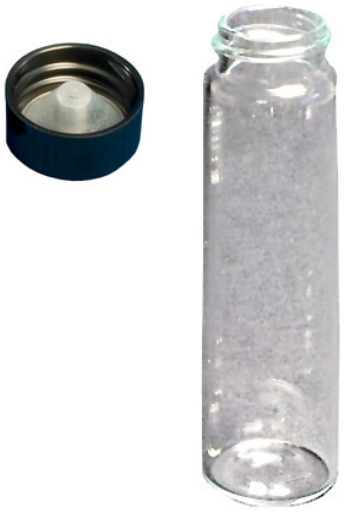


**Figure 1.** Screw-cap vial (on right) with poly-seal cap (on left).

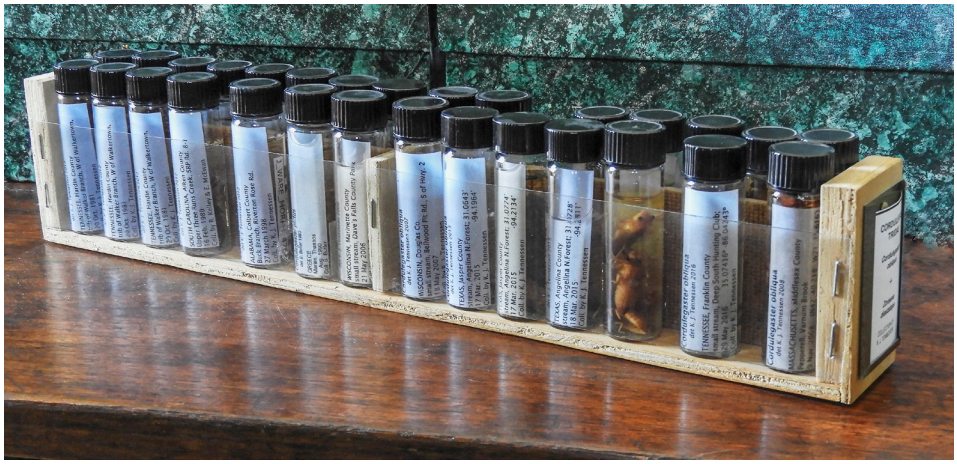


**Figure 2.** Example of a home-made wooden rack containing screw-cap vials.

Libellula semifasciata
**det K.J. Tennessen 2021**

**ILLINOIS, McHenry County**
**Glacial Park Conservation Area, Ringwood**
**Leatherleaf Bog**
**25 May 2020 42.419435° -88.327462°**
**Coll by M.C. Garrison**

Vials can be stored in vial racks (Fig. 2), which can be purchased or made from available materials. Racks should be stored in drawers that block out light. Exposure to light will bleach pigments and nymphs will become quite pale over time. After a few months of preservation, the alcohol in some vials may become discolored, especially if numerous specimens are stored therein; we recommend replacing the alcohol if that happens.

In summary, exuviae and nymphs preserved with the methods presented here have many benefits, especially regarding quality of condition and superior longevity of the specimens. Such specimens are great subjects not only for identification but also for molecular and morphological taxonomic study. Properly preserved nymph and exuviae collections serve as valuable sources for future gathering of many kinds of data.

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*Ken Tennessen has published over 80 technical papers on Odonata. His recent book,* Dragonflies Nymphs of North America, *was published by Springer in 2019.*