SAMPLING PROTOCOL FOR ODONATA LARVAE

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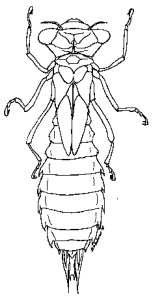


Larval odonates are aquatic, and can be found in just about every type of aquatic habitat. Below is a brief summary of the methods and materials used to find larval Odonata.

Materials Used for Larval Collection

Being well-supplied and organized for aquatic sampling requires some planning and certainly more materials than when collecting for adults. Below is a list of required and recommended items. Consult your MOS Handbook or ask MOS members for sources to purchase these materials.

1. Net, sieves and pans. Several types are recommended. D-nets are the best, all-purpose net for both lotic and lentic habitats. They can be used in kick-seining for flowing waters, and are excellent for reaching difficult areas, such as underneath overhanging vegetation, undercut banks, from piers, and from the edge of water. A high-quality net with approximately 0.5-mm mesh works best. Kick-seines are very good in flowing waters, and can be inexpensively constructed with door screening nailed to several planks of wood. They are difficult to use in lentic waters, as one must move around in order to capture dislodged organisms, and are even more difficult to use in areas thick with vegetation or other obstructions. Small dip nets are also useful, especially for small habitats such as tiny pools, seeps and springs. White sorting pans are very useful for sorting organism – the white background makes it easy to find organism moving among the inevitable debris. Finally, USGS round steel sieves (0.25 – 1.00 mm mesh diameters) and mesh from kick-seines are useful for sorting through material from nets and seines. Odonates generally will begin to move about when water drains through the mesh, and can thus be easily identified and picked.

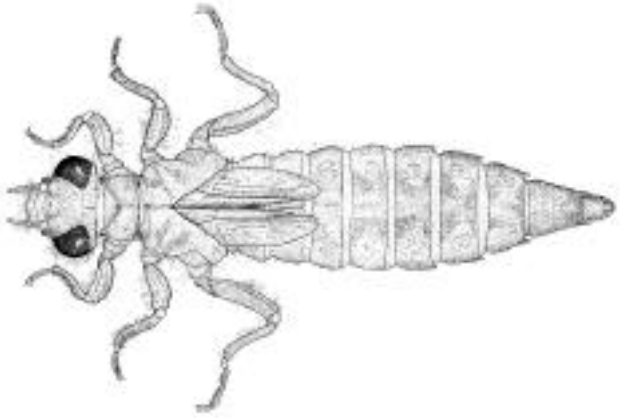
2. Alcohol. Preferred is ethyl or grain alcohol (ETOH), but this is difficult to legally obtain without permit. However, isopropanol can also be used, but this tends to dehydrate specimens and make then stiff and brittle. When merely preserving all sampled material, it is best to use 95% alcohol, as water from collected debris will add considerable water to the container. Too much water will allow fungi to develop upon specimens. Also, larval Odonata are predacious, and sometimes take several minutes to die in alcohol and thus can damage other specimens (particularly if you are collecting aquatic organisms other than Odonata). It may be a good idea to avoid putting too many specimens in one container. At least separate Zygoptera from Anisoptera, and the former has fragile caudal gills which are easily broken off and lost from the specimen, and which are very important diagnostic features. When returning to the lab to sort, place the specimen in 70% ETOH (or other alcohol) and add a few drops of glycerin, which will help prevent desiccation. Contact MOS for obtaining ETOH for collection purposes ONLY. BioQuip also sells ETOH that is doctored with an poisonous and odorous additive (this is required by law). Shipping a liquid is also costly.

3. Vials, jars or whirl packs. 4 dram vials, 1 or 2 ounce jars are ideal. Plastic vials can also be used, and are less likely to break. Good quality lids (e.g., polyseal) are recommended. Do not forget to mark containers to identify collection location, date and collector. Whirl packs are an excellent idea when one is taking many samples, as these are disposable, light-weight and generally do not leak. One can easily write locational data on them. Finally, I also use 0.25 dram ("quarter-dram") shell vials for coenagrionids. Three of these can be placed in one 4 dram vial, and are very useful to protect these delicate creatures and their fragile gills from being damaged from movement. These can be stoppered with cotton, which can be inexpensively purchased from cosmetic sections of stores (the thick ones for facial makeup are the best).

4. Clothing and gloves. Although the weather may be warm, the water may be a lot colder than you think. If you accidentally become wet, there is a chance of hypothermia, particularly if the weather suddenly changes. Bring a towel and spare set of dry clothing. And how to prevent getting wet? A good set of chest or hip waders are indispensable, and rubber waterproof aquatic sampling gloves (Bemar) are also recommended. These are particularly a good idea if one has doubts about water quality. And because water – particularly lakes – reflect light, don't forget sunscreen during the summer.

5. Forceps. Finer tipped forceps are preferred, particularly for more fragile (e.g., Zygoptera) specimens.

6. Notebook, preferable water-resistant (e.g., rite-in-the-rain), and **pencils** (more resistant to rain than ink, and also durable in alcohol). Pigma Micron ink pens (01 recommended) also work well, but allow about a minute to dry before immersing in fluid. Also, don't forget **labeling paper** for making labels!



Procedure for Collecting Larval Odonata

Below is an outline on how to collect for larvae. Effective and useful collecting is not merely finding the organism and preserving it in a vial of alcohol for posterity. Rather, you are a naturalist and scientist, not a hunter - observe the environment in which the specimen was taken. This way, you can make a really *useful* contribution to our knowledge and, at the same time, truly appreciate the function and beauty of the organism in study.

Sampling Methodology

1. Site information. Note the type and name of habitat you are sampling – spring, seep, brook, stream or river; a pool, pond, fen, bog and lake. Provide location data including state, county, and some locality reference, such as a road or distance from a town. Other useful information is either latitude-longitude and/or township-range-section. This can be obtained from a USGS topographical map, road atlases and the newer GPS units. Note the date and

name of collector. If you have the ability, note important features such as the type of aquatic and nearby terrestrial vegetation, the condition (human impact) of a habitat, flow conditions (low or baseflow, or high flow), etc. Refer to the MOS Handbook for more information.

2. Sampling Notation. Differentiate between samples from a particular locality. For example, if you sampled from an undercut bank, a riffle, a run, and a backwater area with aquatic vegetation, a bog pool, among cattails or algae, or wave-portion of a lake, you can really improve our knowledge by noting where particular odonates were collected by separating individual samples, instead of pooling them all in one container. Make separate references to these different habitats in your field notebook.

3. Sampling Method. Below is a general methodology for sampling for odonate larvae. Small pools, springs and seeps are best sampled with a small dip-net, with contents being placed in a pan or screen and gently sorted. These can be fragile ecosystems, so do not recklessly tread in the water, or sample too many times in one particular area. Streams and rivers are best sampled with a D-net or kick-seine. Place the net downstream about 1 foot from your feet, and then with your feet disturb the substrate. Organisms are then dislodged and collected by the net or screen. If using a D-net, empty the container into a pan or screen and pick the organism. The D-net can also be used to in a similar fashion to sample from underneath undercut banks, and also swept through aquatic vegetation growing in slow-moving or still-portions of the stream or river. Samples from sandy or silty areas from both lakes and streams are best sampled with the D-net, with contents emptied onto a screen or sieve. Also, do not forget to sample among *and* underneath woody or leafy debris accumulations, for these habitats often harbor a great number of odonate and other aquatic insect larvae. If you need any further information.

4. Preservative. Place the specimen in a vial containing alcohol. Do not place too many different kinds of organisms (or odonates themselves) in a container. It takes some time for these organisms to expire, and they may trash about or attempt to feed upon one another. Thus, a specimen may be damaged, making future identification more difficult. If a lot of debris is placed in the container with the organism, it is probably best to use 95% alcohol to compensate for dilution. Replace with 70% alcohol back in the lab.

5. Identification. Larvae of Michigan Odonata can be identified to species using an on-line www key at the following address: http://insects.ummz.lsa.umich.edu/ michodo/test/home.htm. For other identification resources, contact Mark O'Brien or Ethan Bright. Identical specimens should be placed in individual vials with a label noting the identity (Family, Genus and Species) and Locality (State, County, Habitat and Locality, Date, Collector and Field Notebook Reference Number). This information should then be made available to the MOS so that we can include this information in our computer database.

Brief Survey of Aquatic Habitats

There are many kinds of aquatic systems, but these can be classified as either *lentic* (standing-water) or *lotic* (running-water).

Lentic systems range from temporary pools to large, deep lakes, and tend ecologically to be more self-contained, with recycling occurring within the basin. Types of lentic systems are governed by climate, geology and basin shape. These range from temporary pools that can arise from depressions filling from rain or an elevated water table, to ponds in which aquatic vegetation is found throughout the lake, to bogs in which are covered by a mat of floating sphagnum, and lakes which there are large sections of open water and great differences in water chemistry and temperature. Lentic habitats are divided into zones:

1. *pleuston* (surface film)
2. *limnetic zone* (open water to the depth of photosynthetically-effective light penetration
3. *profundal zone* (area below the limnetic zone (usually below 10 m) where light penetration is inadequate for plant growth; *and*

4. *littoral zone* (shallow region with light penetration to the bottom, or benthos, and characterized by macrophytic (rooted aquatic plants and macroalgae) growth.

For reasons of productivity and food availability, predator avoidance and emergence needs, almost all larvae of lentic Odonata are found in the littoral zone. Zygoptera and Aeshnidae, which are clingers, and some Libellulidae, will be found among the aquatic vegetation; Gomphidae, Corduliidae, Macromiidae and most Libellulidae are sprawlers or burrowers among the substrates. Some Coenagrionidae, Lestidae and Libellulidae are adapted to develop in small, temporary pools. These are all best sampled with the D-frame or dip net, depending on the size of area being sampled.

Lotic habitats are a direct result of landscape geomorphology, and result simply from water precipitated upon the land being drained by gravity to lower elevations. Sources of water for lotic systems include direct input from precipitation, surface and subsurface drainage, and groundwater. Water eventually collects into channels to form seeps and springs, creeks and streams, and ultimately rivers. A myriad of factors work to form river channels and the ecosystems they influence. Rivers respond to changes in discharge and sediment load, and these factors control a rivers' response in adjusting channel form – and habitats. Seeps and springs points at which groundwater reaches the surfaces, and can be a mere trickle of water that appears as wet ground, or a forceful stream of water from the ground or hillside source. Several odonate larvae are found in these habitats (e.g., *Tachopteryx, Cordulegaster*). Brooks, stream and rivers usually provide more diverse habitats for larval Odonata. The riffle-pool sequence forms as part of a regularly-spaced alteration between areas of erosion and deposition within the stream channel. Riffles are areas of particulate deposition (during bankfull discharge), and during low flow, because water must rise over riffles, mean water velocity is higher. Clingers such as *Argia*, *Boyeria*, *Basiaeschna* may be found here, but generally few odonate larvae are found here (though many other aquatic insect taxa). However, riffle areas with larger stones and rocks often have silt and sand accumulated about them, and these should be sampled thoroughly. *Ophiogomphus*, *Cordulegaster*, and other sprawlers and clingers are found here. Pools and runs are characterized by finer substrates, greater depth and lower velocity. During high flow, these are areas of erosion. Areas of finer substrates, including sand bars after river bends, attract burrowing and sprawling Gomphidae, and other sprawlers such as *Cordulegaster*, *Hagenius*, Macromiidae and many Libellulidae. Flow and channel characteristics are also the forces creating other important stream habitats, such as depositional areas of low flow and floodplains that often support aquatic vegetation, log jams (where water power is insufficient to transport it out of the channel), and variously shaped stream banks and undercuts. These stream and river habitats perhaps provide for the greatest number and diversity of odonate larvae.

Final Word

After some initial hesitation, you'll find aquatic sampling is a lot of fun. However, it is by nature a procedure of disturbance. We need to keep disruption to these habitats to a minimum. Whenever safe, try to enter a sampling point from land, rather than moving continually within aquatic habitats. This should be always done in seeps, springs and small pools. This may not be possible in larger streams and rivers, particularly if the current is strong, and in larger lakes. In this case, move about slowly, trying to disturb the substrate as little as possible. Finally, it is not necessary to take *everything*: in fact, you can learn a lot by releasing the organism back into the water and watching what it does. Examine how larvae propel themselves through the water, and how gomphids, macromiids, and cordulegastrids attempt to conceal themselves within the substrate, or how aeshnids and zygopterans cling to

substrate surfaces. Also, if you have a large enough container, think about perhaps keeping these alive in an aquarium or shallow pan for a few days. Although perhaps not as beautiful as the adult stage, aquatic larvae have a different set of colors and hues that look quite stunning underneath the microscope. Collect other aquatic organisms as food, and then examine how odonate larvae feed – you'll find it as exciting, if not more, than that of the adults. Unless you have access to an air pump, release them back to where they were collected within a few days.

If you have any questions or need any particular resource so make your studies possible, feel free to contact Ethan Bright or Mark O'Brien. We can also recommend various texts and journals that will considerably help in appreciating larval odonate biology and ecology, and the aquatic habitats in which they live.

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