

# Chapter 20

## Sampling Methods for Aquatic Insects



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### 20.1 Introduction

Aquatic insects are important elements in the structure and function of freshwater ecosystems because they contribute to organic matter processing, nutrient cycling, and energy flux and serve as food for vertebrate and invertebrate predators in aquatic food webs. They comprise a highly varied group of animals that are dependent on water habitats at some stage in their life cycles (Table 20.1). Aquatic insects are ubiquitous in the lakes, streams and rivers, springs, and wetlands of the world. The habitats where they can be found are varied, including hyporheic zones, bottom sediments, air-water interfaces, temporary systems, and brackish, salty, and hyper-saline waters.

Aquatic insect collection methods vary depending on the freshwater habitat type (lentic or lotic) and substrate type (e.g., sand, stones, leaves, and vegetation). Moreover, sampling aquatic insects can be qualitative or quantitative, depending on the type of study performed. Depending on the study objectives, researchers can collect individual specimens from single habitats a single time or entire assemblages across river basins, ecoregions, or continents through use of long-term systematic surveys (Oliveira and Pes 2014; USEPA 2016; Ligeiro et al. 2020). Additional concerns must include sample site location, sampling frequency (e.g., daily, monthly, yearly), and numbers of samples, true replicates, pseudo-replicates, and subsamples (Hughes and Peck 2008).

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**Table 20.1** Insect orders with aquatic forms

Order	Common names	Aquatic development phase (excluding egg)
Ephemeroptera	Mayflies	Nymphs
Odonata	Damselflies and dragonflies	Nymphs
Orthoptera	Grasshoppers and crickets	All
Plecoptera	Stoneflies	Nymphs
Hemiptera	True bugs	All
Mecoptera	Scorpionflies and hangingflies	Larvae
Neuroptera	Lacewings	Larvae
Megaloptera	Dobsonflies and alderflies	Larvae
Coleoptera	Beetles	All
Trichoptera	Caddisflies	Larvae and pupae
Lepidoptera	Moths	Larvae and pupae
Diptera	True flies	Larvae and pupae
Hymenoptera	Wasps	Larvae

The collection of aquatic insects can be done with a wide variety of equipment and methods, from simply hand-lifting rocks at the bottom of a river or examining aquatic vegetation. Sampling efficiency of aquatic insects is significantly increased when hand nets, kick nets, dredges, and other inexpensive apparatus is used. After a sample has been collected, labeled, stored, and safely transported to a laboratory, samples should be washed over standard sieves (e.g., 250  $\mu\text{m}$ , 500  $\mu\text{m}$ ) to remove organic debris and leaf detritus, algae, and sediment particles, which facilitates the screening of aquatic insects (Fig. 20.1). In general, aquatic insect samples are fixed with 70% alcohol and preserved for later taxonomic identification using stereomicroscopes. Eventually, slides are prepared for the identification of small-size morphological structures and observation of gills and lamellae and other structures not visible under stereomicroscopes (Fig. 20.2). Lastly, DNA barcoding has been used to identify insects, but the necessary DNA barcode libraries are just beginning to be developed for the Neotropics (Nichols et al. 2019).

## 20.2 Freshwater Habitats and Sampling Devices

### 20.2.1 Lotic

Shallow lotic ecosystems can show high substrate diversity and are generally rich in aquatic insect species (Fig. 20.3). Kick nets (D-shape net with 0.3–0.5 m aperture and 1 m x 1 m square of 500  $\mu\text{m}$  coupled to a 1.5 m wooden handle) are used to obtain semiquantitative data (Fig. 20.4a–d), whereas Surber samplers (a 0.3 x 0.3 m quadrat linked to a 1 m x 1 m square of 500  $\mu\text{m}$  net) offer quantitative samples (Fig. 20.4e–h). Both methods require in disturbing bottom sediments (e.g., cobbles,



**Fig. 20.1** Field and laboratory sediment wash over standard sieves

gravel, sand, leaf deposits) and catching organisms in a net downstream. Besides, a researcher can collect stones and pebbles by hand to visually inspect and collect the aquatic insects with tweezers, transfer them to a flask, and take them to the laboratory for further taxonomic identification (Fig. 20.9c–d). When the collected material



**Fig. 20.2** Slide preparation for taxonomic identification of morphological structures using a microscope in laboratory

has sediment, leaf debris, or organic matter left in the sample, it should be washed (Fig. 20.9a) transferred to a white tray and manually sorted live invertebrates are transferred to vials in the laboratory (Fig. 20.9b). Ideally, sediment samples have to be collected from downstream to upstream, to avoid drift addition.

### **20.2.2 Lentic**

Lentic ecosystems can be roughly divided between littoral and limnetic zones (Esteves 2011). Littoral habitats form a transition zone between aquatic and terrestrial ecosystems, concentrating much of the biodiversity of a lake (Kutyła 2014; Gownaris et al. 2018). To sample aquatic insects in the littoral zone, D-shape nets are used for semiquantitative sampling. This methodology is similar to the one used in lotic ecosystems, consisting of disturbing bottom sediments, with the difference being that due to the lack of a strong downstream current, it is usually necessary to push the sediment to the net or sweep the net through the sediment plume. Corer samplers (8–20 cm diameter acrylic cylinder tube) are used for quantitative sampling, gathering sediment samples of a known area (Fig. 20.5a–d).

Pelagic habitats usually need the assistance of a boat to be sampled, due to the depth. Eckman-Birge and Petersen dredges (a clamshell bucket with two opposing jaws for the collection of a sediment sample) are the most common samplers in these conditions, being able to acquire sediment samples in deepwater ecosystems,



**Fig. 20.3** Examples of running waters in reference conditions

but can be also used in the littoral zone (Fig. 20.6a–c). Additional samples using D-net can be collected in littoral habitats (Figs. 20.4b and 20.6g–h).

### 20.2.3 *Hyporheic Zone*

The hyporheic zone (Greek *hypo* and *rheos* = under and river) is the connecting ecotone between surface and groundwater and is functionally part of both fluvial and groundwater ecosystems (Mugnai et al. 2015). Some aquatic insects can live in



**Fig. 20.4** Pictures of field samplings using D-nets (a–d), drift net (e), and Surber sampler (f–h)

the hyporheic zone to avoid competition or predation, as a nursery, or to escape seasonal flooding and droughts (Dole-Olivier et al. 1997; James and Suren 2009). Larvae of some Coleoptera, Diptera, Ephemeroptera, and Plecoptera and adult forms like elmid and staphylinid coleopterans are frequently observed in hyporheic zones. The larvae are generally confined to the upper layers, but some species penetrate deeper and are important components of the hyporheic fauna (Mugnai et al. 2019).

To sample aquatic insects in hyporheic zones, several techniques can be used (Fig. 20.7) including the Karaman-Chappuis technique for gravel bed streams, where a hole is dug in the bank of a river and the water is filtered; Bou-Rouch pump modified, where a perforated bar is inserted directly into the riverbed and water is pumped through a membrane; standpipe cores, where perforated bars are inserted semipermanently into a riverbed and the water is pumped periodically using a hand

pump; freeze-cores, where a freezing medium is used to freeze and extract part of a riverbed; artificial substrates and baited; and baited and un-baited traps buried in the riverbed allow the collection of specimens. All these methods substantially differ in efficiency, financial cost, and field effort (Hahn 2005; Malard et al. 2002).

#### 20.2.4 Springs

Springs are natural points of water resurgence and are threatened and poorly known freshwater environments (Malard et al. 2002). These environments are subject to deforestation, trampling by livestock, and human recreational activities. These habitats can be very heterogeneous from a geomorphological point of view, amount of water provided, aquatic vegetation, and water chemistry.

From biotic point of view, in general, the benthic and nektonic insect fauna is relatively scarce due to the characteristics of the water (usually low pH, nutrient, and dissolved oxygen), but some are specialists for crenon habitats such as some Diptera (Chironomidae), Ephemeroptera (Baetidae), Plecoptera (*Leuctra*), and Trichoptera (Lepidostomatidae) (Dobrin and Giberson 2003; Ilmonen and Paasivirta 2005).

To sample aquatic insects in springs, it is advisable to use a 10 × 10 cm D-shape net (Rosati et al. 2016), artificial substrate for benthic and nektonic fauna, and modified Hess sampler (Dobrin and Giberson 2003). For interstitial organisms, the gear and techniques described for hyporheic habitats can be used (Malard et al. 2002).

#### 20.2.5 Marine, Brackish, and Inland Saltwaters

Aquatic insects occasionally live in waters with greater than 0.5‰ salinity. These environments are represented by brackish waters, which include mixtures of coastal freshwater with marine water as estuary, mangrove, coastal lagoons, etc., with a variable salinity between 0.5 and 32‰. Saline inland waters consist of saline lakes occurring typically in arid or semiarid regions with a variable salinity between 0.5 and 300‰. The marine water is constituted by intertidal areas and neritic or oceanic environments with salinity between 34 and 37‰.

Species that have occupied marine and brackish environments are currently constituted by a few hundred species belonging to the Hemiptera, Dermaptera, Hymenoptera, Coleoptera, and Diptera. Some few species of Lepidoptera, Odonata, and Trichoptera may live in environments with salinity up to 15‰ (Ward 1992). Intertidal or benthos specimens may be collected by hand or using samplers depending on the nature of substrata (kick samplers, Surber sampler, grab samplers, corer, etc.). Neustonic (small organisms that inhabit below the surface of a body of water), and nektonic (actively swimming aquatic organisms in a body of water) species are best sampled with a handheld plankton net screwed to an extendable handle and



**Fig. 20.5** Field sediment sampling using a corer sampler

obtained by snorkeling or SCUBA diving (Thorp and Covich 2010; Merritt and Cummings 1996).

## 20.2.6 Habitats Outside Water Bodies

### 20.2.6.1 Phytotelmata

The word phytotelmata is derived from the Greek *phyton* and *telm* = plant and pond, consisting in small waterbodies of hollow trees or inflorescences such as bromeliads. Thienemann (1954), Kitching (1971), and Greeney (2001) divided this habitat into six major types: tree holes, leaf axils, bowers, modified leaves, fallen vegetative





**Fig. 20.6** Field sediment sampling using Eckman-Birge dredge (a, b, c), Petersen dredge (d, e, f), and D-net (g, h) at the littoral region of a lentic habitat



**Fig. 20.7** Example of field equipment used to sample in hyporheic zones

parts (such as leaves or bracts), and fallen fruit husks. Stem rots, traditionally not included, are considered as transition zones between phytotelmata and other similar habitats (Greeney 2001). This habitat is heterogeneous and is generally considered as temporary but often being available all year round. From a dimensional standpoint, they are usually less than 200 ml, even those of 30 ml or less being quite common, to at least 45 l.

Phytotelmata occur in a wide range of ecosystems from subarctic to tropics in tree holes of deciduous woodlands, in Sarraceniaceae and Nepenthaceae plants, and in tropical plants that can retain water such as bromeliaceans and bamboos. These habitats are most common in tropical and subtropical areas and are utilized by a wide range of arthropods. Besides insects, mites, entomostracods (Copepoda, Ostracoda, Cladocera), brachiurans, crabs, and tartigrads are often found in phytotelmata habitats. Seventy families from 11 orders of insects have been reported living in phytotelmata habitats. That includes truly aquatic insects such as Diptera, Coleoptera, and Odonata (as Pseudostigmatidae and Megapodagrionidae) that are best represented, but Hemiptera, Trichoptera, and Plecoptera are also common. Besides that, semi-aquatic species and terrestrial species foraging in or around phytotelmata habitats are included (Greeney 2001; Frank and Lounibos 2009).

The interest in the phytotelmata habitat, such as in some plants as Sarraceniaceae, Nepenthaceae, and bromeliaceans, can be destructive, due to invasive methods that result in the dissection and removal of the plant. Water-sucking devices of various types can be used such as laboratory pipettes, cooking basters, syringes or syringes with tubes can be used to collect in this environment with good results (Derraik 2009; Jocque et al. 2010).

### 20.2.6.2 Hygropetric and Madicolous Habitats

Hygropetric and madicolous are two habitats characterized by a tiny layer of water. The word hygropetric derived from the Greek *hydro* and Latin *pedra* = water and stone. It is constituted by the rock environment always covered by a subtle veil of water usually due to the splash of water from waterfalls or turbulent waters. Madicolous habitats are characterized by a flowing, often permanently, thin layer of water (<2 mm), over many types of surfaces, usually limited to mountain areas and restricted in size. In these environments, rich in organic muck and mosses, can be found specialized species in the Coleoptera (Torridincolidae, Hydrophilidae, Dytiscidae), Diptera (Ceratopogonidae, Chironomidae, Dolichopodidae, Psychodidae, Stratiomyidae, Thaumaleidae, Tipulidae), Ephemeroptera (Heptageniidae), Odonata (Megapodagrionidae), and Trichoptera (Hydroptilidae), besides semiterrestrial organisms (Ferrington et al. 1995; Hájek et al. 2019; Miyairi and Tojo 2007; Tennessen 2010). Aquatic specimens can be collected by hand, or with small hand nets (Thorpe and Covich 2010), flying adults are collected by sticky traps or emergence traps (Shimabukuro et al. 2015).

### 20.3 Drift Samples

Insects and other aquatic invertebrates are transported downstream in a phenomenon known as drift. The entry of invertebrates into the water column can be active or passive and may be the result of several factors, such as (a) changes in water flow and velocity, (b) presence of predators, (c) changes in the water physical and chemical characteristics, and (iv) redistribution of invertebrate populations as a function of competitive pressures (Brittain and Eikeland 1988). The transport of invertebrates downstream is not constant and varies with the season, day to day, and at different times of the day. Differences in the densities of drift organisms may also vary for each species and their propensity to drift at different stages of the life cycle or when insects are emerging. Invertebrate drift is of great importance to the functioning of aquatic ecosystems as it is a primary mechanism for invertebrate redistribution and colonization (Brittain and Eikeland 1988; Naman et al. 2016).

Techniques and methods for sampling insect drift are generally simple, and different equipment can be used. The most widespread method has been the use of a frame (square or rectangular) (Fig. 20.4e) or tube mouth fitted with a long net (~0.5–2.0 m) which has a removable container at the end. Drift nets must be placed in the stream with the net face perpendicular to the direction of flow and anchored with iron bars driven into the substrate (Fig. 20.8a–c). The top edge of the frame must be above the water surface, and the bottom edge close to the stream bottom, but clear of the bottom to ensure that only drifting insects enter the net, avoiding insects crawling directly into the net. The mesh size of the nets will depend on the objectives of the study, but typical mesh size used is between 200 and 500  $\mu\text{m}$ . Smaller mesh size nets can clog rapidly and can only be deployed for short amounts of time to avoid backflow.

It is important to measure the volume of water passing through a drift sampler. The net filters a column of water with a known cross-sectional area. Using a flow meter near the mouth of the sampler and the cross-sectional area, it is possible to calculate the volume of water flowing through a drift sampler. With the volume filtered and the number of invertebrates collected, it is possible to calculate the drift density (i.e., the number of invertebrates per unit volume of water) and the drift rate (the number of invertebrates passing a sampling point in unit time) (Allan and Russek 1985).

The sampling period can vary from a few minutes to several hours depending on the sampling station characteristics and the aim of the research. It is important to monitor the net during the sampling because the net may become clogged with organic detritus and thus produce a backflow that causes a decrease in sampling efficiency.

## 20.4 Sampling Aquatic Insect Adults

Flying adults of aquatic insects can be sampled using ultraviolet (UV)-light traps, mercury vapor lights, Malaise traps, sweep netting riparian vegetation, and rearing from immature stages. These sampling methodologies are unselective and provide big samples including mostly terrestrial nocturnal insects.

Specific techniques to sample emerging aquatic insect adults include enclosed channels (Wartinbee and Coffman 1976), floating emergence traps (LeSage and Harrison 1979), hand screen collectors (Usinger 1956), light trap (Southwood 1978), madicolous trap (Shimabukuro et al. 2015), Mundie pyramid trap (Mundie 1956), pan trap (Grigarick 1959), stationary screen trap (Hamilton 1969), sub-aquatic light traps (Aiken 1979), surface film samplers (Coffman 1973), and window trap (Chapman and Kinghorn 1995). All these sample methods differ in construction effort, cost, and microhabitat utilization.

The sampling of emerging adults is mostly influenced by some environmental factors such as seasonality, temperature, and moon phases (Ivković et al. 2013). Aquatic insect adults can also be obtained by direct association between larval and adult forms (Edmunds et al. 1976; Merritt and Cummings 1996).

## 20.5 List of Supplies for Field Sampling

The organization of the necessary material is an essential phase for any field sampling. It is essential to anticipate the needs of the sampling, as usually forgotten equipment can cost a day's worth of work or even more: (1) plastic buckets; (2) 250 or 500  $\mu\text{m}$  sieves (filters 60–90 micron pore for hyporheic); (3) plastic bags, wash bottles, and 70% ethanol; (4) current meter; (5) Surber (Fig. 20.4f–h), D-net (Figs. 20.4b and 20.6g–h), drift nets (Fig. 20.8a–c), Eckman-Birge (Fig. 20.6a–c), or Petersen (Fig. 20.6d–f) dredges; (6) metal holding rods; (7) permanent marker pens and labeling paper; and (8) forceps.

## 20.6 Laboratory Procedures

After field sampling, the collected samples are taken to the laboratory. The first procedure is washing (Fig. 20.9a) and sorting that involves the separation of the aquatic invertebrates from the inorganic and organic matter collected with the sample. This is a time-consuming procedure but essential. Normally this procedure is done using lighted tables, plastic trays, and forceps (Fig. 20.9b). Flotation using salt or sugar is commonly used to remove small larvae (but not for mollusks) in sandy sediments. After samples have been sorted, the following step is to identify and



**Fig. 20.8** Pictures of the use of a drift net in a headwater stream



**Fig. 20.9** Laboratory processing activities of sediment samples: (a) washing, (b) sorting, (c, d) identifying aquatic insects



**Fig. 20.10** Scheme of a reference collection of aquatic insects

count the organisms through the use of taxonomic keys (Fig. 20.9c, d). The results are then analyzed by different procedures depending on the research questions, aim, and experimental design. All aquatic insect forms must be preserved in a Reference Collection, with field sampling method, number of individuals, and taxonomic identification and registered in a public institution (Fig. 20.10).

## 20.7 Specimen Preparation

To prepare aquatic insects, most specimens consist of juvenile phases but can also often include pupae in the case of Holometabola forms. To conserve larvae and pupae, several authors suggest adding to the 70–80% alcohol a few drops of glycerol; to conserve adult phases, specimens can be stored in a dry way, properly pinned or glued to cards depending on size (Lincoln and Sheals 1979). To pin speci-

**Table 20.2** Pinning scheme for different aquatic insect taxa groups

Group	Position of pin	Wing
Large Coleoptera	Right helittrae	Not spread
Large Ephemeroptera	Center of mesothorax	Not spread
Large Heteroptera	Center of scutellum	Not spread
Slender Heteroptera	Center of prothorax	Not spread
Large Hymenoptera	Right forewings	Not spread
Large Lepidoptera	Through middle at thickest point of thorax or just behind base of forewings	Spread
Large Odonata	Through middle at thickest point of thorax or just behind base of forewings	Spread
Large Plecoptera	Center of mesothorax	Spread
Large Trichoptera	Center of mesothorax	Spread

mens, it is necessary to follow standard procedures according to the taxonomic group (Table 20.2).

Despite the possibility of storing dry adult specimens, several aquatic insect research laboratories have in the last decades chosen to conserve these specimens wet, due to the necessary physical space to store specimens and the skill and time necessary to pinning.

## 20.8 Species Identification

Species identification in biodiversity surveys has two major aspects. The first is the identification of species not known from the taxonomic point of view with the purpose to describing and naming them, defined as taxonomy. This activity should follow the precepts illustrated by Winston (1999) in the book *Describing Species: Practical Taxonomic Procedure for Biologists* and the rules dictated by the International Code of Zoological Nomenclature (ICZN).

The second activity aimed at quantifying biological diversity and to develop ecological works aimed at identifying species already known taxonomically known as parataxonomy. The term parataxonomy was introduced by Jansen et al. in 1993 coming from the term parataxonomist (or “biological diversity technicians” in some parts of the world) the specialist involved in the sampling collection of natural history or inventory data and in the activities of specimen sampling, sorting, and identification. For this activity, unlike taxonomy, identification at the species level is not strictly necessary, and often in ecological works, different levels are used such as genus or family, technically defined as operational taxonomic units (OTUs), recog-



nizable taxonomic units (RTUs), or parataxonomic units (Pus) or morphospecies (Krell 2004; Oliver and Beattie 1993, 1996a, b).

To aid the parataxonomy identification activity in the last decades, many specialized publications have been produced by condensing taxonomic work on identification keys, often in the form of juxtaposed dichotomous keys, but also as grouped, identified, graphical, or combined dichotomous keys. More recently, multiple-entry interactive identification electronic keys have been introduced. Such keys illustrate orders or families or brings together various taxonomic groups (e.g., insects, macroinvertebrates, etc.) in the form of identification manuals.

Regarding geographical area, identification keys and identification manuals are heterogeneous, ranging from local (state, microregion, river basin) to large extent publications (North America, South America, Europe). From the point of view of the amount of published works for area, there are notable differences. In the northern hemisphere areas, such as the United States or Europe, there is a large amount of available bibliographic material. On the other hand, in other areas, as Southern Hemisphere (Africa and South America or some places in Asia), bibliographic specific works are scarce or even nonexistent. The absence of specific bibliographic material may lead the researcher to use manuals and keys from other geographic regions to try to identify the specimens found, which is not always possible and generally inappropriate.

Another problem of identification using dichotomous keys, applied to aquatic insects and the most commonly used in biomonitoring programs of water bodies, is related to the continual updating of scientific information as new species and/or cases of synonyms and homonyms are discovered and new identification keys are published. In this case, it is important to point out that the use of the most up-to-date bibliographic material in research may create a problem of identification standardization precluding the possibility to compare the work with previous studies or result in distortions of results between researchers from different teams or even within the same team. Therefore it is important to maintain a library of taxa names to facilitate cross-referencing of data.

## 20.9 Conservation and Curatory

Biological samples naturally tend to degrade with time. Curatory is the set of actions that involve physical and/or chemical actions, routinely implemented, to minimize or avoid physical or biological damage of stored specimens in the collection and preserve the associated information (Herholdt 1990; Horie 1986).

Ultraviolet light, produced either by lamps or direct sunlight, can damage dry specimens (Rose and Torres 2009; Simmons and Munhoz-Saba 2005), and these sources can be avoided using specific adhesive filters. In addition, damage can be caused by some biological factors as the beetle *Anthrenus* spp. (Dermestidae) and some fungi. These pests can be controlled by temperature, humidity, and chemical preservation with paradichlorobenzene. The biggest problem with wet specimen

conservation is the loss of preservative fluid (e.g., 70% alcohol) that must be periodically added and replaced to ensure correct concentration.

## 20.10 Conservation Implications of Aquatic Insects

As insect biodiversity is threatened in all parts of the world (Sánchez-Bayo and Wyckhuys 2019), it is of paramount importance to train students, researchers, and the general public about the importance of aquatic insects. Specific sampling methods for different types of aquatic ecosystems are available at reduced prices and enable qualitative and quantitative sampling of species and estimations of their abundance. Many of these aquatic insects are bioindicators of water quality (Bonada et al. 2006) and have been widely used in environmental monitoring programs and development of multimetric indices (Silva et al. 2017) and as environmental education tools in citizen science activities (França et al. 2019).

The conservation of freshwater ecosystems is necessary to ensure water supply for multiple human uses and ecosystem services but we have a fundamental duty to ensure adequate habitat and conditions for the maintenance of aquatic insect species. These organisms, by their participation in food chains and detritus decomposition, linking different compartments in aquatic ecosystems through energy flow and nutrient cycling in the riparian meta-ecosystems (Callisto et al. 2019) reflect environmental quality in entire river basins, ecoregions and nations (USEPA 2016).

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