
# LIFE AQUATIC WARBLERS ON THE MOVE

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# Entomological protocols for food availability assessment at AW stopover sites

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# Table of contents

# Executive summary

The main prey species of the Aquatic Warbler were identified through targeted studies. Based on these findings, two entomological sampling protocols were developed to assess food availability across five AWOM project sites. In habitats characterized by medium to high dense vegetation, a cornet trap will be used. This interception trap effectively captures both flying insects and insects developing within the vegetation. In areas dominated by low-lying vegetation, a vacuum trap combined with a biocenometer will be used. Numerous studies have demonstrated the suitability of this method for sampling the target arthropod groups, although its effectiveness is influenced by vegetation height and density.

# 1. Introduction

During its spring and autumn migration across Western Europe and North Africa, the Aquatic Warbler relies on a network of wetland stopover sites where it can rest and replenish its energy reserves. The suitability of these sites is critical to the species' ability to successfully complete its migration to breeding and wintering grounds. Assessing and regularly monitoring stopover site quality is essential, both in terms of food sources and fattening opportunity, as well as confirming their overall suitability.

Several approaches can be used to evaluate the feeding value of a stopover site. Habitat assessments provide information on the availability of vegetation types preferred by the species and are relatively straightforward to implement on the field. However, because such assessments do not directly measure prey abundance, they may not accurately reflect actual food availability or overall site quality. Another approach involves estimating fattening rates, based on the assumption that better food resources lead to greater increases in body mass of the bird. While informative, this method is constrained by the scarcity of the species and the need to recapture individuals within a short period at the same site, limiting the number of possible measurements. A more direct approach consists of assessing food availability for the species through direct sampling of the faunal group on which it feeds – namely arthropods – based on current knowledge of the species' diet.

In order to perform the food availability assessment at stopover sites, the Aquatic Warbler diet must be known. Several studies have examined the species' diet through analyses of faecal samples, using both chitinous remains and DNA metabarcoding techniques. These studies have significantly improved knowledge of the prey consumed by this insectivorous species. Because the ecology and behavior of arthropods vary among species and groups, field sampling protocols must be designed to primarily target the species or insect groups most relevant to the Aquatic Warbler. In addition, sampling methods (entomological protocols) must be adapted to the habitats in which they are deployed while remaining sufficiently standardized to allow for comparisons and replication among different sites and habitat types.

The implementation of entomological protocols provides both qualitative information on prey occurrence and quantitative information on prey abundance. Additionally, when assessing food resources for a migratory bird, the biomass ingested is equally important. The Aquatic Warbler must not only encounter its preferred prey in sufficient numbers but also ingest enough biomass to accumulate energy for onward migration. Thus, particular attention must be paid to the methods used for biomass estimation.

Within the framework of the LIFE Aquatic Warblers on the Move (LIFE AWOM) project, these protocols will be implemented at a minimum of five sites during both spring and autumn migration periods. The objective is to assess food availability across the project areas from the perspective of the ecological requirements of the Aquatic Warbler.

This report presents the entomological protocols identified, based on expert assessment, as the most suitable for achieving this objective within the LIFE AWOM project.

## 2. Objectives for food availability assessment

At a LIFE AWOM scale, the main objective is to obtain information about the overall entomofauna availability at least at 5 sites. In the proposal, this objective is described as the establishment of “a relationship between the food availability and habitat structure”. Thus, entomological sampling must be performed in a certain way so that the vegetation parameter doesn't introduce any significant bias. To do so, partners will sample entomofauna per major vegetation type.

In addition to this objective, some partners will also focus on assessing food availability as part of the evaluation of the success of the restoration work performed on the site. This question also requires sampling per major vegetation type, before and after restoration.

To address these two objectives, and given the structural differences between major types of vegetation, two different protocols will be implemented depending on the vegetation type.

## 3. Results from Aquatic Warbler diet studies

The design of the entomological protocols must take into account the dietary preferences of Aquatic Warbler to ensure that the selected methods effectively sample the main arthropod prey groups consumed by the species.

Over the past 15 years, several studies have investigated the diet of the Aquatic Warbler. These studies have primarily relied on faecal analyses, either through the manual identification of chitinous arthropod remains or through DNA metabarcoding techniques.

Studies based on the manual identification of chitinous remains have been published for four French stopover sites: Audierne Bay (Kerbiriou et al., 2011), the Gironde Estuary (Musseau et al., 2014), the Seine Estuary (Provost et al., 2010) and the Brière Marsh (Marquet et al., 2014). Additionally, at one site in Spain: Laguna El Villar (Miguélez et al., 2016).

Results from the French sites were broadly consistent, highlighting the predominance of Diptera, Hemiptera (including both Heteroptera and Homoptera), and Spiders in terms of both prey abundance and occurrence. Depending on the site, Hymenoptera, Coleoptera, Orthoptera, and Odonata also represent significant components of the diet.

The results obtained at the Spanish site showed similar patterns. The most important arthropod groups, both in terms of occurrence (O) and proportion of prey number (A), were spiders (O = 91.7%; A = 21.5%), Heteroptera (O = 75.0%; A = 21.5%), Homoptera (O = 83.3%; A = 18.5%), and Diptera (O = 66.7%; A = 12.3%). Hymenoptera (O = 58.3%; A = 10.8%), Coleoptera (O = 50.0%; A = 9.2%), and Orthoptera (O = 16.7%; A = 3.1%) also represent a substantial part of the diet.

Most recently, metabarcoding analyses were conducted in France in 2024 and 2025 as part of the French Species Action Plan (SAP), and in Spain in 2025 within the framework of LIFE AWOM. The first results of the 2024 study in France, based on 65 faecal samples collected across 13 sites, confirmed the trends identified by previous studies (Alonso et al., 2026, in preparation). The most frequently detected arthropod groups were Diptera, Hemiptera, and Araneae, with notable contributions from Collembola, Lepidoptera, and Hymenoptera. The detection of Collembola is particularly noteworthy, as this group cannot be reliably identified through the analysis of chitinous remains alone.

Laboratory results from the 2025 French study, also based on 65 faecal samples collected at 13 sites, have not been fully analyzed yet by the time of writing. In Spain, the 2025 metabarcoding study was conducted during both spring migration (6 faecal samples collected, 5 analyzed) and autumn migration (9 faecal samples collected, 5 analyzed).

Based on these studies, it is essential that the entomological protocols implemented at the LIFE AWOM scale should efficiently sample Diptera, Hemiptera, and Araneae, while also providing robust coverage of Hymenoptera and Coleoptera.

# 4. Entomological protocols

Two entomological protocols will be implemented, depending on vegetation structure.

The main protocol relies on the use of a cornet trap, an interception trap derived from the Malaise trap and developed by Sarthou (2009). Owing to its low-structure design, the cornet trap captures an insect assemblage comparable to that obtained from the Malaise trap, while also allowing for the sampling of a greater variety of insects living in the vegetation layer, such as Hemiptera, Coleoptera, and Orthoptera (Uhler et al., 2022).

The second protocol is based on vacuum sampling using a biocenometer. This method is particularly effective for collecting arthropods, including Hemiptera, Diptera, Hymenoptera (excluding Formicidae), vegetation-dwelling spiders, and certain beetle groups, such as Curculionidae. Its effectiveness has been demonstrated in a range of habitats, including flooded areas such as rice paddies (Perfect et al., 1983; Brook et al., 2008; Sanders & Entling, 2011; Zou et al., 2016).

## 4.1 Sampling using the cornet trap

### 4.1.1 Type of habitats targeted

Since the objective of this study is to compare food availability for the Aquatic Warbler across different vegetation types, the placement of cornet traps is directly linked to habitat structure. Thus, habitat mapping must be conducted at each site prior to trap deployment to identify the best locations.

Cornet traps should be installed within homogeneous vegetation units, provided that the structure is high. For habitats with low homogeneous vegetation, the vacuum trap protocol should be applied instead.

The vegetation types targeted for cornet trap sampling include:

- Medium to high wet meadows (*Scirpus*, *Juncus*, *Carex*, and similar species);
- High and dense stands of *Phragmites* and *Cladium*;
- Mix between reed beds and wet meadows;
- Medium to high couch grass vegetation.

As interception traps, cornet traps must be positioned to block insect movement corridors. Such traps are typically placed along the edges of high vegetation habitats, where vegetation structures act as natural movement corridors (Sheikh et al., 2016). Proper placement is critical, as even minor changes in trap position can significantly affect the capture efficiency. Consequently, consultation with an experienced entomologist is recommended when determining trap locations.

Within the LIFE AWOM project, traps should be placed within homogeneous habitats to allow for meaningful comparison across sites. To standardize trapping conditions, an artificial corridor will be created in front of each trap by mowing the vegetation. The mown corridor should extend a full width equal to that of the trap and continue over a length of 10 meters.

### 4.1.2 Trap design and conception

Cornet traps are not commercially available as complete units and must therefore be constructed from individual components (see below). The only pre-manufactured component that can be purchased is the arthropod collection system, consisting of a double-bottle collector and its attachment mechanism for mounting on a wooden pole.

The main trap components include:

- **Insect-proof netting**: a mesh size of 180µm will ensure comprehensive arthropod collection.
- **Flexible tubing**: used to form the arch-shaped trap entrance through which the arthropods will reach the collector.
- **Bottle collector**: a double-bottle system commonly used with Malaise traps. The upper bottle is empty and functions as an airlock, while the lower bottle is filled with 96% undenatured ethanol for insect preservation.
- **Heavy-duty sewing thread and bias tape**: used for assembling and reinforcing the netting structure.
- **Wooden pole**: supports the bottle collector.
- **Polyester webbing**: used to create attachment loops for securing the trap.
- **Ground attachment system**: where site conditions permit, tent pegs can be used to secure the trap. Alternatively, waterproof fabric can be sewn along the base of the trap, extending beyond the bottom edges, to allow rocks or other weights to hold it firmly against the ground.

The estimated cost of constructing a single trap is up to 127.65€ (**Annex 1**). However, several materials, such as insect-proof netting and sewing thread, are supplied in quantities sufficient for multiple traps. Hence, the average cost per trap decreases as the number of traps constructed increases (**Figure 1**).

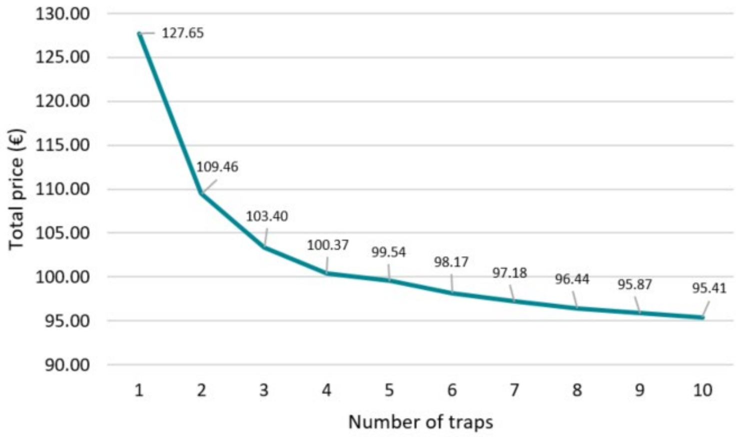


**Figure 1:** Total price for the material to sew cornet traps, depending on the number of traps to be sewn.

### 4.1.3 Number of replicates

Cornet traps are unidirectional interception traps. To sample arthropod movement in both directions, two cornet traps should be installed back-to-back at each sampling location within a site, forming a single cornet trap unit.

The total number of sampling locations at each site will depend on the number of vegetation units identified during habitat mapping.

One cornet trap unit (i.e., two back-to-back traps) should be deployed within each vegetation unit identified.

### 4.1.4 Timing and duration of sampling

Cornet traps should be deployed for at least the **entire month of August**, corresponding to the post-breeding (post-nuptial) migration period of the Aquatic Warbler.

Collection bottles should be retrieved and replaced every 10 days throughout the sampling period.

## 4.2 Vacuum sampling using a biocenometer

### 4.2.1 Type of habitats targeted

Unlike cornet traps, vacuum sampling is better suited to habitats with low vegetation. Dense vegetation can reduce sampling efficiency by obstructing airflow and limiting the capture of arthropods (Brook et al., 2008). Taller vegetation also reduces the proportion of certain captured taxonomic groups, such as Hemiptera. On the other hand, Diptera, for example, appear to be less affected by vegetation height (Henderson & Whitaker, 1977 ; Hossain et al., 1999).

Vacuum sampling will therefore be carried out in homogeneous low-growing habitats, including:

- Low wet meadows (small species of *Juncus*, *Carex*, grasses, and similar species);
- Low-lying pond-edge communities;

- Saltmarsh vegetation (*Halimione*, *Limonium*, *Suadea*, and *Salicornia*);
- Subhalophilic hay meadows.

The use of a biocenometer enables to better define a fixed area, allowing for the estimation of absolute abundances. It also reduces the escape of arthropods that may react to the relatively high noise level produced by the vacuum device before sampling begins (Henderson & Whitaker, 1977; Zou et al., 2016; Nageleisen & Bouget, 2020; Agere et al., 2021).

Differences in sampling efficiency between sites have been found to be most pronounced for aphids, springtails, millipedes, ants, and thrips. In contrast, variation is generally lower for Diptera, Auchenorrhyncha (suborder of Hemiptera), Heteroptera, spiders, and Hymenoptera (Sanders & Entling, 2011).

## 4.2.2 Equipment and Materials

### *4.2.2.1 Equipment specifications*

Studies have shown that a modified leaf blower can be just as effective as, if not more, than a conventional D-Vac, while also being more maneuverable and cost-efficient (Wilson et al., 1993; Stewart et al., 1995; Zou et al., 2016). Sampling efficiency for Diptera, Hymenoptera, and most Homoptera (Auchenorrhyncha) is comparable between the two systems, while the modified leaf blower may provide higher capture rates for Coleoptera and spiders (Wilson et al., 1993).

Accordingly, a modified leaf blower, such as a cordless Black&Decker vacuum/blower (BCBLV3625L1-QW; suction volume: 8.8 $m^3$/min), will be used.

The required equipment includes:

- Black&Decker vacuum/blower (BCBLV3625L1-QW; suction volume: 8.8 $m^3$/min);
- Battery charging station and spare battery;
- Fine-mesh socks to retain sampled/vacuumed arthropods.

To standardize the sampled area and to contain the arthropods from escaping, a biocenometer will be used. The biocenometer consists of a quadrat with raised edges and a removable fabric/piece of cloth on top to cover it.

Based on field trials conducted in the Aiguillon Bay (France), the biocenometer dimensions will be 80x80 cm with a height of 60 cm. These dimensions provide a trade-off between reducing sampling variability and minimizing arthropod escape. While smaller sampling areas generally increase the variability in the results, they also facilitate more efficient arthropod collection and reduce losses associated with microtopographic variations.

### *4.2.2.2 Operation on the field*

For each replicate, the procedure should be the following:

**1.** Place the biocenometer over the vegetation to be sampled, ensuring that all edges touch the ground to prevent arthropods from escaping.

**2.** Attach fine-mesh sock to the end of the vacuum suction tube to collect all the arthropod to be vacuumed.

**3.** Vacuum the entire enclosed area for a period of two minutes.

**4.** Upon completion, remove the sock and securely tie in the opening.

**5.** Label the sample with the operator's name, replicate number, location, and date.

**6.** Store sample in a cool box for processing.

#### *4.2.2.3 Number of replicates*

During each field visit, three replicate samples will be collected within each identified low-growing homogeneous habitat type.

Sampling locations will be selected by randomly placing the biocenometer within the target habitat.

The total number of samples collected at site will depend on the number of vegetation units identified during habitat mapping.

#### *4.2.2.4 Timing and duration of sampling*

Sampling will be conducted **three times during August**:

- Early August;
- Mid-August;
- Late August.

This will represent a total of 9 replicates per vegetation unit over the sampling period.

## 4.3 Sorting and identification

Insects will be sorted and identified manually to enable biomass analyses. Although DNA metabarcoding would facilitate rapid and highly precise taxonomic identification, this approach does not preserve information on individual body size, which is required for biomass estimation.

The taxonomic resolution will be as follows:

- Diptera and spiders will be identified to family level;
- All other insects will be identified to the order level.

Where resources permit, the remaining material will be submitted for eDNA analyses. This would maximize the value of the samples at a site scale.

## 4.4 Biomass analysis

Arthropod biomass is a key indicator of food availability for insectivorous bird species and is at least as important as arthropod composition when assessing habitat quality for the Aquatic Warbler. To estimate biomass, each individual arthropod will be measured, and dry biomass (mg dry weight) will be calculated using Hodar's (1996) regression tables which relate body size to dry weight.

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# Annexes

## Annex 1: Equipment needed for the confection of cornet traps, and prices associated

| Material | Packaging | Supplier and link (example) | | Quantity needed for 1 trap | Price |
|---|---|---|---|---|---|
| Insect proof netting, 180µm mesh | 2.2 x 5m (11 $m^2$) | Magasin Vert | https://www.monmagasinvert.fr/filet-biocontrol-insecte-2m20-x-5m-1820022.html | 0.9 $m^2$ | 26.20 € |
| Tube that can be bent to form an arch | 2m, ⌀ 16mm | Leroy Merlin | https://www.leroymerlin.fr/produits/tube-multicouche-nu-16-en-barre-de-2m-equation-84850965.html | 1 | 3.89 € |
| Double-bottle collector | 1 | Cahurel Entomologie | | 1 | 81.60 € |
| Strong sewing thread | 200 m | Mondial Tissus | https://www.mondialtissus.fr/bobine-de-fil-super-resistant-blanc-54619.html | 20 m (overestimated) | 5.19 € |
| Bias tape | 20 m | Tissus Price | https://www.tissus-price.com/fr/bobine-de-biais/15091-bobine-de-biais-20-m-blanc-14-3008477810143.html | 5 m | 4.99€ |
| Wooden pole | 2.5 x 2.5 x 150 cm | Castorama | https://www.castorama.fr/piquet-en-bois-carre-blooma-en-pin-l-25-x-l-25-mm-x-h-150-cm/3663602731474_CAFR.prd | 1 | 2.99 € |
| Polyester webbing | 1 m | Mondial Tissus | https://www.mondialtissus.fr/sangle-polyester-35mm-blanc-288741.html | 1 | 2.79 € |
| Alcool to fill in the bottle collector | 96% undenatured ethanol will be used | | | | |
| System for attaching the trap | Depend on the situation on the site : tent pegs, cable support system... | | | | |
| | | TOTAL | 127.65 € (taking into account the fact that some packages can be used to build multiple traps) | | |