# *AESHNA JUNCEA* SAMPLING PROTOCOL

## SUMMERS 2025-2026

**Marie Lamouille-Hébert, 2025**

*Many thanks to Guillaume Doucet, Nina Gouze, Pauline Pompa, Emmanuel Jaulin, Philippe Lambret, Maxence Forcellini, OPIE Odonata group and Glenn Yannic for their invaluable contributions (figures, choice of material or thoughts on protocol).*

You have received this sample collection and return kit (5) because you have had prior discussions with me, Marie Lamouille-Hébert. You are a confirmed odonatologist and have one or more sample collection sectors defined together.
If this is not the case, or if you have any questions, please contact me: marie.lamouille-hebert@inrae.fr and 0668520773.

### What equipment do you need for the field?

- This **protocol**
- A mobile **phone** for entering data directly in the field, or a paper form to complete upon returning online
- An Odonata **net**
- A **botanical magnifying glass**
- A pair of small **scissors**
- An isothermal bag containing **5 tubes** and a frozen ice pack

### Where to collect samples?

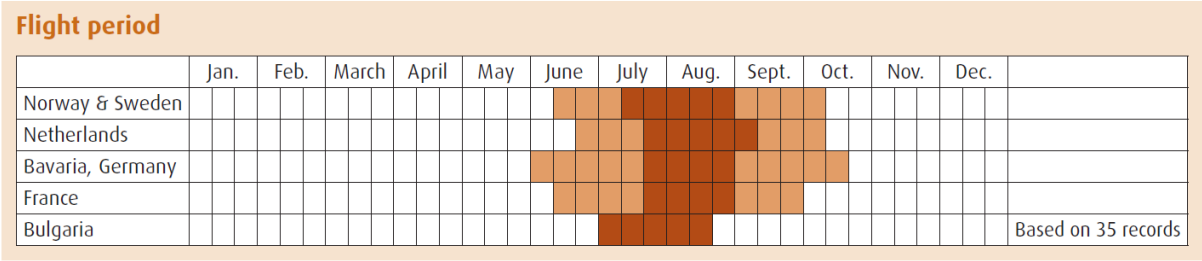
In each sector where you will be collecting, your objective is to collect **five individuals**. A sector is defined as a coherent geographical area, such as a cirque, plateau or valley. Collections must be restricted to the agreed sector and must be at least 20 kilometres away from any other collection sector assigned to a different collector.

These individuals (described below) are **located in one (if there is only one) or more ponds** in the agreed area.

### When do you collect them?

Specimen collection will take place **mainly in June, July and August** of 2025 and 2026. The peak emergence times of *Aeshna juncea* may vary from one area to another. The periods shaded in dark orange/red in Table 1 are the ones to be favoured. No collection period is imposed. **Observers are free to collect data at any time during the summers of 2025 and 2026.**

**Table 1.** *Aeshna juncea* flight periods (Boudot & Kalkman, 2015)




## Which individuals do you need to collect?

**A single individual is placed in each tube. So 5 tubes for one sector, so 5 individuals.**

**I only open the tube containing the alcohol once I have collected the sample (exuvia or dead individual). Be careful not to spill the alcohol. If possible, I place it vertically in the bag. This is to prevent too much alcohol from escaping.**

Once the sample has been placed in the tube, **ensure that it is tightly closed** before returning it to the isothermal bag containing the partially frozen ice pack...

The preferred option is fresh exuviae or parts of a dead individual. If this is not possible, the live leg option described below can be used as a last resort.

***1. Fresh exuviae:*** you have witnessed the emergence, or the emergent is still present near the exuviae. Alternatively, you collect exuviae daily and can confirm that they are less than 24 hours old.

Below is the key (Fig. 1) and the *Aeshna juncea* exuviae identification table (Fig. 2) to help you confirm identification and avoid possible confusion. Both documents were produced by Guillaume Doucet for this protocol.

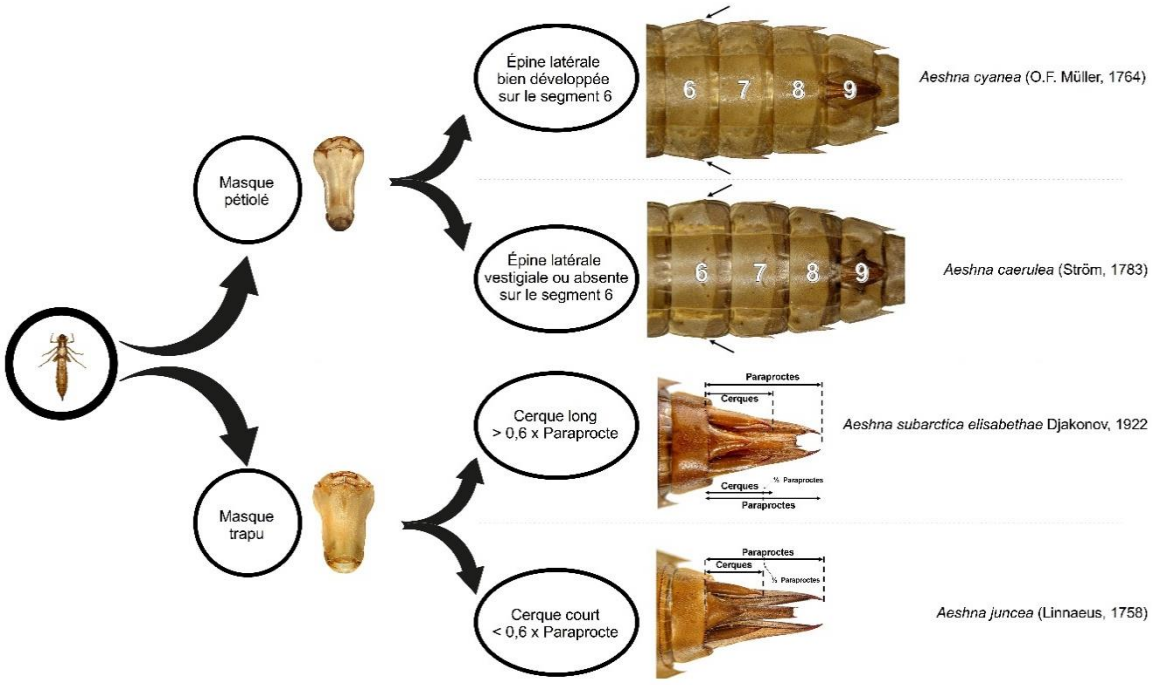



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**Figure 1.** This identification key for *Aeshna juncea* exuviae is intended to avoid confusion with those of *A. cyanea*, *A. caerulea* and *A. subarctica*. This key was produced by Guillaume Doucet as part of the protocol for collecting *A. juncea* samples for the CIMaE project.

| | | *A. caerulea* | *A. cyanea* | *A. juncea* | *elisabethae*<br>*A. subarctica* |
|---|---|---|---|---|---|
| Taille (mm) | | 36 à 40 | 38 à 48 | 37 à 45 | 38 à 43 |
| Épines latérales | | 7 à 9 | 6 à 9 | (6) 7 à 9 | 7 à 9 |
| Longueurs<br>Cerques / paraproctes | | ≈ 0,4 | ≈ 0,5 | 0,45 à 0,55 | 0,6 à 0,7 |
| Longueurs<br>Épines latérales 9/<br>segment 10 | | 0,4 à 0,5<br>  | ≈ 0,5<br>  | 0,4 à 0,5<br>  | 0,4 à 0,5<br>  |
| Masque | L / l.min | 2,7 à 2,8 | 3 à 3,1 | 2 à 2,2 | 1,95 à 2,1 |
| | l.max / l.min | 1,9 à 2 | 2,1 à 2,3 | 1,7 à 1,8 | < 1,7 |
| | Forme | Pétiolé<br>  | Pétiolé<br>  | Trapu<br>  | Trapu<br>  |

**Figure 2.** Comparison table of the exuviae of *A. juncea*, *A. cyanea*, *A. caerulea* and *A. subarctica*. This key was produced by Guillaume Doucet as part of the protocol for collecting *A. juncea* samples for the CIMaE project.

### *2/ Part or all legs*

***a) from a dead individual***
***b) from a living individual***
*(If you are interested, you can read more about the non-lethality of this operation on butterflies here: [https://resjournals.onlinelibrary.wiley.com/doi/10.1111/icad.12024](https://resjournals.onlinelibrary.wiley.com/doi/10.1111/icad.12024))*

The adult identification key (Fig. 3) below can be used to confirm the identification of *Aeshna juncea* and avoid possible confusion.

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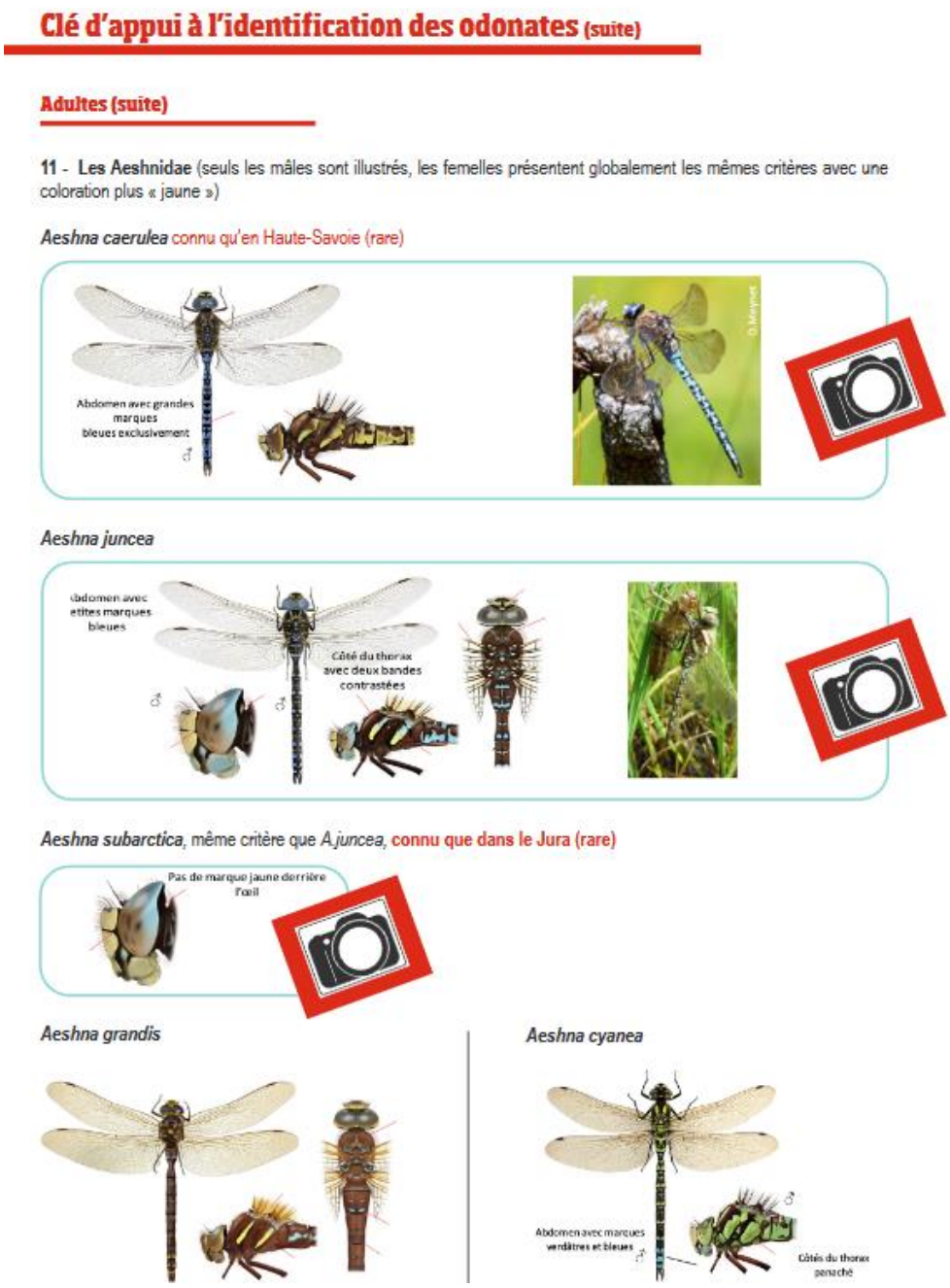


**Figure 3.** Comparaison of *A. juncea*, *A. cyanea*, *A. caerulea* and *A. subarctica* adults. This is an extract from the key produced by Marie Lamouille-Hébert as part of the CIMaE project. (Lamouille-Hébert, 2021).

After capturing **a dead specimen, attach several intact legs**. If the individual is still alive, cut off part of the central leg, as illustrated by Philippe Lambret in Fig. 4 below.

- Hold the specimen with its wings folded, facing your palm, between your middle and index fingers (Fig. 4, top).
- Position the medial tibia so that it dips into the Eppendorf-type tube containing absolute ethanol. Hold the tube with the same hand, between your thumb and ring finger. If necessary, use the end of a pair of small scissors in your other hand (see Fig. 4, bottom left).
- Cut the tibia at the highest point, just below the femur, so that the lower part of the leg falls into the tube (Fig. 4, bottom right).

**Figure 4.** Cutting the tibia from the leg. This was set up by Philippe Lambret for the *Aeshna juncea* sample collection protocol as part of the CIMaE project. It is shown here with *Sympetrum fonscolombii*.

## What information should be collected and where?

The following information must be recorded **for each specimen collected**:

- location (X, Y) in WGS84 reference coordinate system
- the collector's name
- the date of collection
- sample type (e.g. fresh exuvia, leg)
- the tube number in which the sample is placed (The number is the right way around when the cap is on top)
- email address

If possible, enter this information directly into the project-specific form on your **Android phone**.

### *1) Collect data on paper and enter it online when you return*

If you use paper, enter the data in the table below and submit it via the following link when you return from the field: https://central.sicen.fr/-/single/1p4WBgWj2fh3btHt0H7jIkawIsUEbId?st=kSXmMfTcuZxT0mUIPEu$y0GOAIBjAt4ChJlP0hbV4DhmnJEuCSD0FIgmEK8GdXw$ . You can change the language of the form using the button at the top right.

| Collector's name | X | Y | Reference coordinate system | Date | Sample type (fresh exuvia: E /leg: L) | Tube number |
|---|---|---|---|---|---|---|
| | | | | | | |
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***2/ Collecting data directly on the telephone form in the field***

Install the form on your phone before going out into the field, when you have an internet connection. **You can, of course, fill in the form offline**.

**Step 1:** Download the « **ODK collect** » application onto your Android phone from the PlayStore or from this link: https://play.google.com/store/apps/details?id=org.odk.collect.android&hl=fr

**Step 2:** Launch the application, then scan the QR code below to access the 'DNA sample collection' form. Once you have done this, you will be able to fill in the form even without an Internet connection.

**When I get back from the field, what do I do with my samples?**

When I return from the field, I remove the ice pack from the isothermal bag. I put the ice pack in the freezer and the tubes in the fridge at 4°C. If I leave them in the bag, I leave the bag open in the fridge.

When I have collected my five individuals from the sector, I can send back my samples. I put the frozen ice pack back into one of the compartments of the isothermal bag, and the tubes into the other compartment. The isothermal bag should then be placed in the stamped envelope. Post the envelope before daybreak on Monday if possible to reduce transit time and ensure that it does not remain in the mailbox over the weekend.

***THANK YOU FOR TAKING PART IN THIS PROJECT! IT WOULD NOT HAVE BEEN POSSIBLE WITHOUT YOU.*** **Marie ☺**

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