LIFE AQUATIC WARBLERS ON THE MOVE

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Aquatic Warbler (Acrocephalus paludicola) Fat reserve measurement protocol

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Authors:

- Mallaury LACOMBE, Bretagne Vivante
- Christine BLAIZE, Bretagne Vivante
- Romain LORRILLILÈRE, CRBPO

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Abbreviations

AWOM	Aquatic Warblers on the Move
MLS	Minimum length stopover

Executive summary

Some biometric measurements are needed to calculate elements linked to fuel accumulation in birds. Within a stopover site, body condition, fuel deposition load, and daily fuel deposition rate can be calculated, and adiposity and pectoral muscle can be scored. A single capture is sufficient to calculate the body condition index or to score adiposity and pectoral muscle, whereas a recapture is needed for the other calculations. Based on measurements done while ringing, they make it possible to assess birds' fattening and some elements linked to migratory behaviour. Within the LIFE AWOM project, these calculations will be mainly used to assess stopover site quality for the Aquatic Warbler.

1. Introduction

The Aquatic Warbler is Europe's most endangered continental migrating passerine. It migrates from eastern Europe, where it breeds, to western Africa, where it winters, stopping in several countries in western Europe and northern Africa to rest and replenish its reserves. The main sectors for autumn migration stopovers are now well known in most of the countries concerned, but knowledge needs to be deepened in other countries, located mainly in Northern Africa.

In Belgium, the bird is mainly found along rivers and the Flemish coast (Van Hove *et al.*, 2007), as in France, where it is encountered largely along the Channel and Atlantic littoral (Jiguet *et al.*, 2011). The same tendency is observed in Spain, where the stopover sites are located in the northern half of the country, and in Portugal. Morocco also seems to be an important country for Aquatic Warbler migration, and studies and prospecting are ongoing there. To date in this country, the bird has been detected more during the spring migration than the autumn migration. Information from Morocco and other countries suggests that the Aquatic Warbler tends to take another path in spring migration. However, less is currently known about spring migration than about autumn migration, which may be partly due to the birds migrating faster just before breeding (De By, 1990). During this period, the birds appear to be travelling more through the eastern coast of Spain and the Mediterranean area in France (Le Nevé, 2013). Although the location of these stopover sites, spring and autumn combined, has been studied for some time, further work is needed to improve knowledge about the quality of the different sites, and thus their functionality for the species.

The LIFE AWOM, focused on the Aquatic Warbler in stopover and wintering sites, aims to develop a comprehensive, coherent and climate resilient flyway site network for the species. In order to reach this general objective, there is a need to assess the extent and quality of habitats and the importance of staging and wintering sites for the Aquatic Warbler. To do so, diverse methods will be implemented, regarding habitat, food availability and migration monitoring. One of these methods concerns fat reserve measurements and calculations.

Within this framework, the present document details the different elements to be measured on individuals and the methodology of data analysis. Half of this document concerns fat reserve measurements, which aim is to bring an additional metric to assess quality of stopover sites. The other half explores birds' body condition analysis, whose aim is broader, informing about migratory behaviour.

2. Important Note

Calculations presented are based on measurements made directly on the birds. Since the Aquatic Warbler is a protected species, its capture and manipulation require authorization from the competent authorities.

In addition, the data is obtained through ringing activities. It is important to note that ringing activities depend on the rules and procedures issued by the national ringing center of the country concerned, and this center must provide the authorization to perform such activities.

3. Definitions

Different metrics can be calculated, based on the measurements taken from individuals in the field, which all refer to the replenishment of energy reserves by the individual during migration, at a stopover site.

Fuel deposition load : percentage of variation between initial and final body mass, for a single bird at the same stopover site.

Daily fuel deposition rate : amount of fuel deposited per day.

Minimum length stopover : minimal duration of a bird's stopover at a site, calculated on the basis of the dates of capture and recapture.

Body condition index : standardized mass of a bird at a specific point in time, projected with tarsus, wing chord and bill length.

4. Influencing Factors

Several factors can influence body condition and fattening in migratory birds, the most important one being food availability on stopover sites. Also, for many species, there is a difference between adults' and juveniles' fattening kinetics (Ellegren, 1991; Woodrey, 2000; Heise & Moore, 2003; Yosef & Chernetsov, 2004; Choi *et al.*, 2009). This kinetics may also differ depending on individual corporal condition (Maitav & Izhaki, 1994; Schaub & Jenni, 2000; Yosef & Chernetsov, 2004).

Such an age-related difference in body condition has been specifically observed in the Aquatic Warbler with the juveniles being in better condition than the adults in Portugal (Neto *et al.*, 2010), but the opposite occurring in France (Musseau *et al.*, 2014; Hemery *et al.*, 2017). This difference could be explained by an earlier onset of the fueling period of adults in France, and thus a shorter migration duration linked to their dominance and experience. Further south in Portugal and later in the season, juveniles may then have to catch up with the adults and fatten up comparatively faster (Neto *et al.*, 2010). Results in France also highlight that birds' fuel deposition load is inversely proportional to their body condition with lean juveniles fattening significantly faster than fat birds (Musseau *et al.*, 2014; Noualhier, 2024). At Gironde estuary, lean juveniles have been found to fatten up to 23 times faster than fat birds (Musseau *et al.*, 2014). This might be due to a hormonal regulation mechanism affecting feeding behavior (Long & Holberton, 2004).

For some trans-Saharan migrants, fattening behaviour, especially the amount of time dedicated to the exploitation of trophic resources, also appear to be influenced by day length (Bauchinger & Klaassen, 2005; Pokrovsky *et al.*, 2021; Engert *et al.*, 2023). In France, the fattening strategy of the Aquatic Warbler also seems to be influenced by latitude and by a gradient in the distribution of trophic resources exploited by the species (Hemery *et al.*, 2017; Noualhier, 2024).

However, it should be kept in mind that fuel deposition load is not entirely linked to the stopover site quality (e.g., food availability) for the species. Indeed, bad weather conditions, for example, may affect the foraging opportunities for individuals, and therefore, their fuel deposition loads, without calling into question the initial quality of the stopover site (Ormerod, 1989).

5. Fat Reserve Measurement

5.1 Calculations

Two metrics, calculated on the basis of biometric data, make it possible to assess birds' fat reserves; **fuel deposition load** and **daily fuel deposition rate**. Analyzing an individual mass variation through time at a stopover site makes it possible to obtain information on stopover site quality through food availability. To do so, two conditions must be met for an individual at a stopover site:

- **Recapture data** must exist for the selected birds, in order to estimate the mass gain between the first and the second capture at an individual level.
- **A 48-hour gap** between the capture and recapture data is needed for calculations. Indeed, it is considered that with a shorter gap, the parameters to be measured are still too influenced by the stress suffered by the bird during the first capture, which generally induces a decrease in mass (Schwilch and Jenni, 2001).

5.1.1 Fuel deposition load

The calculation of a fuel deposition load for a single individual on a single stopover site makes it possible to compare individual corporal masses between two captures, which requires some **biometric** and **spatiotemporal** data.

- Biometric data:
    - **Mass**, measured to the 1/10 gram.

- Spatiotemporal data, to link calculations to stopover sites:
    - **Ringing date** (DD/MM/YYYY).
    - **Ringing time** (HH:MM). If time is not known, leave the cell empty.
    - **Country** where the ringing action is performed. Acronyms on Euring website.
    - **Region** where the ringing action is performed. Acronyms on Euring website.
    - **City** where the ringing action is performed (France, Spain, and Switzerland only). Must be written in capital letters, without special characters nor abbreviations.
    - **Lieu-dit (placename)** where the bird is ringed. Even if it is an open field, it is essential to keep the exact same name for the same ringing station over time.
    - **Latitude**. Indicated in decimal degrees WGS 84.
    - **Longitude.** Indicated in decimal degrees WGS 84.

If the two prerequisites are met, the **fuel deposition load** is calculated as follows :

$$Fuel\ deposition\ load = \frac{Final\ body\ mass - Initial\ body\ mass}{Initial\ body\ mass} \times 100 \text{ without unit}$$

Initial and final body mass are measured respectively during the first and the second capture. If the fuel deposition load is positive, the bird fattened between the two captures, indicating the relatively good quality of the site. If it is negative, a decrease in fat reserves occurred. In order to better discuss the results, minimum length stopover may also be taken into consideration.

5.1.2 Daily fuel deposition rate

The daily fuel deposition rate makes it possible, unlike fuel deposition load, to take into consideration the time between capture and recapture. The refueling kinetics, hence included in the calculation, enables a better evaluation of the quality of stopover sites.

Daily fuel deposition rate is calculated as follows:

$$Daily\ fuel\ deposition\ rate = \frac{Fuel\ deposition\ load}{Minimum\ length\ stopover} \text{ in days}^{-1}$$

Note: the minimum length stopover does not correspond to the duration between the date of arrival and of departure of a bird at stopover sites, but to the time interval between captures.

The value obtained gives the mean standardized mass intake per day, during the minimum length stopover (MLS). However, as this calculation is an average, it must be considered with caution, as it can mask possible different foraging and fuel deposition strategies on the different days of the MLS, depending particularly notably on birds' body condition (Musseau *et al.*, 2014, see section 6).

5.2 Adiposity: a complementary qualitative metric

Adiposity is measured according to the scores described by Kaiser, 1993 (see Annex 1). Ranked from 0 to 8, this parameter can also be used to provide details about birds' fattening on stopover sites.

Adiposity can be used as a complementary element for fat measurement. Although its analysis is more subjective and less precise than the calculations described above (Redfern *et al.*, 2004), it allows for more exploitation of the dataset. Indeed, the main advantage of this method is that, unlike fuel deposition load and daily fuel deposition rate, it can provide information based on a single capture. For example, a bird captured on a stopover site with an adiposity score of 8 has necessarily fattened on this site.

6. To go further: Body Condition Analysis

Body condition has generally been defined in a very broad sense to indicate the ability of an individual to cope with present and future physiological stress, and therefore, the ability to enhance fitness (Carrascal *et al.*, 1998). Individuals' body condition index makes it possible to study various parameters, regarding the individuals own characteristics or their behavior. Indeed, this calculation may reflect morphological variation and/or be an indicator for sex determination for certain species (Lislevand *et al.*, 2009). The body condition may also condition migratory behavior, such as stopover frequency or wind selection (Duijns *et al.*, 2017). Orientation behavior appears to also vary depending on the individual's body condition. Indeed, fat birds tend to cross ecological barriers such as the Mediterranean Sea or the Sahara Desert, whereas lean birds avoid them by detouring from the principal migratory direction, to orient parallel to the coast line or to search more profitable stopover sites (Sandberg and Moore, 1996; Korner-Nievergelt *et al.*, 2002; Schmaljohann & Naef-Daenzer, 2011) Finally, reproductive outcomes and apparent survival can be predicted by body condition (Blums *et al.*, 2005; Benson & Bednarz, 2010; Heath *et al.*, 2011; Anderson *et al.*, 2019). It seems that fat individuals observed during spring migration are more likely to be captured again during autumn migration, suggesting an increased mortality of lean birds (Duijns *et al.*, 2017; Anderson *et al.*, 2019).

In order to assess birds' body condition, two different types of metrics can be used. A **statistical analysis of body condition** can be carried, based on the standardization of birds' mass by their structural size. **Qualitative metrics** linked to fat and pectoral muscle can also be used to measure birds' body condition.

6.1 Statistical analysis of body condition

The goal of this statistical analysis is to measure a bird's fat reserve level at a given time, based on its mass. However, the bird's mass itself is not an ideal indicator of the fat reserve level, because it depends largely on its size (Connell & Odum, 1960; Pearson, 1971). Hence, body mass must be standardized using biometric data measured while ringing, to rule out the effect of the bird's size. Such biometric data include tarsus length, wing chord length, bill length, head length, or tail length.

Analyses based on several coupled biometric data are generally a better predictor of mass (Gosler *et al.*, 1998). It is also necessary to have the best possible ratio between the ideal number of biometric measurements and the possibility of implementing them at ringing stations across Europe. Hence, three measurements will be retained: length of the tarsus, wing chord length, and bill length.

Hence, in order to be able to launch the statistical analysis and to further discuss the results, biometric and spatiotemporal data are required.

- Biometric data:
  - **Mass**, measured to the 1/10 gram.
  - **Length of the tarsus**, measured to the 1/10 millimeter (Fig. 1)

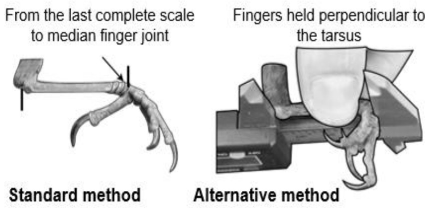

Figure 1 : *Measurement of the tarsus length*

- Length of the wing chord, measured to the ½ millimeter (Fig. 2)

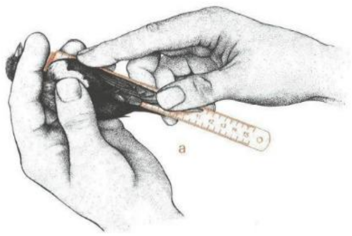

Figure 2 : *Measurement of the wing chord (D. Ornith.-Ges., 2011)*

- **Length of the bill (bill to skull),** measured to the ½ millimeter (Fig. 3)

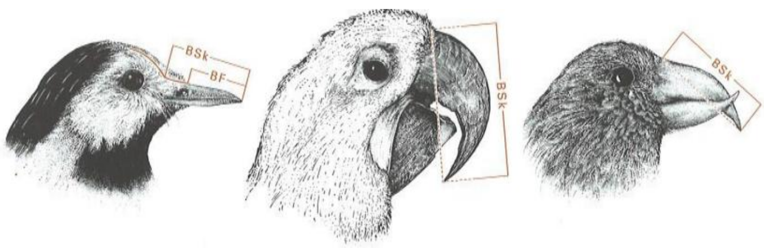

Figure 3 : *Measurement of the bill length, from bill to skull (D. Ornith.-Ges., 2011)*

- Spatiotemporal data: in order to link analyses to the migratory route, the same data as for fuel deposition load are required.

Tarsus, wing and bill length will then be included as covariates in statistical models, in order to account for the effect of size on bird mass. Attention will have to be paid to the correlation of the variables linked to birds' size.

6.2 Adiposity and pectoral muscle scores: complementary qualitative metrics

Adiposity and pectoral muscle scores, assigned at a specific point in time, can also be used to assess birds' body condition. Concerning pectoral muscle, the score is assigned according to the method described by Bairlein, 1995 (see Annex 2), which is ranked from 0 to 4. Adiposity can be used to assess birds' body condition, in addition to being used in complementarity with fat reserve measurement. Regarding its methodology, refer to part 5.2 of this document.

Fat and pectoral muscle should increase in mass in preparation for migration, particularly in species flying long distances at once (Sandberg & Moore, 1996; Redfern *et al.*, 2004). During migratory flight, a bird's muscles are likely to be used as the source of protein at the same time as its fat reserves are used as an energy source (Evans & Smith, 1975; Bairlein, 1995). In addition, the rapid consumption and replacement of pectoral muscle on a short time scale suggests that it may also be used as an energy source during migration (Lindström *et al.*, 2000). This utilization of extra muscle mass for fuel (Redfern *et al.*, 2004) occurs especially when both glycogen and lipid reserves are nearly or completely exhausted (Carrascal *et al.*, 1998). Hence, the longer the migratory flight, the greater the extent of hypertrophy to be expected. Fat score may also influence the decision to embark on migration and the choice of migratory direction (Sandberg & Moore, 1996).

Although fat and pectoral muscle mass vary in parallel, they appear to be independent measures of body condition (Evans & Smith, 1975; Carrascal *et al.*, 1998; Redfern *et al.*, 2004).

As adiposity and pectoral muscle scoring are qualitative methods, there could be observer bias. This may concern the pectoral muscle more than the fat scores. For the latter, scores are relatively easy to assign, as there is a high level of agreement between ringers. Conversely, pectoral muscle score assignment appears to be rather more difficult (Redfern *et al.*, 2004).

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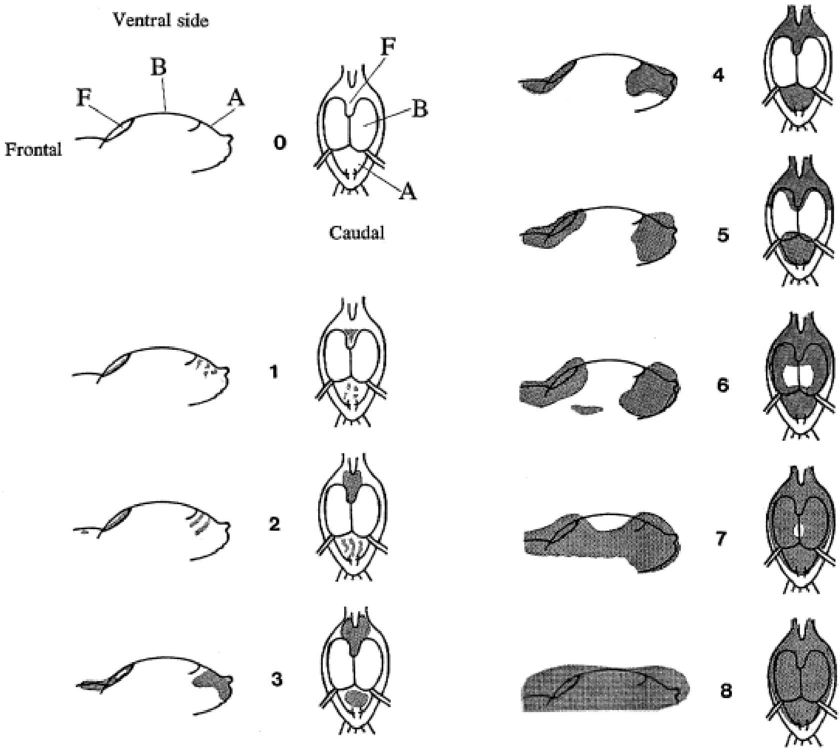
8. Annexes

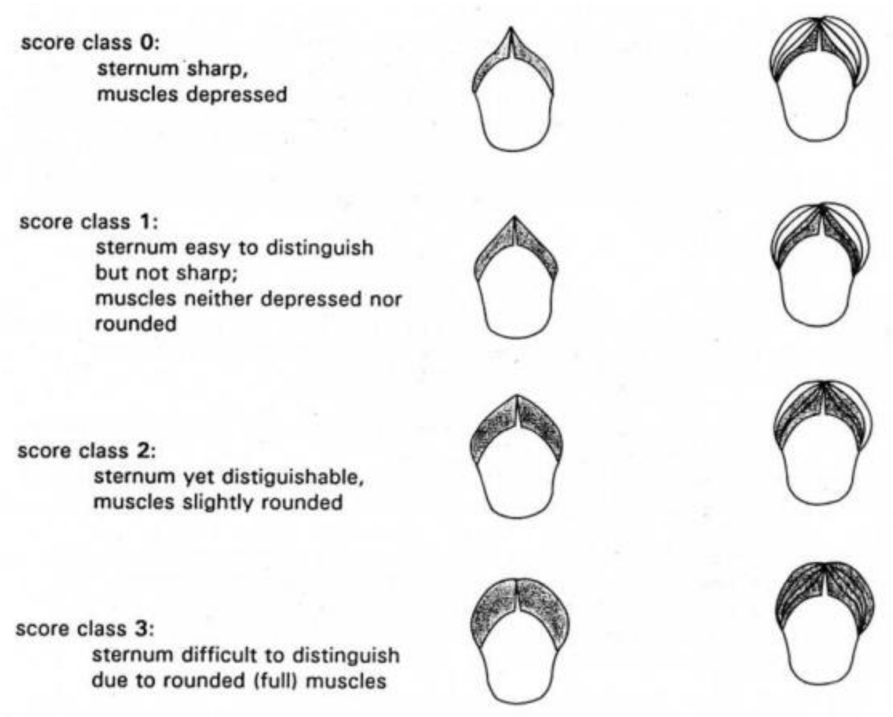
Annex 1 : Adiposity measurement (Kaiser, 1993)



Main fat score classes (0-8). F = Furcular depression (interclavicular depression), B = Breast muscles, A = Abdomen, stippled = fat

Main class	Subclass	Furcular depression	Abdomen	Color of the considered areas
	0.00	no fat	no fat	dark red
0	0.25	barest trace, very narrow stripe	fat deposits not yet delimited	red
	0.50	small stripe	fat deposits not yet delimited	red
	0.75	wedge-shaped	small trace, patchy	light red
1	1.00	wide wedge	trace, very small, stripes around intestinal loops (<1 mm)	light red
	1.25	half of furcular depression is covered	trace, stripes 1 mm wide	yellow-red
	1.50	almost completely covered with fat	trace, stripes smaller than intestinal loops	yellow-red
	1.75	small amount, almost completely covered with fat	wide stripes (2 mm)	yellowish
2	2.00	completely covered, shape deeply concave	slips of visceral fat, area between intestinal loops completely filled	light yellow
	2.25	completely covered, shape deeply concave	some subcutaneous lipid, not yet forming a pad	light yellow
	2.50	completely covered, shape deeply concave	very small pad	light yellow
	2.75	completely covered, shape deeply concave	small pad, at least 2 or 3 intestinal loops still visible	light yellow
3	3.00	moderate fat reserves cover ends of interclavicles	flat pad, one loop still visible	light yellow
	3.25	concave	slightly rounded pad, one loop sometimes visible	yolk-yellow
	3.50	still concave	slightly bulging, loops completely covered	yolk-yellow
	3.75	almost filled	bulging	yolk-yellow
4	4.00	filled up to distal portion of interclavicles	conspicuously bulging (2–4 mm)	-
	4.25	filled up to distal portion of interclavicles	further increase in bulge (4–5 mm)	-
	4.50	filled up to distal portion of interclavicles	abdominal structures completely covered and bulging	-
	4.75	slightly bulging with central depression (concave)	abdominal structures completely covered and bulging	-
5	5.00	convex bulge	extreme convex bulge, increasing thickness	-
	5.25	just covering flight muscles from either furc. or abdomen	extreme convex bulge, increasing thickness	-
	5.50	covering border of flight muscles a few mm	covering border of flight muscles a few mm	-
6	6.00	covering flight muscles by several mm	covering abdominal part of flight muscles by several mm	-
	6.50	fat reaches flight muscles from sides of wings		-
	6.75	fat covering flight muscles conspicuously		-
7	7.00	three quarters of flight muscles covered		-
	7.25	large rounded fat-free area in middle of breast		-
	7.50	small rounded fat-free area (red)		-
	7.75	very small fat-free area still visible		-
8	8.00	flight muscles not visible, fat layer covers underside/ventral side of the bird completely		-

Annex 2 : Pectoral muscle measurement (Bairlein, 1995)



Drawings by Göran Walinder, Falsterbo B.O., based on studies on live birds trapped for ringing and a few dissected ones.