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# Diet and fuelling of the globally threatened aquatic warbler at autumn migration stopover as compared with two congeners

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## Abstract

The effective conservation of aquatic warbler *Acrocephalus paludicola*, one of the most threatened western Palaearctic migratory passerines, requires good knowledge of its ecological needs at stopover sites. In particular, identifying its diet, which controls the accumulation of fat reserves during migration, facilitates the selection and management of adequately protected areas. Further key information includes the relationship between prey species abundance and habitats of aquatic warbler on stopover. We performed standardized mist netting in the Audierne marshes (western France) during 12 years, which resulted in the capture of 1200 aquatic warblers, and provided measurements for mass gain and the collection of faeces to infer the birds' diet. Invertebrate sampling was carried out in the three main Audierne marsh habitats (reed bed, fen mire and meadow). In order to go beyond prey digestibility bias, we also studied two closely related *Acrocephalus* species, present at migration stopover sites during the same period. We found that the diet composition of aquatic warbler observed at migration stopover sites is based on large-sized prey (Odonata, Orthoptera, Lepidoptera). Like sedge warblers, aquatic warblers put on weight during migration stopovers (daily mass gain = 0.38 g). This increase in weight suggests that the aquatic warblers might have adopted a strategy for long-distance migration with few stopovers only. Owing to great differences in diet, conservation management for the threatened aquatic warbler at stopover sites should not rely on existing knowledge about sedge and reed warblers. Similarities in the diet of aquatic warbler between nesting areas and migration stopover areas and the relationship between habitat and prey abundance suggest that fen mires play an important role in the quality of the foraging habitat at stopover sites.

## Introduction

A decline in long-distance migratory songbirds has been repeatedly observed. The causes of this decline are numerous: climate change (Both *et al*., 2006), degradation of wintering, breeding habitats (Robbins *et al*., 1989), or loss and fragmentation of stopover site (Hutto, 1998). Recently, the vital importance of the presence and quality of migration stopover sites to en route songbirds has come to the forefront of avian conservation (Petit, 2000). Long-distance migration requires exceptional reserves. Migratory songbirds must rest and deposit fat reserves in restricted stopover. There, the often high density of birds together with heavily depleted food supplies lead to a severe competition both within and among species (Newton, 2004). Accordingly, it is known that high-quality habitats at stopover sites and a preserved network of stopovers should be considered an essential component of strategies for the conservation of migratory bird populations (Newton, 2004; Ktitorov, Bairlein & Dubinin, 2008). However, the quality value of a site may differ among species and the reserve managers need explicit recommendations.

The aquatic warbler *Acrocephalus paludicola* is a rare long-distance migratory bird species and is considered one of the most threatened western Palaearctic migratory passerines (Collar, Crosby & Stattersfield, 1994). Its populations suffered an important decline mainly due to the loss in their breeding habitat (Dyrcz & Zdunek, 1993; Kozulin, Vergeichik & Stepanovich, 2004). In addition, like many insectivorous birds that breed in northern Europe and winter in sub-Saharan Africa, aquatic warbler crosses wide ecological barriers, which requires long uninterrupted flights fuelled by large fat deposits. The migration strategy includes departure date, flight duration, habitat and diet selection, and is known to be under considerable selection pressure (Bairlein & Totzke, 1992). Northern aquatic warbler populations migrate through western Europe in autumn, chiefly visiting marshes in the Netherlands, Belgium and western coastal regions of France. France hosts the largest number of individuals in migration (Julliard *et al.*, 2006). However, important, rapid losses in marsh areas have occurred on its migratory route: 50% of marsh areas in France were lost in the 1970–1990 period (Bernard, 1994); 40% of freshwater wetlands were destroyed or degraded in the Netherlands in only a 10-year period (Holland *et al.*, 1995).

As highlighted in the European Action Plan (Heredia, 1996), the effective conservation of these threatened migratory passerines requires a thorough description of its ecological needs at stopover sites. Yet, to the extent that we are aware, few studies have analysed aquatic warblers' diet, and these studies have focused on the breeding period only. Unfortunately, the ecological needs and the network of stopover sites of aquatic warbler cannot be derived from information on congeners, as species within the *Acrocephalus* genus can exhibit very different migration strategies (Bibby & Green, 1981). The direct observation of aquatic warbler feeding on stopover is hardly possible due to the rarity of this bird and to poor visibility in marsh habitats. In addition, indirect studies of diet through faeces analysis are hindered by differential prey digestibility between preys. To circumvent these difficulties, we chose to compare faeces of aquatic warbler and two more common congeners known to exhibit differential strategy (reed warbler, *Acrocephalus scirpaceus*, and sedge warbler, *Acrocephalus schoenobaenus*) within the same stopover area. This comparison revealed diet specificities of aquatic warbler with the underlying assumption that digestibility bias is equal among the three closely related species. We then identified the taxa that made a major contribution to the diet of each species and the taxa that distinguished the diet of aquatic warbler from the two other warblers. In addition, we studied the correlations between aquatic warbler's main prey and habitat.

The strategies that underpin long migratory distances differ among species. Some birds – such as reed warblers – are known to move in many short steps, while others – like sedge warbler – negotiate the same distance in a few jumps with very long flights (Bibby & Green, 1981; Bensch & Nielsen, 1999). Consequently, physiological requirements and ecological and time constraints are different. Indeed, moving in a series of short flights requires smaller fat reserves on board. The comparison of mass gain during stopovers between aquatic, reed and sedge warblers is thus expected to inform us on strategies underlying long migratory distances. This information is of conservation concern because moving in a series of short flights requires many different suitable stopover sites en route. In this case, the removal of one site is less tragic, as these 'hoppers' can easily move to the next site. However, for species exhibiting long-haul flights, the disappearance or degradation of a critical stopover site would seriously impair migration.

# Methods

## Focal species

Aquatic warbler is a globally threatened species (Collar *et al.*, 1994) whose breeding range shrank dramatically during the last decades. The species disappeared from its former breeding grounds in Austria, Belgium, France and the Netherlands (Bargain, 1999). The European population comprises 13 000–21 000 singing males, which were mostly found in Belarus, Ukraine and Poland (Aquatic Warbler Conservation Team, 1999). Despite yearly fluctuations, there is strong evidence that the aquatic warbler population keeps declining in Europe (Birdlife International, 2004).

## Study area

The study was carried out in the Audierne marsh (western France, W4°19′14 0229 N47°55′15 0881). Three main vegetation types dominated the landscape from the coast to the inland: reed bed, fen mire and hygrophilous meadow. Reed beds surrounded the coastal lake and were dominated by the common reed *Phragmites australis*; the water table was above ground level for most of the year. Fen mire comprised medium herbaceous vegetation (up to 1 m), and in summer, the water table was only a few centimetres above ground level and sometimes dried up. Fen mires were dominated by numerous plant species including *Scirpus* spp., *Juncus* ssp., *Eleocharis* spp., *Iris pseudacorus*, and *Oenanthe* spp. Hygrophilous meadows were grazed extensively and were dominated by *Agrostis* spp. and *Dactylis glomerata*.

We performed standardized mist netting between 1988 and 2006 (same mist-net type, localization and functioning period), which resulted in the capture of up to 60 000 sedge warblers, 26 000 reed warblers and 1200 aquatic warblers (for more details on the method used, see Bargain, Vansteewegen & Henry, 2002). Owing to technical constraints, that is, mist netting could not be set up in fen mires or meadows, we were only able to capture aquatic warbler on reed beds; however, mist nets were localized close to the fen mire of <100 m (for more detail on localization and habitat, see Bargain *et al.*, 2002). The Audierne marsh is known as an important national breeding ground for reed warbler, whereas sedge warblers hardly ever breed in these marshes. However, sedge warblers that transit at the site during migration period represent 2% of the European breeding population (Bargain *et al.*, 2002). Moreover, this area is likely to constitute a major world stopover for aquatic warbler (Julliard *et al.*, 2006).

## Faecal analysis

The diet of the three warblers was assessed by faecal analysis. Between 2001 and 2004, we collected 128, 78 and 28 samples of aquatic, sedge and reed warbler faeces, respectively (with just one faecal sample by bird), during ringing operations in August and September. In order to collect faeces, we placed birds in special bags with a plastic-coated bottom, 15 min before their release. Identifiable chitinous fragments were counted in each sample with the aim to estimate the minimum number of individuals of each taxonomic group (e.g. four Odonata wings were counted as one individual). This method likely led to some bias in diet evaluation, because soft-bodied or small preys are less readily detected. However, Davies (1977*a*,*b*) demonstrated that there is a strong correlation between prey remains in the faeces and the composition of the true diet in other insectivorous passerines.

## Identifying the specificity of the aquatic warbler's diet

We first conducted a canonical correspondence analysis (CCA; Palmer, 1993) in order to evaluate the contribution of each prey species to the diet composition of aquatic, reed and sedge warblers. Furthermore, we used the apportionment of quadratic entropy (APQE), an analysis that allows diversity decomposition according to a given hierarchy (Pavoine & Dolédec, 2005). Here, the hierarchy comes from *Acrocephalus* faeces and prey species in each faeces. This analysis evaluates (1) whether the diversity in diet composition was higher among faeces within warbler species than expected randomly (within-species diversity in diet composition); (2) whether it was higher between faeces among warbler species than expected randomly (among-species diversity in diet composition). The significance of this hierarchy was tested using the permuting approach ($n = 1000$). Given that diet data mostly came from 1 month in 1 year (Table 1), we restricted these analyses (CCA and APQE) to August 2003 diet data, although similar results were obtained with the full dataset.

## Relationship between aquatic warbler's prey and habitat

To increase our knowledge on aquatic warbler's foraging habitat selection, we combined three semi-quantitative invertebrate sampling methods among the three major habitats of the Audierne marsh: (1) we made a pitfall trap, with an unattractive conservative liquid, in order to assess invertebrate density-activity in the ground. However, as pitfall traps collected few of aquatic warblers' preys, they were not detailed in this study; (2) we used a yellow bowl trap for invertebrates collected in a medium level of vegetation (two stations per habitat, one bowl trap per station, collection after 4 days of operation, total of 15 samplings per habitat); (3) we performed a standardized sweep net in order to collect invertebrates in the upper part of the vegetation (two samplings per habitat, walking a 25 m distance, carried out the same day for the three habitats). Variations in prey abundance among habitats were assessed using a Student's *t*-test with *P*-values adjusted for multiple comparisons using Hochberg (1988) correction.

**Table 1** Number of faecal samples collected for each warbler species across months and years in Audierne marshes

| | Aquatic warbler | Sedge warbler | Reed warbler |
|---|---|---|---|
| 2001 | | | |
| August | 9 | 1 | – |
| September | – | 1 | – |
| 2002 | | | |
| August | 11 | – | – |
| September | 12 | – | – |
| 2003 | | | |
| August | 50 | 64 | 21 |
| September | 11 | 3 | 2 |
| 2004 | | | |
| August | 32 | 8 | 5 |
| September | 3 | 1 | – |
| Total | 128 | 78 | 28 |

## Comparing diet diversity of aquatic, sedge and reed warblers

We assessed prey richness within each warbler's diet, using faeces. Taking into account closeness in terms of phylogeny or mass, the fairly similar prey digestibility could be considered a robust assumption for the three warblers studied. However, equal detectability of all prey species is probably not met. For example, beetles are probably more detectable than Diptera. Hence, estimating diet richness using the classic cumulative curve approach is inappropriate. We therefore used statistical methods derived from capture–-recapture approaches. However, instead of capturing individuals, we capture species; and instead of assessing population size, this approach provides an estimator of community size, here, prey species richness. This method relies on a table with faeces samples as columns, species as rows and presence–absence as entries, which constitutes the 'capture histories matrix'. This approach models richness with heterogeneous species detection probabilities. Prey species richness was estimated with the jackknife estimator (Burnham & Overton, 1979). For more detail on methods, see recent studies (Lekve *et al.*, 2002; Selmi & Boulinier, 2003; Kerbiriou *et al.*, 2007) addressing richness estimation and detection probabilities from species count data and using COMDYN software (Hines *et al.*, 2003). As reed warblers had the smallest faeces sample sizes, we performed 50 random re-samplings of faeces samples for each warbler to obtain identical sample sizes of faeces ($n = 10$) (i.e. 50 'captures histories' matrix constituted by 10 'captures' events) before the assessment of detectability and richness. Species richness between warblers was compared using the Student's *t*-test with *P*-values adjusted for multiple comparisons using the Hochberg correction.

## Comparing mass gain strategies during stopover

To compare mass gain strategies across the three warbler species, we analysed changes in body mass between capture/recapture events within a same year and stopover site. Between 1988 and 2006, ringing operations were conducted during the post-breeding migration period: from early July to late September (Bargain *et al.*, 2002). Whenever the weather permitted, the ringing station was opened for a total of 77 effective days per year (SE ± 4 days; extreme: 44; 115). Each captured bird was ringed and when safety time between capture and release was not overtaking, birds were weighed and aged (two classes: adult and young, i.e. born within the year). When birds were captured several times within a day, we retained the first measure only. For each bird captured more than once, we recorded the change in body mass between two capture events (the vast majority of individuals were recaptured only once, which generated one data point per individual). At the Audierne marshes, we collected a total of 6724 body mass changes for sedge warbler, 6470 for reed warbler and 47 for aquatic warbler. We used generalized linear models (GLM, with *F*-test in order to account for over-dispersion) to analyse whether body mass change was explained by the number of days between two capture events. Important factors are known to affect the body mass of bird in migration such as age. Moreover, we expect changes in mass during a day or over the seasons (Schaub & Jenni, 2001). In addition, the mass gain of insectivorous bird could also vary across years due to great variations in prey availability. In order to limit biases due to variations in bird mass in the daytime, we only considered data from 7 to 11 AM. Indeed, during this period we did not detect any significant difference between the time of capture and the time of recapture (respectively for the sedge, aquatic and reed warbler, $F_{1,2480} = 0.39$, $P = 0.53$; $F_{1,58} = 0.84$ $P = 0.36$; $F_{1,3150} = 2.59$, $P = 0.11$). In addition, there was no significant interaction between the day and the time of the day (respectively for the sedge, aquatic and reed warbler $F_{1,629} = 1.53$, $P = 0.18$; $F_{1,279} = 0.01$, $P = 0.91$; $F_{1,694} = 174.38$, $P = 0.15$). The other factors, age, season (i.e. day of the year) and year were included in GLM modelling with each variable tested adjusted to all the other variables.

As possible differences in mass gain are expected between birds with different mass, we used relative mass gain ($G'$) instead of gross mass gain to illustrate the relationship between mass change and stopover duration.

$$G' = \frac{(Mr - Mc)}{Mc}$$

$Mc$ is the mass measured during the first capture and $Mr$ is the mass measured during the recapture. In order to cure heteroscedasticity in GLM analyses, we log transformed $Mr$ and $Mc$. In order to distinguish reed warbler breeders from migrants, we then used the same GLM analysis on birds for which the foreign origin was known (birds ringed during the breeding season in another country, $n = 23$). For aquatic warbler, we used the entire national data in order to test the existence of regional differences in mass gain. Yet, complementary data came from Sandouville (W0°19′15 N49°29′51), Chenac-Saint-Seurin-d'Uzet (W0°49′58 N45°29′59) and Frossay-Le Massereau (W1°55′54 N47°14′41), where the same standardized mist-netting protocols were carried out.

## Results

### Taxa that make a major contribution to the diet of aquatic, reed and sedge warblers

In the faeces samples, we recorded a total of 1731 prey items. In terms of prey abundance, the diets of aquatic and reed warblers were dominated by Diptera (38 and 54%, respectively) and aphids (21 and 22%, Table 2) whereas that of sedge warbler was dominated by aphids (67%), followed by Diptera (17%). Using a predictive model of the relationship between body length and invertebrate group mass (Ganihar, 1997), the contribution of Odonata, Araneida, Orthoptera, Diptera and Lepidoptera to consumed biomass was 43, 13, 12, 9 and 8%, respectively, for aquatic warbler. For reed warblers, Diptera represented 33% of consumed biomass, aphids 16% and Hymenoptera 15%. For sedge warbler, aphids represented 48% of consumed biomass, Odonata 12% and Diptera 10% (Table 2).

### Taxa that underlined the specificity of aquatic warbler's diet, when considering abundance

The CCA approach revealed that Lepidoptera, Araneida, Orthoptera, Odonata, Coleptera and Atlidae contributed to distinguishing the aquatic warbler's diet from that of the two other warblers (Fig. 1). Aphids mainly contributed to the sedge warbler's diet while wasps, and to a lesser extent, flies contributed to the diet of reed warbler (Fig. 1). These differences in diet composition among warbler species were significant, as shown by the APQE analysis ($P = 0.001$), whereas no significant variation in composition was detected among faeces samples within warbler species ($P = 0.91$).

### Availability of aquatic warbler's prey among habitats

The availability of the five principal preys in terms of biomass (Odonata, Orthoptera, Araneida, Lepidoptera and Diptera) varied across habitats (Fig. 2). The abundance of Araneida species was significantly higher in fen mires than in pasture ($P < 0.001$ whatever the sampling method) or in reed beds ($P = 0.04$ for bowl trap and $P < 0.001$ for sweep net). The abundance of Odonata was higher in fen mires than in pasture ($P = 0.002$ for bowl trap and $P = 0.04$ for sweep net), but did not differ from reed beds ($P = 0.06$ for bowl trap and $P = 0.21$ for sweep net). Orthoptera abundance was high in both fen mires and pasture but no difference could be detected between the two habitats whatever the method used ($P = 0.11$ and $P = 0.71$ for bowl trap and sweep net, respectively). No significant difference was found between habitats for Diptera abundance ($P > 0.20$ whatever the sampling method used). Lepidoptera (moth) were almost exclusively collected in fen mires.

### Diet diversity of aquatic, sedge and reed warblers

Significantly fewer preys were found in aquatic warbler faeces (4.9 preys per faeces sample; SE = 0.4) than in sedge

**Table 2** Percentage of each arthropod group found in faeces samples of aquatic warble *Acrocephalus paludicola*, sedge warble *Acrocephalus schoenobaenus* and reed warble *Acrocephalus scirpaceus*

| Taxa | CCA abbreviation | Aquatic warbler (*n*=571) | Sedge warbler (*n*=1027) | Reed warbler (*n*=173) |
|---|---|---|---|---|
| Opilinioda (*Leiobucnum* sp.) | | 0.2 | 0 | 0.6 |
| Araneida total | | 13.8 (13) | 3.3 (8) | 5.8 (14) |
| Araneida indeterminate | *AraInd* | 10.3 | 2.1 | 4.0 |
| Araneida Araneidae (*Larinoides cornutus*) | | 0.4 | 0 | 0 |
| Araneida Clubionidae (*Clubiona* sp.) | *AraClu* | 1.9 | 0.3 | 0.6 |
| Araneida Lycosidae | | 0.2 | 0.1 | 0.6 |
| Araneida Tetragnathidae (*Tetragnatha extensa*) | *AraTet* | 1.1 | 0.6 | 0 |
| Araneida cocoon | | 0 | 0.2 | 0.6 |
| Coleoptera total | | 5.8 (5) | 3.1 (7) | 4.0 (9) |
| Coleoptera indeterminate | *ColInd* | 2.3 | 2.1 | 3.5 |
| Coleoptera Altisidae | *ColAlt* | 1.4 | 0.3 | 0.1 |
| Coleoptera Cantharidae | | 0.2 | 0 | 0 |
| Coleoptera Carabidae | *ColCar* | 1.1 | 0.1 | 0.6 |
| Coleoptera Curculionidae | | 0.9 | 0.5 | 0.3 |
| Coleoptera Histeridae | | 0 | 0.1 | 0 |
| Diptera total | | 37.5 (9) | 16.6 (10) | 53.8 (33) |
| Diptera Indeterminate | *DipInd* | 31.7 | 15.1 | 49.7 |
| Diptera Dolichopodidae | *DipDol* | 4.7 | 1.3 | 2.9 |
| Diptera Syrphidae | *DipSyr* | 0.7 | 0 | 0.6 |
| Diptera Tipulidae | | 0.4 | 0.1 | 0 |
| Diptera Nematocera | | 0 | 0.1 | 0.6 |
| Diptera Brachycera | | 0 | 0.3 | 1.2 |
| Heteroptera total | | 1.8 (1) | 3.1 (4) | 2.3 (3) |
| Heteroptera indeterminate | *HetInd* | 1.1 | 0.1 | 2.3 |
| Heteroptera (*Hydrometra stagnatorum*) | *HetHyd* | 0.7 | 3.0 | 0 |
| Homoptera total | | 21.0 (6) | 66.7 (48) | 22.0 (16) |
| Homoptera (*prob. Hyalopterus pruni*) | *HomAph* | 18.6 | 66.6 | 21.4 |
| Homoptère (Cicadelloidae) | *HomCic* | 2.5 | 0.1 | 0.6 |
| Hymenoptera total | | 4.0 (2) | 6.0 (8) | 11.6 (15) |
| Hymenoptera indeterminate | *HymInd* | 2.8 | 5.1 | 6.9 |
| Hymenoptera Chrysidae | *hymChr* | 0.2 | 0.6 | 1.2 |
| Hymenoptera Ichneumonidae | *HymIch* | 1.1 | 0.4 | 1.7 |
| Hymenoptera Formicidae | | 0 | 0 | 1.7 |
| Lepidoptera total | *LepInd* | 4.7 (8) | 0.1 (0) | 0.6 (2) |
| Odonata total | | 8.4 (43) | 0.9 (12) | 0.6 (8) |
| Odonata indeterminate | *ZygIsc* | 1.8 | 0 | 0.6 |
| Odonata (Coenagrionidae) | *ZygIsc* | 3.0 | 0.5 | 0 |
| Odonata (Coenagrionidae *Ischnura elegans*) | *ZygIsc* | 3.7 | 0.4 | 0 |
| Orthoptera total | | 2.8 (13) | 0.3 (3) | 0 (0) |
| Orthoptera (*Chorthipus* sp.) | | 0.7 | 0 | 0 |
| Orthoptera (*Conocephalus discolor*) | *OrtCon* | 2.1 | 0.3 | 0 |

For each group of taxa, the percentages of biomass are given in brackets.
CCA, canonical correspondence analysis.

warbler faeces (13.2 preys; SE = 1.7) ($P<0.0001$). Yet, no significant difference was found between the number of preys of aquatic warbler and reed warbler (6.2 preys; SE = 0.8) ($P = 0.52$).

According to the species richness estimate assessed with the jackknife estimator, the aquatic warbler had a less diversified diet (16.9 species; SE = 1.3; on average in 10 faeces) than the other two warbler species (reed warbler: 22.2 species; SE = 2.5; sedge warbler: 28.8 species; SE = 4.6; $P = 0.02$ and $P = 0.007$, respectively). The average detection probability was generally high (0.77; SE = 0.07 for aquatic warbler, 0.72; SE = 0.02 for reed warbler and 0.72; SE = 0.04 for sedge warbler) and not significantly different across warbler species (GLM, $F_{2,147} = 1.58$, $P = 0.20$).

Finally, aquatic warblers consumed larger preys (average 9.2 mm; SE = 0.4) than reed (5.1 mm; SE = 0.3; *t*-value = 7.31, $P<0.0001$) and sedge warblers (4.6 mm; SE = 0.3; *t*-value = 4.57, $P<0.0001$).

## Differences in mass gain strategies of aquatic, sedge and reed warblers during stopover

Significant differences in mass between age classes were detected for the tree warbler studied: 10.99 g SE = 0.02 for

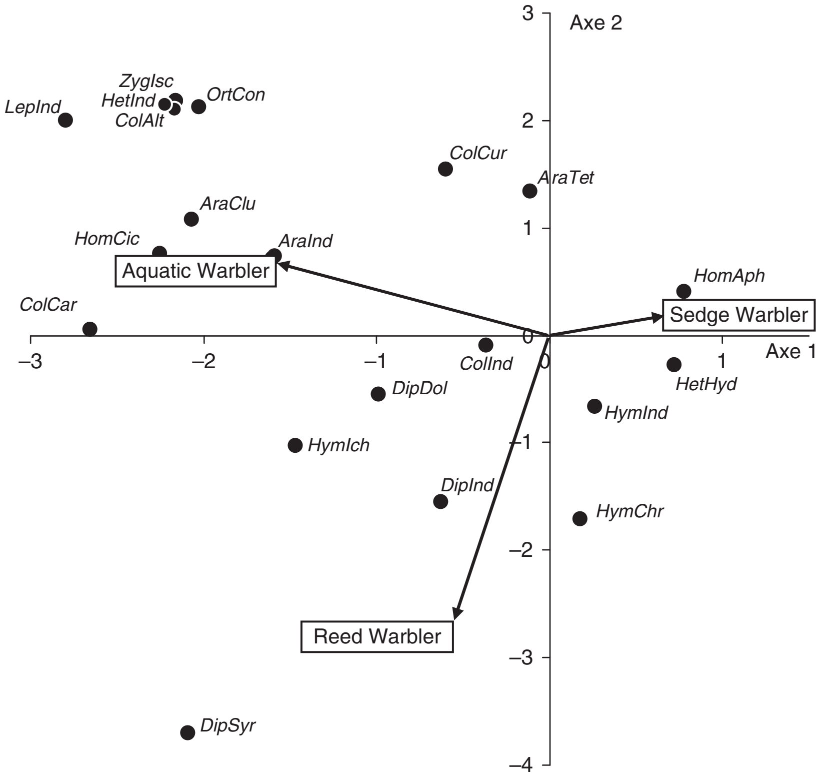

**Figure 1** Specificity of each Warbler's diet assess using a canonical correspondence analysis, ordination of preys [Axis 1 (28%), Axis 2 (5%)]. *AraInd*, Araneida indeterminate; *AraClu*, Araneida Clubionidae; *AraTet*, Araneida Tetragnathidae; *ColInd*, Coleoptera indeterminate; *ColAlt*, Coleoptera Altisidae; *ColCar*, Coleoptera Carabidae; *ColCur* Coleoptera Curculionidae; *DipInd*, Diptera Indeterminate; *DipDol*, Diptera Dolichopodidae; *DipSyr*, Diptera Syrphidae; *HetInd*, Heteroptera indeterminate Heteroptera; *HetHyd*, Hydrometra stagnatorum; *HomAph*, Homoptera Aphid; *HomCic*, Homoptère Cicadelloidae; *HymInd*, Hymenoptera indeterminate; *hymChr*, Hymenoptera Chrysidae; *HymIch*, Hymenoptera Ichneumonidae; *LepInd*, Lepidoptera indeterminate; *ZygIsc* Odonata Coenagrionidae; and *OrtCon*, Orthoptera *Conocephalus discolor*.

young and 12.02 g SE = 0.06 for adult GLM, $F_{1,6709} = 448.44$; $P < 0.0001$ for sedge warbler; 10.97 g SE = 0.01 for young and 11.20 g SE = 0.03 for adult $F_{1,6195} = 112.83$; $P < 0.0001$ for reed warbler; 11.31 g SE = 0.03 for young and 11.78 g SE = 0.17 for adult $F_{1,1093} = 7.70$; $P = 0.006$ for aquatic warbler. The number of days between two capture events was significantly influenced by the age class for sedge wabler ($F_{1,6709} = 15.92$; $P < 0.0001$) and reed warbler ($F_{1,6469} = 303.52$; $P < 0.0001$) but not for aquatic warbler ($F_{1,46} = 0.22$; $P = 0.65$).

Except for the estimate of mean daily mass gain and Fig. 3, all the analyses were carried out on relative mass gain ($G'$) with log transformation. No correlation between the relative mass gain and the number of days spent was detected for reed warbler (Table 3, Fig. 3). As there was probably a small proportion of local reed warbler breeders captured and recaptured that could have induced bias because they were not in migration behaviour (birds involved in late reproduction or in partial moult), we performed the same analysis on a subset of data including reed warblers known to be migrating due to foreign ring identities. Again, no correlation could be detected ($F_{1,20} = 2.51$; $P = 0.13$, and moreover, the trend was slightly negative – 0.05 g/days).

In contrast to reed warbler, the mass in sedge and aquatic warblers increased according to the number of days spent on the Audierne marshes migration stopover (Table 3, Fig. 3). According to the linear regression between gross mass gain and time spent between capture and recapture, the mean daily mass gain was 0.21 g SE = 0.01 for sedge warbler and 0.38 g SE = 0.06 for aquatic warbler.

When all French data of aquatic warblers' mass gain are considered, no impact of year, season or age is detected ($F_{16,68} = 1.33$; $P = 0.20$; $F_{1,68} = 1.83$; $P = 0.18$ and $F_{1,68} = 0.31$; $P = 0.57$, respectively). In addition, no variation among the main sites where aquatic warblers were captured (Audierne marsh, Sandouville, Chenac-Saint-Seurin-d'Uzet and Frossay/Le Massereau) as detected ($F_{14,68} = 1.12$; $P = 0.35$). However, the same pattern of mass gain in relation to stopover duration as observed in Audierne is noted ($F_{1,68} = 6.59$; $P = 0.01$).

Mass gain varied significantly across the years for sedge and reed warblers (Table 3). Yet, there was no sign of unconditionally good or bad years, as yearly differences depended on the species: daily mass gain was significantly larger in 1993, 2000, 2003 and 2004 for sedge warbler, but significantly lower in 1991, 1994, 2000, 2002, 2003 and 2005 for reed warbler.

## Discussion

### Diet specificity

The diet composition of aquatic warbler observed at the migration stopover sites of Audierne marshes is similar to that observed by Schulze-Hagen, Flinks & Dyrcz, (1989) in the species' breeding areas: the diet predominantly consists of Araneida, Diptera and Coleoptera (30, 22 and 15%, respectively, in Schulze-Hagen's study and 14, 38 and 6% in this study). Small numbers of larger prey species such as Orthoptera, Lepidoptera and Odonata are also reported in

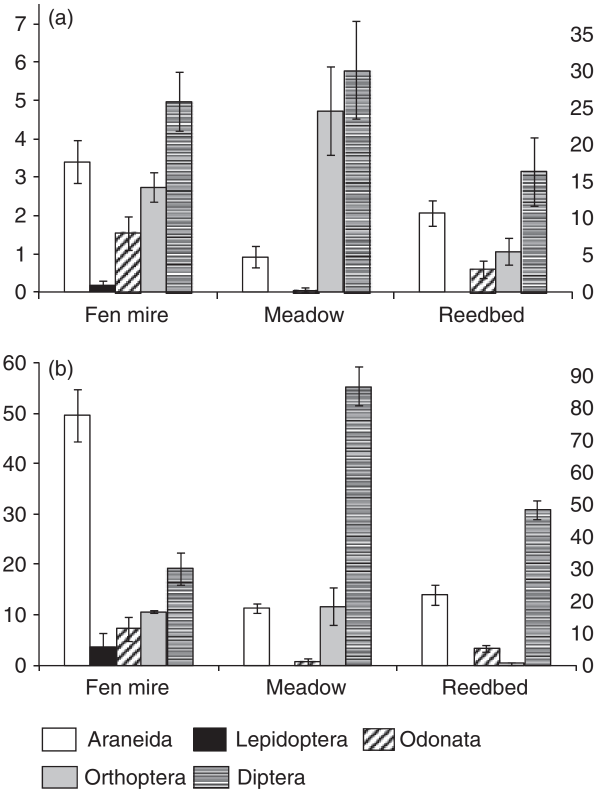

**Figure 2** Variation of abundance of the main aquatic warbler *Acrocephalus paludicola* prey categories among the three main habitats [units: number of individuals collected, (a) bowl trap, (b) sweep net, errors bars represent standard errors, left axis represents Diptera abundance].

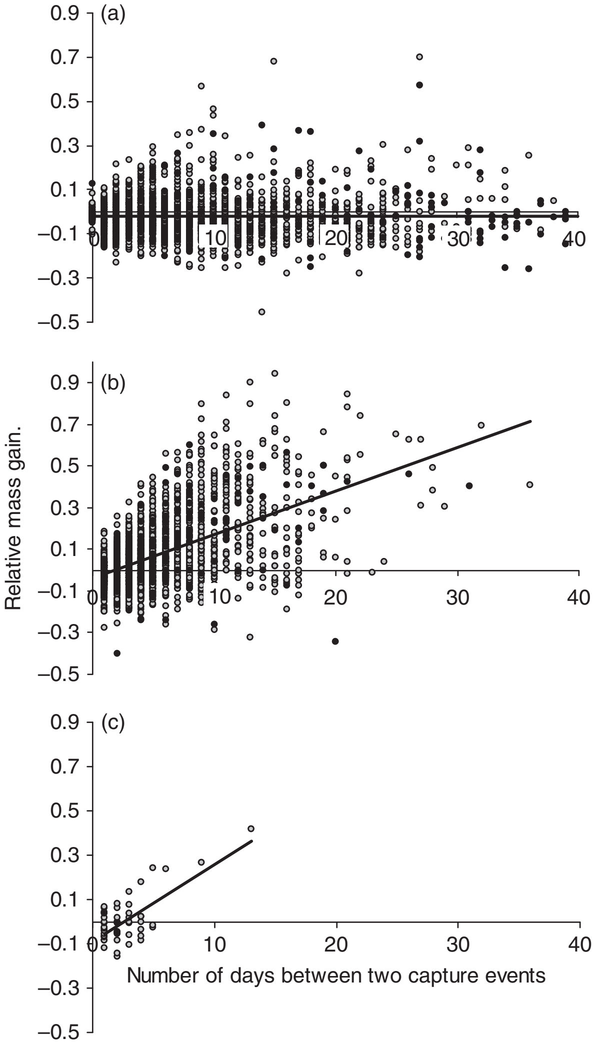

**Figure 3** Mass gain strategies of the reed warbler *Acrocephalus scirpaceus* (a), the sedge warblers *Acrocephalus schoenobaenus* (b) and the aquatic warbler *Acrocephalus paludicola* (c), during autumn stopover in Audierne Bay marshes. Adult measures are shown in black circles, juvenile in grey circles. Mass in ordinate are expressed in relative mass gain (*G'*) and in abscissa the number of days between two capture events.

both studies. Both studies also concur on the average large size of prey: 9.2 mm at Audierne marshes versus 8.4 mm (Schulze-Hagen *et al.*, 1989). Leisler (1985) found 12.1 mm prey sizes at breeding sites. The major difference between the Schulze-Hagen and colleagues' study and ours is the presence of caterpillars in the former study, whereas none were detected here, which is probably due to the scarcity of such prey in late summer when aquatic warblers visit the stopover site. Although large prey species (Odonata, Araneida, Orthoptera) are found in small numbers (25% of total preys) in the aquatic warbler's diet, they significantly contribute to the total biomass consumed (68%). These three large prey groups only represented 23 and 20% of consumed biomass for sedge and reed warbler, respectively. Owing to the potential differences in prey digestibility, the value of this result is mainly qualitative and the strength of the result lies in the comparison between warbler species. Accordingly, diet of aquatic warblers differs only slightly between the breeding and the migration period but its diet is definitely different from that of the two other warblers.

Similarly, the diet composition of sedge warbler estimated at the stopover site of Audierne marshes matched previous studies. The large contribution of aphids was already observed in the diet of sedge warblers in various breeding areas (Koskimies & Saurola, 1985; Leivits & Vilbaste, 1990; Chernetsov & Manukyan, 2000) and on migration stopover (Bibby & Green, 1981). Furthermore, observed aphid outbreaks around the study site (Bargain *et al.*, 2002) are consistent with years of increased mass gain. However, a lot of alternative preys have been inventoried, (Chernetsov & Manukyan, 2000) including Diptera, Coleoptera, Hymenoptera and Araneida, which is consistent with our results: among the three warbler species, the diet of sedge warbler presented the highest prey species richness estimate.

Reed warbler also exhibited a diverse diet, which was yet centred on Diptera, and to a lesser extent, Hymenoptera and

**Table 3** Factors that influenced the relative mass gain

| | Aquatic warbler | Sedge warbler | Reed warbler |
|---|---|---|---|
| Influence of number of days after first capture | $F_{1,32}=27.72$; $P<0.0001$ | $F_{1,6689}=2479.42$; $P<0.0001$ | $F_{1,6450}=0.11$; $P=0.73$ |
| Influence of year on mass gain | $F_{11,32}=1.32$; $P=0.25$ | $F_{17,6689}=11.97$; $P<0.0001$ | $F_{1,6450}=7.99$; $P<0.0001$ |
| Influence of season (day of the year) | $F_{1,32}=0.61$; $P=0.44$ | $F_{1,6689}=74.38$; $P<0.0001$ | $F_{1,6450}=77.38$; $P<0.0001$ |
| Influence of age on mass gain | $F_{1,32}=0.01$; $P=0.91$ | $F_{1,6689}=22.99$; $P<0.0001$ | $F_{1,6450}=0.02$; $P=0.87$. |

Each variable tested was adjusted to the other variables.

aphids. This type of diet composition was also observed by Bibby & Green (1981), Evans (1989), Grim & Honza (1996), Rguibi Idrissi, Lefebvre & Poulin (2004) and Grim (2006). Once again, average prey size in the reed warbler's diet measured in this study (5.1 mm) was close to that observed by Leisler (1985), 5.4 mm, or Rguibi Idrissi *et al.* (2004), 4.5 –5.4 mm.

The major part of the prey biomass in the aquatic warbler's diet that was distinct from the diet of the two other warblers was recorded in fen mires rather than reed beds. Spider families found in the aquatic warbler's diet, such as *Clubionidae*, *Araneidae* and *Tetragnatidae*, and the absence of Lycosidae or Gnaphosidae, indicated that aquatic warbler did not forage on the ground level of vegetation (according to the functional group requirements of the families described in the literature; Duffey, 1962; Roberts, 1985; Marc & Canard, 1997).

## Mass gain

In Audierne's marshes and three other French marshes, Aquatic warblers' mass gain strategies were very close to those of sedge warblers: they both exhibited a significant increase in body mass during their stopover, suggesting the accumulation of fat reserves. Sedge warblers, which migrate earlier and more rapidly than reed warblers, seem to accumulate fat in northern France or southern England and fly almost directly to West Africa over Iberia. In contrast, reed warblers migrate more slowly, thus over a longer period and break up the journey by refuelling (Bibby & Green, 1981; Bensch & Nielsen, 1999). Nevertheless, results from other stopover sites would be necessary to conclude that the aquatic warbler conducts a few-stop migration strategy as sedge warbler.

## Conservation concerns

As regards the diet specificity of aquatic warbler, the choice and management of protected stopover areas for this species cannot only be based on existing knowledge on sedge and reed warblers. Moreover, according to the possible mass gain strategy and our initial knowledge on the stopover network of aquatic warbler (important refuelling and few migration stopovers), this species is thus expected to be impacted more by the degradation or loss of any important refuelling stopovers during migration. The current stopover known to be used by the aquatic warbler are thus of great importance for the conservation of this species. During the nesting period, the aquatic warbler is a habitat specialist species, preferring fen mires characterized by a mesotrophic level, a water table near the soil surface and intermediate vegetation height and density (Kovacs & Vegvari, 1999; Kozulin & Flade, 1999; Kozulin & Krogulec, 1999; Schaefer *et al.*, 2000; Kozulin *et al.*, 2004). As aquatic warblers are captured in reed beds, certainly, this vegetation plays a role for stopover; however, our study underlined that higher abundance of several prey species occurs in fen mires. In addition, the first results found in France with radio-tagged birds in stopover migration also indicated that fen mires are very used by aquatic warblers (P. Provost, C. Kerbiriou & F. Jiguet unpubl. data). This habitat plays an important role in allowing the complete life cycle of aquatic warbler's prey. Fen mire vegetation maximizes the abundance of large Orthoptera prey *Conocephalus discolor* (Baldi & Kisbenedek, 1997; Szövényi, 2002; this study) and the densities of Clubionidae and Tetragnathidae (Cattin *et al.*, 2003; this study).

However, fen mires in western European coast (i.e. the aquatic warbler migration route) are localized at the margin of reed beds due to hydrological constraints. The main threat for these small areas of fen mires is firstly direct human destruction such as drainage and agriculture (pasture or maize culture). A second threat is the encroachment of shrubs in marsh edge and reed vegetation of open wetlands (Kozulin & Krogulec, 1999). In European Atlantic stopover sites, mostly comprising large areas of common reed, conservation measures should therefore aim at maintaining areas of medium vegetation height (50–100 cm). Restoration management, such as clearing, should focus on marsh edges that are often colonized by shrub willow associated with common reed. However, reed cutting, especially cutting for commercial reasons, appears to affect the arthropod communities with, for instance, observed decreases in some passerine birds' prey, such as Coleoptera and Araneida, together with increases in other prey, such as aphids (Schmidt *et al.*, 2005). To minimize negative effects, reed cutting should be restricted to small areas, connected with uncut areas, thereby allowing arthropod recolonization (Schmidt *et al.*, 2005). In addition, the creation of small ponds near reed beds is expected to provide habitat patches with exceptional densities of Diptera (Brunel *et al.*, 1998) and Odonata.

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