

Breeding Ecology, Moults and Migration of
Savi's Warblers, *Locustella luscinioides*, in
Portugal

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This thesis is dedicated to my parents, José Guilherme Simões Neto and Maria Graciete Nunes Malha Simões Neto, who have always fully support me throughout my long student life.

Abstract

The breeding, moult and migration strategies of a Portuguese population of the little-known Savi's Warbler, *Locustella luscinioides*, were studied and compared with other populations and species. The biometrics, the variation in body condition during the breeding season, the breeding phenology and success, the variation in egg and clutch size, and the nest-site selection were described. In both sexes, mating and breeding success benefited from early arrival and breeding; females produced enough fledglings to balance the typical mortality rates of passerines. Although this species depend on pure reeds to feed, the preferred nest-sites were located in high and dense *Juncus* and *Rubus* with thick litter; and predation was greater in sites with less litter.

The phenology, sequence and extent of the post-juvenile and post-breeding moults were also described. Later-moulting birds retained more primaries and tended to start moult in an outer primary. This was interpreted as an adaptation to replace as many as possible of the most important flight feathers in the time available.

The winter moult extent was determined by analysing the stable isotope signatures of the wing feather generations present in birds captured in spring. This is a novel application of this technique. Carbon, Nitrogen and Hydrogen isotope ratios of feathers grown in Europe differed from those grown in Africa and were used to classify the origin of unknown feathers. This species has a suspended post-breeding moult and a suspended or arrested pre-breeding moult, a strategy which was not been described previously.

The data from birds captured in Portugal were analysed to assess the phenology of the post-breeding migration, fuel deposition rates and flight range. Many of the adults were able to reach the winter quarters without refuelling, which suggests that potential staging sites were bypassed by birds; as has been predicted for time-selected migrants. The possibility that juvenile migration might be energy-selected could not be excluded, as these birds left the breeding quarters with only enough fuel to reach the next staging site.

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CHAPTER 1

General introduction

1.1 Life-history trade-offs and the annual cycle of birds

Life-history trade-offs represent the cost paid in terms of fitness when a beneficial change in one trait is linked to a detrimental change in another. The most prominent life-history trade-off involves the cost of reproduction, which may be paid in survival or future reproduction (Stearns 1989). At least two different and non-exclusive causal mechanisms have been proposed to explain how current reproductive effort might impose these costs. First, high reproductive investments may suppress the immune system leading to increased risk of parasitism and diseases (Gustafsson *et al.* 1994). Second, reproductive effort may result in depletion of energy and time available for self-maintenance in terms of reconstruction of somatic tissues (Dawson *et al.* 2000, Nilsson & Svensson 1996).

In birds, the investment in new feathers is of fundamental importance for an individual's chances of survival. The process of moult is also one of the most demanding activities during the annual cycle both in terms of time and energy (Klaassen 1995, Murphy & King 1992). The cost of moult involves the production of new feathers, increased thermoregulatory cost caused by impaired feather insulation and increased flight cost due to the reduced wing area (Ginn & Melville 1983, Lindström *et al.* 1993, Pennycuick 1975, Swaddle & Witter 1997). As both the overlap between breeding and moult, and the poor quality plumage resulting from a high moult rate in late breeders lead to fitness costs, birds seem to face both physiological and ecological trade-offs between the investments in breeding and moult (Dawson *et al.* 2000, Hemborg 1999, Hemborg & Lundberg 1998, Hemborg & Merilä 1998, Hinsley *et al.* 2003, Nilsson & Svensson 1996,

Svensson & Nilsson 1997).

A similar trade-off seems to occur between moult and migration, as both activities are very demanding in terms of time and energy, and also because the moult of flight feathers causes a detrimental effect on aerodynamic performance (Hedenström & Sunada 1999, Klaassen 1995, Lindström *et al.* 1994, Swaddle & Witter 1997, Williams & Swaddle 2003).

In short-lived passerines, it is reasonable to assume that when these activities are in conflict, priority is given to breeding, and probably the time needed for successful breeding is hard to change. Therefore, when time is short, passerines have to adjust moult, migration or both. There are three possible mechanisms of adjustment: (1) they could speed up or restrict the extent of moult or migration, (2) they could let the activities overlap in time, and (3) they could skip or postpone one of the activities (Lindström *et al.* 1994). These mechanisms, particularly the latter, are responsible for the variation in the way birds fit breeding, moult and migration into their annual cycle.

1.2 Conflict between breeding, moult and migration

In birds, breeding, moult and migration are generally thought to be mutually exclusive in time. In species that live in markedly seasonal environments, the conflict between these activities often arises at the end of the breeding season because all residents and many migrants have a complete moult after breeding, or a partial moult in juveniles (Jenni & Winkler 1994). This is particularly true if the breeding season was long, for instance in individuals that had replacement clutches, and at high latitudes, where there is little time between the end of breeding and the decline in food abundance

in autumn.

It is well known that birds at higher latitudes moult more rapidly than those at southern latitudes and sometimes become flightless (Jenni & Winkler 1994). In addition, migratory birds, which are more constrained in time than residents, also tend to moult more quickly (Jenni & Winkler 1994). In fact, some species, populations or individuals increase the speed of moult when the time is short, although this may have a detrimental effect on feather quality and survival (Dawson 1998, Dawson *et al.* 2000, Hall 2002, Hall & Fransson 2000, Hinsley *et al.* 2003). In other species, there is a negative relationship between the extent of the post-breeding moult and date, which means that birds may also restrict moult when the time available is short (as in the Whitethroat, *Sylvia communis*, Hall & Fransson 2001). Regarding migration, in some species the fuelling rate and potential flight range of migratory birds increases throughout the season, which seems to show that birds are also able to increase the speed of migration later in the season. Therefore, at least some birds are able to speed up moult and migration and also to restrict moult.

Although breeding and moult are generally thought to be mutually exclusive in time, a number of species may ‘overlap’ these activities to some degree (Hemborg 1999, Jenni & Winkler 1994, Sanz 1999, Svensson & Nilsson 1997). However, although the overlap between breeding and moult occurs in time-constrained birds, the extent and frequency of the overlap does not seem to depend on the available time, particularly in females, as there is no relationship with latitude in long-distance migrants (Hemborg *et al.* 1998, 2001). Under experimental conditions, an overlap between post-breeding moult and pre-migratory fuelling may also occur (Lindström *et al.* 1994), even though the additional mass will hinder the flight performance of a bird whose manoeuvrability is already

reduced because of feather replacement (Hedenström 1992). Although the partial post-juvenile moult may overlap to a large extent with migration in Reed Warblers, *Acrocephalus scirpaceus*, the complete adult moult very rarely overlaps with migration (Herremans 1990 and references therein). Hence, in some species there are overlaps between breeding and moult and, to a lesser extent, moult and migration.

Although it may not be possible to postpone migration, it is possible to postpone moult, for instance when the time between breeding and migration is short. In fact, comparative analysis suggested that winter moult evolved several times within the family Sylviidae in association with the increase in migratory distance (Kjellén 1994, Svensson & Hedenström 1999). That is, when time during the post-breeding period became shorter as the populations colonised areas further north, some species postponed the complete post-breeding moult (which was the ancestral character) to the winter period. This might also happen at the individual level in extreme cases when time available for moult is short.

Thus, evidence strongly suggests that when breeding, moult and migration are in conflict, there are several non-exclusive mechanisms of adjustment which act at the individual, population or species level. Moult seems to be the activity most often adjusted and, in fact, the variation in the way in which moult fits into a bird's annual cycle is far greater than that of breeding and migration.

1.3 The moult strategies

The moult strategies are very variable at the individual, population and species levels (Jenni & Winkler 1994), and their evolution seems to depend on the other activities of the annual cycle (Figuerola & Jovani 2001, Hall 2003, Svensson & Hedenström 1999).

As mentioned earlier, in all adult resident passerines, the annual complete moult takes place shortly after the breeding season, but this is not always the case for migrants (Jenni & Winkler 1994). Migratory passerines have a large variety of moult strategies that cope in different ways with breeding and migration. These strategies include: (1) complete (i.e. involving all feathers) summer (or post-breeding) moult, which may or may not be followed by a partial (i.e. involving only a variable number of body feathers, wing coverts and tertials) winter (or pre-breeding) moult, (2) complete pre-breeding moult, which may be preceded by a partial post-breeding moult, (3) seasonally divided moult of remiges, which includes various forms of moult suspension (i.e. an interrupted moult that is resumed from the point of interruption in the subsequent moult period) within primaries or secondaries, (4) arrested (i.e. an interrupted moult that is not resumed in the subsequent moult period) post-breeding moult within the remiges followed by a complete winter moult, and (5) complete biannual moult (reviewed by Jenni & Winkler 1994).

The functional and evolutionary significance of the different moult strategies has been the subject of much interest, but our understanding of the ecological factors that influence the moult patterns is still limited (Hall 2003, Jenni & Winkler 1994, Svensson & Hedenström 1999). In fact, there is still no natural classification of the moult strategies (but see Howell *et al.* 2003), mostly because of their variability and the lack of information about the complementarity of the different moults that take place during the annual cycle (particularly in migratory birds which are rarely retrapped between breeding and wintering quarters). For instance, it is often not known if birds that have a restricted or interrupted post-breeding moult resume the same moult in the winter quarters or have a new moult altogether (Jenni & Winkler 1994). Also, in migratory species that have a very restricted post-juvenile moult and a complete first pre-breeding moult, it is not known

whether the winter moult is homologous to the post-juvenile moult of resident birds.

In order to understand the way in which moult fits into the annual cycle, and the ecological factors that influence its variation, the breeding, moult and migration strategies should be studied in the same population, and compared with other populations and species. This was attempted in this study.

1.4 Aims

The general objective of this study is to describe several aspects of the breeding ecology, moult and migration of a Portuguese population of Savi's Warblers, *Locustella luscinoides*, and to compare them with other populations and species. The Savi's Warbler, and the other *Locustella* warblers, has been little studied, particularly in the southern part of its distribution, as it is very skulking and occurs in a dense habitat. However, some aspects of its life history are unusual, in particular the extreme individual variation in moult sequence and extent, and its very long breeding season. The study of all these aspects in a single population seems to be essential to understand the annual cycle of a species and the ecological factors that influence the breeding, moult and migration strategies. In addition, it would be very interesting to include this species in comparative analyses of well-known Sylviidae family, for which more data are needed, and so this study contributes to that requirement also.

More specifically, the objectives are:

- to determine the differences in biometry and weight change between the age and sex classes during the breeding season (§Chapter 2);
- to study the factors influencing the breeding phenology and success (§Chapter 3);

- to describe habitat selection with regard to the nest site (§Chapter 4);
- to describe the variation in phenology, sequence and extent of the post-juvenile and post-breeding moult (§Chapter 5);
- to determine the extent of the pre-breeding moult and to assess the Savi's Warbler's moult strategy (§Chapter 6);
- to study the variation in body condition in late summer, the weight dynamics and the phenology of migration (§Chapter 7).

Finally, I intend to interpret the breeding, moult and migratory strategies of the Savi's Warbler and to compare them with other marshland passerines.

1.5 The study species

1.5.1 Taxonomy

The Savi's Warbler, *Locustella luscinioides* (Savi, 1824), is an insectivorous, migratory passerine of the family Sylviidae. Three subspecies are generally recognised. The nominate occurs in North Africa and Europe east to the Balkan countries and the western shore of the Sea of Azov; *L. l. samartica* Kazakov, 1993, from the eastern shore of the Sea of Azov south to the Urals, Volga valley and northern foothills of the Greater Caucasus; and *L. l. fusca* (Severtsov, 1872) occurs in Asia east from Asia Minor and the Emba River (western Kazakhstan) to Mongolia (Cramp 1992).

On the basis of morphology, *Locustella* species are generally considered to be close to the *Acrocephalus* warblers (Sibley & Alquist 1990, Cramp 1992). However, no genetic analysis of this genus was undertaken except by Helbig *et al.* (1999), where *L. luscinioides* was used as an outgroup in a study of the genetic relationships of

Acrocephalus and *Hippolais*. In this study, *Locustella* did not seem to be closer to *Acrocephalus* than *Cettia* or *Cisticola* and, in fact, *Phylloscopus* and *Sylvia* seemed to be closer to *Acrocephalus* and *Hippolais* than the other genera mentioned, but the bootstrap support was poor and so all the basal branches were regarded as unresolved. The relationships between genera in the Sylviidae, and particularly *Locustella*, clearly need to be clarified (see Drovetski *et al.* 2004 for a recent analysis of mitochondrial DNA of *Locustella* and related genera).

1.5.2 Distribution

Savi's Warblers breed in the Palearctic region, occurring from Portugal, NW France and SE England to Mongolia, and from Estonia in the north to Morocco (Cramp 1992, Hagemeyer & Blair 1997, Snow & Perrins 1998).

The winter quarters are poorly known. Although there are a few records of birds wintering north of the Sahara, namely in Algeria, its main winter distribution lies in sub-Saharan Africa north of the forest zone. It seems to occur in a narrow belt extending discontinuously from Senegal to Eritrea. The nominate subspecies is regular in wetlands in Senegal, southern Mali, northern Ghana, northern Nigeria and irregular in southern Mauritania (Cramp 1992, Hedenström *et al.* 1991). Further east, both nominate and *fusca* occur (and presumably *samartica*), being common in Sudan, western Ethiopia and probably regular in eastern lowlands of Eritrea (Cramp 1992). Two records in Ngulia, south-east Kenya, suggests that small numbers may cross the Equator (Pearson 1988).

1.5.3 Population

The European population is estimated to be from 152,363 to 221,293 breeding pairs, being particularly abundant in Romania (70000 to 130000 bp) and Hungary (30000 to 60000 bp) (Hagemeyer & Blair 1997). It seems to be stable in most parts of its

distribution, but a decrease was reported from Britain, Belgium, Netherlands, Portugal, Italy, Croatia, Moldova and Israel, whereas there was a marked increase in Latvia, and a slight increase in Estonia, Poland (stable in recent years) and Ukraine (Snow & Perrins 1998).

1.5.4 Movements

All populations are migratory. West European birds are believed to head south or southwest in autumn (reverse in spring), central and east European birds south-east towards Levant, and Asian birds south-west (Cramp 1992). Hence, there seems to be a migratory divide in central Europe, which is supported by the very late arrivals in northern Denmark in comparison to Belgium, by the absence of records from either seasons in Niger, central and eastern Libya, Chad and western Egypt, and by markedly higher numbers in Levant than in northern Egypt (Cramp 1992). However, there are very few ringing data supporting this hypothesis; two birds ringed in the Netherlands were recovered in eastern France, one ringed in France was retrapped in Barcelona, and a bird ringed in East Germany was recovered in Athens (Aymí & Tomás 1998, Cramp 1992, Dürr *et al.* 1995). There is only one recapture from sub-Saharan Africa: one individual ringed in Barcelona was recovered in Senegal (Aymí & Tomás 1998).

Western populations leave the breeding grounds from August to mid-September and passage is inconspicuous (Cramp 1992). Pre-migratory fattening seems to occur in western France (Bibby & Green 1983). Low abundance at migratory stopovers, even at well-studied sites strongly suggest that migration takes place in long, non-stop flights. It is recorded in small numbers in western Mauritania in September-November. Arrival at wintering sites is rather late, namely southern Mali (November) and Senegal (December), and the whereabouts of late-autumn stopover sites are unknown (Cramp 1992).

Eastern populations are far more conspicuous in Europe during migration, presumably due to the greater numbers of birds, being common in Poland, western Ukraine, Israel and Sinai. Departure from the breeding grounds occurs gradually from September to early October with occasional records in late October (Cramp 1992).

Northward movements begin in February for all populations, and the species is present in Senegal until March and northern Nigeria until April. It occurs on the Algerian coast from the end of February to May. Passage in Switzerland takes place about 10 days earlier than that of the Grasshopper Warbler, *Locustella naevia*, being recorded from late March to the end of May. The northern Moroccan breeding grounds are re-occupied in March, sometimes February, western France and Belgium in early April, southwest Russia in late March, Poland and East Germany from mid-April and Sweden in May (Cramp 1992).

1.5.5 Habitat selection

The Savi's Warbler breeds in the West Palaearctic lowlands across middle and lower middle latitudes between July isotherms of 18 and 32°C, in large swamps, wetlands, flood lands and inundated fringes of fresh or brackish surface waters (Cramp 1992). Its breeding territories are located in extensive stands of reeds (*Phragmites*) in shallow water, often with reed mace (*Typha*) fen sedge (*Cladium*), bur-reed (*Sparganium*), sedge (*Carex*), or club-rush (*Scirpus*) and scattered willows (*Salix*), alders (*Alnus*) and other moisture-loving trees and shrubs (van der Hut 1986, Cramp 1992). It occasionally occurs in sedgy marshes without reeds and in Spain breeds in clumps of rushes (*Juncus*) fringing the marismas of Coto Doñana (Cramp 1992). In Britain, the presence of fens within often sparsely dense reeds seem to be selected (Bibby & Lunn 1982).

Although there is little information from the wintering quarters, they seem to

occur in thickly vegetated wetland, as in the breeding quarters. Cramp (1992) states that in Senegal, it occurs in *Typha* and in Eritrea in dense thickets of *Salvadora persica* by permanent springs in *Acacia* bush; feeding in grass, but also in the open.

1.5.6 Breeding

Several authors have described aspects of the breeding behaviour and ecology of Savi's Warblers, namely Dirkx (1939), Dittberner & Dittberner (1985, 1991, 1992), Mayaud (1930), Mildenberger (1958), Moreau & Moreau (1982), Schiermann (1926, 1928), Suchantke & Schwarz (1961) and others. The most detailed studies on its breeding biology were made by Pikulsky (1986) in Poland and, more recently, by Aebischer & Meyer (1996) and Aebischer *et al.* (1998) in Switzerland. No previous studies have ever been made in the southern part of its distribution.

Male Savi's Warblers arrive in the breeding quarters and start defending territories from late March to late April in Switzerland (Aebischer & Meyer 1998) and from mid-April in Poland (Pikulski 1986). Females arrive one week later than males and start laying at about 20 April in Switzerland and 30 April in Poland (Aebischer & Meyer 1998, Pikulski 1986). The breeding season is very long: in Poland the last nestlings fledged on 28 July (but a case was found in which incubation started as late as 10-15 August, Pikulsky 1986), and in Switzerland on 26 August (Aebischer & Meyer 1998). In Poland, 32% of pairs commenced a second brood after the successful fledging of the first (Pikulsky 1986) and in Switzerland three pairs each had three successful clutches in a season (Aebischer & Meyer 1998). The incubation and nestling periods last 12-13 and 11-15 days, respectively, depending on air temperature and the number of nestlings, and the eggs hatch within 1-3 days of each other, depending on the clutch size (Pikulsky 1986). Aebischer & Meyer (1998) estimated incubation periods of 11-14 days and nestling

periods of 11-14 days. Females undertake most of the incubation and both sexes feed the nestlings (Pikulsky 1986).

The nest is a well-concealed cup built of dead water-plant leaves and is built by both sexes. Eggs are laid daily, most frequently during the morning. Clutch size varies from 3 to 6 eggs (average 5.0) in Poland (Pikulsky 1986) and 3-5 eggs, average 4.1, in East Germany (Schierman 1926). Aebischer & Meyer (1998) found an average clutch-size of 4.74 eggs per nest. The breeding system seems to be 'facultative polygamous'. In Switzerland, one out of 18 males mated with two females even though a moderately high proportion of the males (21 to 41%) remained unmated during the whole season (Aebischer *et al.* 1996). In Poland, the 48% of males that remained unmated occupied sub-optimal habitats, mainly reeds in deep water and *Caricetum* on unflooded ground (Pikulski 1986). Females do not seem actively to select any measured feature of the male or its territory. Aebischer *et al.* (1996) argue that a simple lottery model assuming habitat preferences of males and non-selection by the females is sufficient to explain the variation in mating success of the males and the correlation between the arrival of males and laying dates. Early-arriving males simply had a higher chance of meeting a female due to the overlap between the arrival of females and males and the slightly biased sex ratio during that period (Aebischer *et al.* 1996).

1.5.7 Moults

Some data on the post-breeding moult of Savi's Warblers in Europe was presented among others by Steiner (1970), Stresemann & Stresemann (1970), Mead & Watmough (1976), Thomas (1977), Muller (1981), Trias *et al.* (1982), Márton (2000) and Galea (2002) and from Africa by Aidley & Wilkinson (1987), Bensch *et al.* (1991), Nikolaus & Pearson (1991) and Stresemann & Stresemann (1970). Despite the small sample sizes, a

huge variation was found in both the sequence and the extent of moults.

Dement'ev & Gladkov (1954, cited by Thomas 1977) state that some *L. luscinioides* have a complete moult before autumn migration in Russia and Steiner (1970) described the post-breeding moult of four birds ringed in autumn in Austria, three of which moulted the primaries ascendantly and descendantly from a centre in the middle of the tract; the fourth just had three old outer primaries so the sequence could not be ascertained. He stated that Savi's Warblers are able to live flightless in late summer in reedbeds, and can afford considerable individual variability in the speed of moult in various parts of the wing, asymmetry in the pattern of moult, and the renewal of a large number of wing feathers at the same time. The moult of the secondaries occurred as in other passerines: following the renewal of the innermost three, the remaining feathers were replaced ascendantly. The tail feathers were shed at very short intervals, which, together with frequent accidental loss of some of them, was responsible for the fact that moult was not perfectly centrifugal. The renewal of the body feathers, with the exception of the head, began at the time of the replacement of the innermost secondaries. Most body feathers, up to seven wing feathers and the whole tail grew simultaneously during the period of flightlessness. Steiner (1970) reported that moult started shortly after the last brood hatched, and that the growing juveniles were still being fed during this period, which should be regarded as a remarkable physiological achievement.

Stresemann & Stresemann (1970) studied six skins of Savi's Warblers, all of which showed a primary moult in the normal sequence. Only one specimen was from the European breeding grounds whereas the others were wintering birds from Sudan.

Mead & Watmough (1976) captured 11 Savi's Warblers in Golegã, Portugal, two of which (one female and one of unknown sex) were caught at the beginning of August

without any signs of moult. Five birds started moulting in the middle of the primaries, two had progressed too far for this to be detected even if it had occurred, and one had delayed the moult of the fourth, fifth and sixth primaries. They considered that the complexity and variability of this species, with adults migrating with old, new and arrested primaries was astonishing. They thought that this could illustrate the ability of individuals with different strategies to maximise their use of potentially superabundant late summer feeding resources, and that the primary moult progressing both directions from the vicinity of primaries three, four or five could enable primary moult to be completed more quickly than in the normal sequence. Thomas (1977) reviewed the literature on Savi's Warbler moult including all the moulting birds described and some new observations from France and Spain; 25 birds in all. He introduced the term *eccentric* to describe the abnormal and unique sequence of moult in the Savi's Warbler. This term was also used later by Jenni & Winkler (1994) but in referring to all birds that started the primary moult in the middle of the tract, independently of the directions of moult. Thomas (1977) concluded that at least 79% of the birds moult *eccentrically* and at least six out of 25 had suspended moult. Some other birds seemed to have suspended moult. However, one of these appeared to have resumed moult after suspension. All those that suspended moult also moulted *eccentrically*, suggesting that this strategy enabled them to moult the most important feathers for flight more quickly (Thomas 1977). On the basis of three retraps presented by Thomas (1997), Cramp (1992) estimated a moult duration of 55 days, whereas Müller (1981) estimated a duration of six weeks and Stresemann & Stresemann (1970) of 5 weeks. Thomas (1977) also suggested that birds suspending moult were not late starters, but the sample sizes were small and taken from widely separated populations in different years.

Trias *et al.* (1982) captured 14 Savi's Warblers in the Ebro Delta, Spain, of which

two adults were moulting, one of them eccentrically and the other was too advanced to know the sequence. The others were juveniles that had the first plumage still growing but one of them was moulting the tertials. This is contrary to Heinroth & Heinroth (1924-26 cited by Cramp 1992) and Müller (1981) who state that Savi's Warblers do not undertake a post-juvenile moult at all. Galea (2002) reported that one bird had a complete moult in Malta, where this species is a rare passage migrant.

In a recent analysis of 135 moult cards of Savi's Warblers trapped in Hungary, Márton *et al.* (2000) divided the data into three groups: the descendent group, i.e., birds with a complete post-breeding moult starting with the innermost primary; the eccentric group, i.e., birds that initiated moult with a central primary and did not show an ascendant wave; and the divergent group, i.e., those that initiated moult from a central primary and showed both the ascendant and descendant waves. The eccentric group was subdivided into subcategories by the initiation locus which varied between primary two and four. The date of moult initiation and the speed of moult increased with decreasing number of primaries involved in moult. Those that retained more feathers started to moult later in the season but finished moulting comparatively early, i.e. the difference in time of the initiation of moult between the groups is larger than the difference in time at the end of moult, suggesting that season constrains the extent of post-breeding moult.

Aidley & Wilkinson (1987) captured six Savi's Warblers in Kano, Nigeria, three had completed their remex moult, one had the two inner primaries growing after moulting the outer ones, and two were actively moulting after suspension. Bensch *et al.* (1991) caught 16 Savi's Warblers in northern Ghana which showed a similar moult variation to that observed in Europe. Moult started with the fourth or fifth primary in four individuals and seven out of nine birds suspended moult, retaining some old primaries and/or

secondaries. The 14 birds that completed or almost completed their moult had, on average, replaced 7.7 primaries (range 5-9 out of 9) and 3.1 secondaries (range 0-6). However, the “fresh” feathers of some of these birds could have been moulted on the breeding grounds. In Sudan, Nikolaus & Person (1991) examined eight Savi’s Warblers, three of which presented a typical suspended moult and the others had old primaries. According to Orden (1964), some Savi’s Warblers may still have growing primaries early in spring, when they arrive in the breeding quarters. The age of the birds studied in the winter quarters is unknown except for one or two birds reported in Cramp (1992).

As a result of published studies, Jenni & Winkler (1994), in their review of moult strategies of European passerines, classified the moult strategy of adult Savi’s Warblers into three groups: one with no post-breeding moult and complete pre-breeding moult (group 10), and two with post-breeding moult complete or involving just part of the primaries and with unknown moult in the winter quarters (groups 11 and 13).

1.6 Study area

This study was carried out at Salreu marshlands (40°43’N, 08°35’W), Estarreja, western Portugal. These marshlands are located in the inner part of Ria de Aveiro, a large coastal lagoon that spreads along the coast for nearly 50 km. Ria de Aveiro belongs to the NATURA 2000 network, but only a small part of it is currently protected (Dunas de São Jacinto), due of its plant diversity. In addition, Salreu is located in an Important Bird Area and a Site of Special Scientific Interest (Farinha & Trindade 1994).

In Salreu, reedbeds occupy an area of 45.8 ha, whereas salt marshes (dominated by rushes, *Juncus* spp.) and rice fields occupy 70.0 and 13.3 ha, respectively (Fernandes 1998). The whole area was occupied by rice fields, but most of them have been

abandoned in recent decades and colonized by marsh plants. Presently, there is a mosaic of reeds (*Phragmites australis*), rushes, reed maces (*Typha latifolia*), club-rushes (*Scirpus maritimus*), all of which can be dominant in particular sites, and scattered willows (mainly *Salix atrocinerea*) and alders (*Alnus glutinosa*).

The reeds, and other plants, are commonly cut for animal bedding by the local community, mainly from July to October, although in some areas they are cut earlier in the year. A dyke separates the marshes from the lagoon, allowing the control of the water level and preventing the entrance of saltwater. The water level depends on human activities in the surrounding rice fields. During March and April and from the middle of August until October, the water level is lowered in order to plough the rice fields and harvest the rice, respectively. In May, fresh water from river Antuã is poured into the rice fields and after October the level rises again through rainfall.

A study site was selected within the Salreu marshlands. In 2000 an area of 21.7 ha located immediately north of Salreu canal was surveyed. In order to increase the sample size, this area was extended to the southern part of the canal in May 2001, then becoming 31.8 ha. This new area is somewhat influenced by the tides because the dyke cannot completely prevent the entrance of the salt water. Here, the proportion of rushes is higher and the reeds only occur in the deeper parts, usually along the ditches.

1.7 General methodology

The data were collected during fieldwork undertaken at the study site from early March to early October in 2000 to 2002, plus a short field season from March to May in 2003.

The study area was visited almost daily from early March (mid-March in 2000) to the end of August in order to record the arrival date of the males, and trap and colour-ring all adults by using mist-nets and a tape-recording of the song. The adults were measured for wing length, 8th primary, tail length and roundness, bill, weight, fat and muscle score. The third tail feather of the right side was sampled, but, in birds where this feather was missing, the left one was collected. The patterns of feather wear of the remiges and rectrices (i.e. the moult contrasts) were recorded in order to study the pre-breeding moult extent. Some data obtained from 1997 to 1999 through ringing were also used even though only wing length, weight and fat (from 0 to 5) and primary moult were recorded. In 2002 and 2003 a small drop of blood was collected from adults and nestlings by puncturing the wing vein and stored in 70% ethanol.

Nests were searched for and visited regularly in order to record their phenology and success. Several habitat features were measured at the nest site as soon as the nests were abandoned. Eggs were marked and their length and breadth measured. The nestlings were ringed and measured as for the adults when they reached about 10 days old. From July onwards, the breeding adults were retrapped when their nestlings were about to fledge, in order to record the overlap between breeding and moult.

From August to early October, constant-effort mist-netting was carried out in three lines within the study area, in which 90 to 126 m of mist-nets were open before sunrise and during five hours. Some ringing was also undertaken in other places to trap as many Savi's Warblers as possible in order to study the post-breeding moult and the pre-migratory fuelling. The moult of adult and juvenile birds was recorded following Ginn & Melville (1983) for remiges and rectrices and using a 0-3 score for each body feather tract.

The growth bars of the tail feathers were measured in the lab using the methodology described by Grubb (1986) and modified by Takaki *et al.* (2001).

In addition, the tail and wing feather samples were used for stable isotope analysis, which was performed at SUERC, Glasgow, in collaboration with Dr Jason Newton. For the Carbon and Nitrogen analysis, approximately 0.55 mg of the ground and homogenised feather sample was folded into a clean tin capsule and combusted in a Carlo Erba C/N/S analyser interfaced with a Finnigan Tracer Matt continuous flow isotope ratio mass spectrometer (CF-IRMS). Hydrogen analysis was performed with an Elemental Analyser Pyrolysis Isotope Ratio Mass Spectrometry (EA-Pyr-IRMS) using 0.75 mg of the feather sample folded in a clean silver capsule.

In order to determine parentage, microsatellite primers were screened at Oxford University, in collaboration with Dr Simon Griffith. This involved a Qelex DNA extraction from Savi's Warblers blood samples and the use of several primers to amplify microsatellite markers in a PCR and polyacrilamide gel electrophoresis to separate and score the alleles. However, due to technical problems, no polymorphic loci were found and so the extra-pair paternity was not studied.

The statistical analysis and data presentation follows Sokal & Rohlf (1995) and Fidell & Tabachnick (2001) and was undertaken with SPSS 10.0 and GenStat 7.1. All statistical tests are two-tailed. Descriptive statistics are mean $\pm$ s.e. followed by range and sample size.

CHAPTER 2

Biometrics and weight variation of Savi's Warblers during the breeding season

Biometrics of breeding Savi's Warblers captured in Portugal are presented. The males were significantly larger than females in all (seven) measurements except tail roundness, but the difference was rather small (2.4 to 3.8%). In a stepwise discriminant analysis including six biometric variables, only 80.1% of the original cases were correctly classified. Adults were 3.2 to 11% larger than juveniles in all measurements except tarsus length. Portuguese Savi's Warblers seem to be slightly smaller than individuals of central European populations, but have longer tails. Breeding females had a greater variance in weight and more fat than males. This resulted from a greater variation in weight and body condition during the breeding cycle associated with fattening up and carrying eggs during the laying period. Females were significantly heavier than males during egg-laying and almost significantly so during incubation. Although females do most of the incubation and are not fed by males, their weight did not decrease during this period. During the nestling period, both sexes decreased in weight, which for females was associated with a decline in fat. It was also shown that both males and females decreased in weight, fat, muscle and lean body mass during the breeding season. The greater changes in muscle found in females might reflect their greater breeding effort and consequent physiological stress.

2.1 Introduction

During the breeding season, the body weight of adult birds, especially of females, is very variable, being influenced by the production of eggs and changes in gonad weight, water, fat and protein stores (Dowsett-Lemaire & Collette 1980, Freed 1981, Moreno 1989a, 1989b, Ricklefs & Husell 1984, Woodburn & Perrins 1997). In fact, before and during laying the growth of gonads and the production of eggs leads to a mass increase of 7-30% in females, whilst males may decrease in weight due to territorial and courtship activities (Moreno 1989a). This often causes the reversal of the usual sexual dimorphism in body size. Immediately after laying, there is a marked drop in weight in females as they are no longer carrying eggs and the reproductive system regresses (Woodburn & Perrins 1997). During incubation, however, two strategies arise: the incubatory mass loss strategy and the incubatory mass constancy; which depend on the share of incubation between the sexes and on the existence of incubation feeding (Moreno 1989a). It was suggested that the decline in adult weight during the breeding cycle, particularly the strong decline that occurs shortly after hatching in females, was adaptive, allowing the parents to spend less energy in flight when feeding nestlings (Freed 1981, Kullberg *et al.* 2002, Norberg 1981). The adaptive hypothesis was supported by experimental evidence in which food provisioning did not affect the weight of brooding females (Hillström 1995, Moreno 1989b). However, Nur (1984) and Moreno & Carlson (1989) showed that an experimental increase in brood size would increase female weight loss and decrease its survival. Therefore, the decline in weight seems to be partly caused by a physiological stress associated with egg laying and parental care (Hillström 1995). As with the breeding cycle, the decline in body weight during the breeding season may be caused by physiological stress or simply by the reduction of the reproductive organs, particularly in single-

brooded species. As the various components of weight were rarely analysed in relation to the breeding cycle (exceptions include studies in which birds were killed to analyse their composition, Woodburn & Perrins 1997), the analysis of variation of weight as well as independent measures of fat and muscle may provide a better understanding of the relative cost of reproduction for males and females. This was partly done by Gosler (1991) for a single-brooded species (the Great Tit, *Parus major*), who showed that besides weight, females decline in muscle during the breeding season thus showing evidence for physiological stress.

The Savi's Warbler, *Locustella luscinioides*, is a long-distance migrant with reduced sexual dimorphism (Cramp 1992). Its skulking habits, dense habitat and insect-like song make them quite difficult to detect. Hence, there are relatively few studies on this species, especially in comparison with other reedbed warblers. The breeding distribution ranges from the Mediterranean to Estonia and from Portugal and western France to Mongolia (Hagemeijer & Blair 1997, Snow & Perrins 1998). The winter quarters lie in sub-Saharan Africa north of the forest zone, where it occurs in a narrow belt extending discontinuously from Senegal to Eritrea (Snow & Perrins 1998).

Few studies have provided data on Savi's Warbler biometrics. Müller (1981) described the biometrics of a German population and evaluated the differences between sex and age classes and the seasonal variation. C. S. Roselaar (in Cramp 1992) presented biometric data from a wide geographical range based on both skins and living birds, but the sample sizes were nevertheless quite small. In addition, Aebischer *et al.* (1996) analysed the relationship between biometrics and arrival date and breeding success, but they did not describe the measurements themselves. A detailed analysis of Savi's Warbler biometrics could be useful for the sex determination of non-breeding birds, and to

evaluate if there is any geographic variation in body size.

The aims of this chapter are twofold. First, to describe the biometric variation of a Portuguese population and compare these data with those of other populations. Second, to determine the factors that influence the weight and body condition indices during the breeding season and evaluate if these indices indicate physiological stress in this multibrooded species.

2.2 Material and methods

2.2.1 Study site

This study was undertaken at Salreu marshlands, which are located in Ria de Aveiro, a large coastal lagoon in Western Portugal (40°43'N, 08°35'W). The habitat consists of a large reedbed where patches of *Scirpus*, *Juncus* and *Typha* occur with scattered alders, *Alnus glutinosa*, and willows, *Salix sp.*. The vegetation is frequently cut for bedding and the water level depends on the stage of development of the rice fields nearby (see §Chapter 1 for more details).

2.2.2 Methodology

From 2000 to 2003, Savi's Warblers were captured by mist-net and marked with a unique combination of three colour-rings and a metal ring issued by the Portuguese Ringing Centre (CEMPA/ICN), two on each tarsus. The males were usually captured shortly after arrival with the use of a tape-recording of the song. Females, however, were only captured when their nests were found (see §Chapter 3). At least 90% of the individuals of each sex that occurred in the study area were trapped and measured, nearly always during the morning. The vast majority of birds, if not all, were local breeders.

The following measurements were taken from each bird: (1) wing length (maximum chord, Svensson 1992), (2) 3rd primary length (numbered ascendantly, Jenni & Winkler 1989), (3) tail length, (4) tail roundness (the difference between the longest and the shortest tail feather), (5) tarsus length, (6) bill length to skull and (7) weight. A 0 to 8 fat score with half points (Kaiser 1993) and a 0 to 2 muscle score with half points (Gosler 1991) were also recorded. Measurements 1–4 were taken with rulers to the nearest 0.5 mm, measurements 5–6 were taken with a calliper to the nearest 0.1 mm and weight with a Pesola balance to the nearest 0.1 g. The weight and wing length of birds captured and ringed at the study site since 1997 were also used (Neto 2003). All measurements were taken by the author. Birds of either age or sex class that had growing or missing outer primaries and tail were not measured for these traits.

Sexing was based on the presence of a well-developed brood patch, which occurs only in females (pers. obs.), and on the behaviour of colour-ringed birds; singing birds were identified as males. The cloacal protuberance was also diagnostic of the sex during the breeding season (Svensson 1992, pers. obs.). Birds were only sexed during the breeding season even though some individuals caught after moult were positively identified as females due to the presence of a brood patch “scar” made of wrinkled abdominal skin. Therefore, all biometrics of known adult males and females are of local breeders during the breeding season. Ageing was based on the wear of the feathers, particularly the remiges, rectrices, primary coverts and alula, and iris colour. Juveniles were readily identified as they had obviously fresher plumage than adults, and had a dark grey iris. Adults were very worn in summer and autumn and the iris colour was grey-brown, brown or light brown. Birds undertaking a complete moult were aged by the same characters if they still had some old feathers. The few birds that had completed their moult and did not retain any feathers were aged only by the iris colour and were all

adults.

As the nests belonging to many of the birds captured were found for another study (§Chapter 3), the exact stage of the breeding cycle was known. The weights and body condition indices of these birds could therefore be related to the breeding stage. Savi's Warblers seem to be sensitive to disturbance only at the earlier stages of the breeding cycle: two nests that were found at the building stage were apparently abandoned due to human interference, but the females started building another nest close by in which they laid eggs. In addition, two nests were also abandoned during egg-laying (one with one egg and the other with two) on the day the females were trapped (§Chapter 3).

2.2.3 Statistics

The statistical analyses follow Sokal & Rohlf (1995) and were performed with SPSS 10.0. All variables were approximately normally distributed (with very low skewness or kurtosis) within each age and sex class. The repeatability of the measurements was calculated using the recapture information obtained in 2000 (Harper 1994, Lessells & Boag 1987). The comparisons of the biometrics between age and sex classes involved the use of Levene tests of equality of variances and *t*-tests assuming equal or unequal variances depending on the result of the first test. Sequential Bonferroni tests were applied in tables of statistical tests (Rice 1989). A discriminant analysis were performed in order to determine which morphological features are important in distinguishing males from females, for which weight, fat and muscle were excluded. Both, direct (complete) and stepwise procedures were used.

A complete and a stepwise multiple regression analysis were used to determine the factors that explain weight variation during the breeding season. However, as most individuals were first captured early in spring, the variation in weight and body condition

indices during the season was evaluated considering all first captures and within and between-year recaptures. Pseudoreplication is unlikely to affect the results because the body condition changes markedly in short periods of time, and any possible effect of the individual would constrain the relationships between season and the dependent variables, making it less likely to obtain significant results. Despite that, in order to control for pseudoreplication, the individual was entered as a random factor in GLM models in which date and sex were independent variables and weight and body condition indices were dependent variables. For this analysis, individuals at the laying stage and those captured later than 31 July were excluded, so that the females captured early in spring that were carrying eggs would not influence the determination of the seasonal variation in weight and body condition indices.

To assess if the decline in weight could be partly explained by the reduction of reproductive organs, the lean body mass was estimated from the regression of muscle, fat and weight ($\text{Weight} = 0.305 (\text{Fat}) + 0.367 (\text{Muscle}) + 13.571$, $R^2 = 0.186$, $F_{[2,265]} = 30.192$, $P < 0.001$). The weight of each bird was subtracted by 0.305 multiplied by their fat score plus 0.367 multiplied by their muscle score.

The breeding cycle was defined as laying period (from three days before laying of the first egg to the day before laying of the last egg), incubation period (from the day in which the last egg was laid until the day before hatching), nestling period and fledgling period (when fledglings were being fed, but the female could be building a second or third nest).

2.3 Results

In total, 348 Savi's Warblers were captured between 1997 and 2003, 73 of which

were caught before 2000. In addition, there were 41 between-year retraps and 92 within-year retraps. The repeatability of the measurements taken in 2000 is presented in Table 2.1.

Table 2.1 – Repeatability of the measurements taken in 2000. *** – $P < 0.001$, ** – $P < 0.01$, * – $P < 0.05$.

Measurement	Repeatability	Statistics
Wing length	0.964	$F_{[15,23]} = 65.40^{***}$
3 rd primary length	0.925	$F_{[14,21]} = 30.43^{***}$
Tail length	0.931	$F_{[14,21]} = 33.35^{***}$
Tail roundness	0.604	$F_{[11,16]} = 4.54^{**}$
Tarsus	0.888	$F_{[15,22]} = 19.23^{***}$
Bill length to skull	0.745	$F_{[14,21]} = 7.99^{***}$
Bill length to feathers	0.422	$F_{[15,21]} = 2.69^{*}$

Bill length to feathers showed only a moderate repeatability, and so this measurement was not taken subsequently. The repeatability of primary length is not greater than that of wing length, as was described by Gosler *et al.* (1995), yet this measurement was taken for future comparison with published data and museum material.

2.3.1 Sex differences in biometrics

The biometrics of adult Savi’s Warblers and the comparison of males and females are presented in Table 2.2. Wing, primary, tail and tarsus lengths were significantly larger in males, whereas bill length to skull was only marginally larger in males. There were no significant differences in weight and muscle score between sexes, but the variance of female weight was significantly higher (Levene’s test for equality of variances: $F = 13.26$, $P < 0.001$).

The overlap between the measurements is quite extensive preventing the sexing of most birds by morphological features. A discriminant analysis between males and females was performed using all the characters except weight, fat and muscle, but only 83.0% of the original individuals were correctly identified. A stepwise procedure correctly

identified 80.1% of the original group cases and included only the variables wing, tarsus, tail length and tail roundness (Table 2.3).

Table 2.2 – Biometrics and statistical comparisons of adult male and female Savi’s Warblers captured during the breeding season. Equal variance t-tests were used for all variables except weight, for which variance was higher in females (see text) and so the unequal variance t-test was used. Data is presented as mean ± S.E.(Range; N). *** – P < 0.001, * – P < 0.05, ns – non-significant. Sequential Bonferroni tests do not change the significance of the comparisons.

Measurements	Adult males	Adult females	Statistics
Wing length (mm)	70.15 ± 0.14 (66.5 - 74.0; 123)	68.03 ± 0.19 (64.0 - 71.5; 62)	8.61***
3 th primary length (mm)	50.65 ± 0.13 (47.0 - 54.5; 123)	49.23 ± 0.16 (46.0 - 52.0; 62)	6.46***
Tail length (mm)	59.43 ± 0.16 (55.0 - 65.5; 121)	57.25 ± 0.27 (51.0 - 61.5; 57)	7.26***
Tail roundness (mm)	16.00 ± 0.16 (12.5 - 20.5; 118)	15.68 ± 0.23 (12.5 - 19.5; 55)	1.13 ^{ns}
Tarsus length (mm)	20.88 ± 0.05 (19.1 - 22.3; 125)	20.38 ± 0.08 (19.0 - 21.9; 66)	5.21***
Bill length to skull (mm)	15.62 ± 0.04 (14.0 - 16.6; 124)	15.42 ± 0.06 (14.3 - 16.6; 65)	2.77**
Weight (g)	14.57 ± 0.07 (12.5 - 16.4; 124)	14.73 ± 0.16 (12.5 - 20.2; 71)	0.87 ^{ns}
Fat score	1.54 ± 0.07 (0.0 - 3.5; 124)	2.12 ± 0.10 (0.0 - 4.0; 66)	4.59***
Muscle score	1.01 ± 0.04 (0.0 - 2.0; 124)	1.11 ± 0.06 (0.0 - 2.0; 66)	1.33 ^{ns}

Table 2.3 – Variables entered in the discriminant analysis (stepwise procedure), Wilks' Lambda and significance test.

Step	Variables entered	Wilks' Lambda	Exact F	df1	df2	Sig.
1	Wing length	0.720	65.647	1	169	0.000
2	Tarsus length	0.669	41.520	2	168	0.000
3	Tail length	0.636	31.795	3	167	0.000
4	Tail roundness	0.614	26.142	4	166	0.000

Table 2.4 – Biometrics and statistical comparisons of adult and juvenile Savi’s Warblers (sexes combined) captured from March to September. Equal variance t-tests were used for tail, tail roundness, bill, fat and muscle, in all other measurements the unequal variance t-test was used. Data is presented as mean ± S.E.(Range; N). *** – P < 0.001, ns – non-significant. The difference in muscle score is not significant after sequential Bonferroni tests.

Measurements	Adults	Juveniles	Statistics
Wing length (mm)	69.43 ± 0.13 (64.0 - 74.0; 200)	67.23 ± 0.17 (64.5 - 70.0; 60)	10.52***
3 th primary length (mm)	50.15 ± 0.11 (46.0 - 54.5; 203)	48.08 ± 0.14 (45.5 - 51.0; 59)	11.45***
Tail length (mm)	58.74 ± 0.15 (51.0 - 65.5; 197)	52.92 ± 0.21 (49.0 - 56.5; 55)	19.12***
Tail roundness (mm)	15.95 ± 0.13 (12.5 - 20.5; 191)	13.43 ± 0.21 (10.0 - 17.0; 56)	9.81***
Tarsus length (mm)	20.70 ± 0.04 (19.0 - 22.3; 211)	20.72 ± 0.06 (19.5 - 21.8; 61)	0.21 ^{ns}
Bill length to skull (mm)	15.55 ± 0.03 (14.0 - 16.6; 209)	14.92 ± 0.06 (13.5 - 16.5; 60)	8.56***
Weight (g)	14.64 ± 0.08 (12.0 - 21.5; 255)	13.55 ± 0.08 (11.8 - 15.7; 88)	7.41***
Fat score	1.88 ± 0.07 (0.0 - 6.0; 211)	1.83 ± 0.14 (0.0 - 4.5; 60)	0.35 ^{ns}
Muscle score	1.08 ± 0.03 (0.0 - 2.0; 211)	1.26 ± 0.07 (0.0 - 2.0; 60)	2.16*

2.3.2 Age differences in biometrics

Adults were significantly larger than juveniles in all measurements except tarsus length (Table 2.4). Juveniles were also smaller (except for tarsus) than adult females, suggesting that the difference between adults and juveniles was not caused by the higher number of males in the adult sample (Tables 2.2 and 2.4). In fact, when the males were randomly sub-sampled to make the ratio of adult birds of known sex even, the significance of the comparisons between juveniles and adults remained the same.

2.3.3 Weight variation during the breeding season

In order to determine which factors influence the variation in weight, multiple linear regressions were used for each sex and age class (Table 2.5). The model for females explains little variation in weight in comparison with the models for males and juveniles, and the only significant variable was muscle score. For adult males and juveniles, tarsus length and fat score were good predictors of weight. In addition, muscle score and, to a lesser extent, wing length also influenced weight in adult males. These results suggest that the best measurement of body size in this species is tarsus length, although in males wing length is also correlated with weight.

Table 2.5 – Multiple linear regression in which weight was the dependent variable and time of day, date, fat, muscle and the main body size measurements were used as independent variables. The F test of the overall model, the R² and the t-tests for each variable are presented. *** - P < 0.001, ** - P < 0.01, * - P < 0.005, all the others are not significant.

	Adult males (n = 123)	Adult females (n = 62)	Juveniles (n = 60)
Model, F	17.092***	2.350*	3.586**
R ²	0.554	0.290	0.395
Constant	-2.550*	-1.259	-2.224*
Date	-0.468	-0.653	-0.879
Time of day	1.749	-0.154	0.217
Wing length	2.543*	1.220	0.649
Tail length	-0.216	0.940	1.936
Tarsus	5.305***	0.832	2.713**
Bill length to skull	1.774	1.540	1.346
Fat score	5.404***	1.045	3.378**
Muscle score	5.725***	2.213*	-0.199

The stepwise multiple linear regressions had similar results to the complete models. For females the variables entered were muscle score followed by wing length ($F = 5.68$, $P = 0.006$, $R^2 = 0.179$) whereas for males the variables were: fat, tarsus, muscle and wing ($F = 31.68$, $P < 0.001$, $R^2 = 0.526$); and for juveniles: tarsus, fat and tail ($F = 8.73$, $P < 0.001$, $R^2 = 0.348$). Because the vast majority of birds were captured during the morning, time of day had no effect on weight in any model (Table 2.5). Season (date) did not seem to influence the weight of the birds either, but this may have resulted from the fact that most birds were first captured early in the season. If the recaptures were taken into account, the weight (see Figure 2.1), fat score (GLM: $F_{[1,65]} = 11.199$, $P = 0.001$), muscle score (GLM: $F_{[1,65]} = 7.270$, $P = 0.009$,) and lean body mass (GLM: $F_{[1,66]} = 10.849$, $P = 0.002$) declined significantly during the season. A significant interaction between sex and date was only found for muscle score (GLM: Date vs Sex: $F_{[1,65]} = 5.147$, $P = 0.027$), because females declined in muscle score more steeply than males.

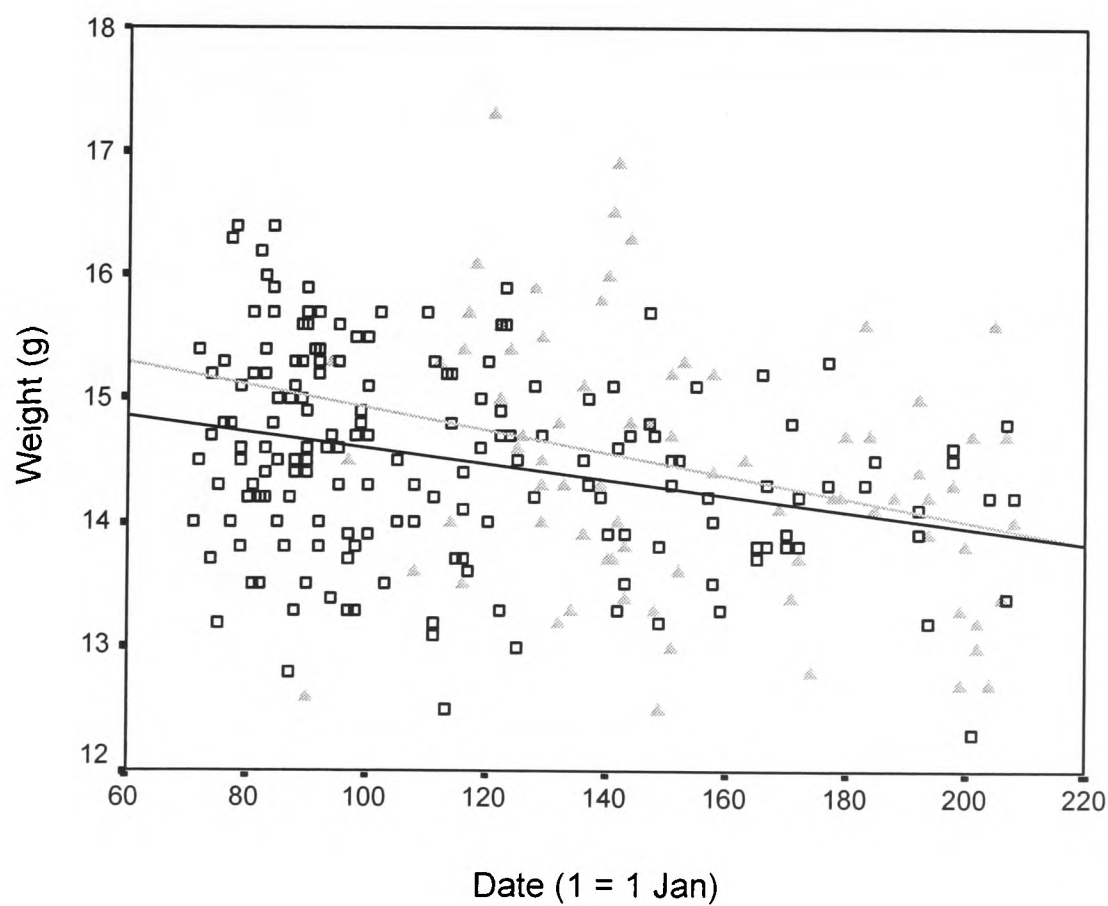


Figure 2.1 – Variation in weight of males (black squares) and females (grey triangles) during the breeding season (all capture events included). GLM analysis: $F_{[1,67]} = 25.529$, $P < 0.001$.

The weight of females was strongly influenced by the breeding stage (ANOVA: $F_{[3,51]} = 17.157$, $P < 0.001$), being heavier during the egg-laying period than during incubation (Tukey's test: $P = 0.009$) and heavier during incubation than during the nestling period (Tukey's test: $P = 0.001$), but no differences were found between the fledgling period and other breeding stages (Figure 2.2). The males, however, did not change their weight during the breeding cycle (ANOVA: $F_{[3,15]} = 0.073$, $P > 0.05$), which explains the higher variance in female weight described above. Females were heavier than males during the egg-laying period (t -test: $t_6 = 3.41$, $P = 0.014$) and almost significantly heavier during incubation (t -test: $t_{16} = 1.979$, $P = 0.065$), but not during the nestling and fledgling stage ($P > 0.05$). A GLM analysis using weight as the dependent variable and breeding stage and sex as independent variables is significant for both variables and their interaction (Model $F = 8.214$, $P < 0.001$; Breeding stage $F = 3.632$, $P = 0.017$; Sex $F = 12.547$, $P = 0.001$; Breeding stage vs. Sex $F = 5.626$, $P = 0.002$).

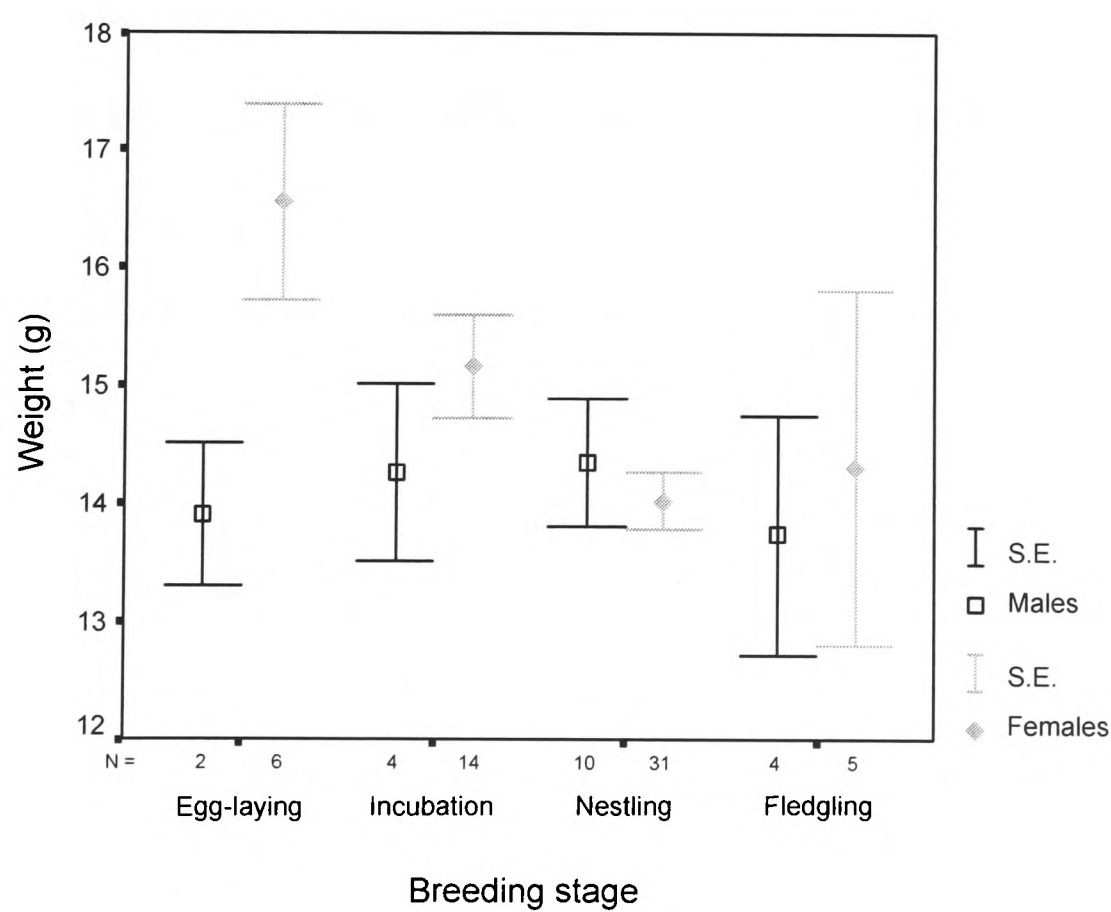


Figure 2.2 – Variation of male and female weight (g) with breeding stage. The average and standard error of the mean for each period is presented as well as the sample size.

The muscle score was also influenced by the breeding stage in females (ANOVA: $F_{[3,51]} = 3.160$, $P = 0.032$), and tended to be higher during incubation than during the nestling stage (Tukey's test: $P = 0.067$), but not in males (ANOVA: $F_{[3,15]} = 0.848$, $P = 0.489$). Muscle score tended to be higher in females than males only during the incubation period (t -test: $t_{16} = 1.923$, $P = 0.073$), but these differences between sexes and breeding stage might reflect sample sizes. In a GLM analysis using muscle as the dependent variable, sex, breeding stage and their interaction were not significant (all $P > 0.05$).

Although the relationship between fat score and breeding stage was not significant for either sex (Females: ANOVA $F_{[3,51]} = 2.211$, $P = 0.098$; Males: ANOVA $F_{[3,15]} = 0.911$, $P = 0.459$), it was much closer to significance in females. Females tended to be fatter than males during the egg-laying stage (t -test: $t_6 = 2.079$, $P = 0.083$) and were significantly fatter than males during the nestling period (t -test: $t_{40} = 4.658$, $P < 0.001$), suggesting that females increased in fat during the incubation. In a GLM analysis using fat as the dependent variable, breeding stage was almost significant ($P = 0.063$) and sex was very significant ($P < 0.001$), but their interaction was not significant ($P > 0.05$).

A GLM analysis using the lean body mass as the dependent variable and breeding stage and sex as independent variables was almost significant for both variables and highly significant for their interaction (Model $F = 6.997$, $P < 0.001$, Sex $F = 3.455$ $P = 0.067$, Breeding stage $F = 2.513$ $P = 0.066$, Breeding stage vs. Sex $F = 5.853$, $P = 0.001$). Therefore, the different weight changes between males and females during the breeding cycle seem to be associated at least partly with differences in the weight loss of the reproductive organs.

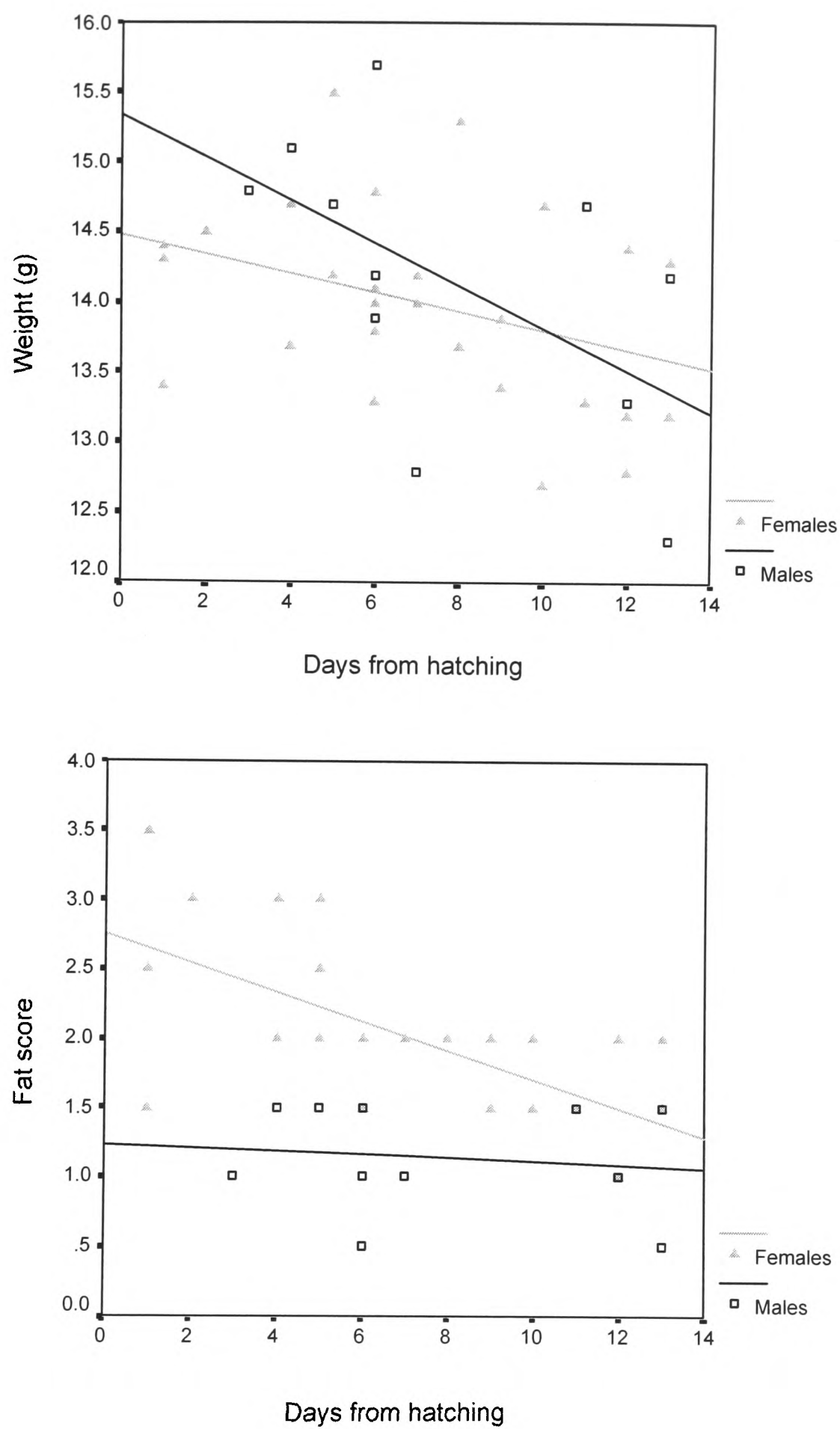


Figure 2.3 – Variation in male and female weight (A) and fat score (B) during the nestling period. The lines represent the linear regressions for the sex classes. A) Males: $\text{Weight} = -0.151 (\text{Days after hatching}) + 15.336$, $R^2 = 0.303$, $F_{[1,9]} = 3.911$, $P = 0.079$; Females: $\text{Weight} = -0.067 (\text{Days after hatching}) + 14.477$, $R^2 = 0.127$, $F_{[1,29]} = 4.229$, $P = 0.049$; B) Males: $\text{Fat score} = -0.001 (\text{Days after hatching}) + 1.234$, $R^2 = 0.014$, $F_{[1,9]} = 0.128$, $P = 0.729$; Females: $\text{Fat score} = -0.104 (\text{Days after hatching}) + 2.749$, $R^2 = 0.392$, $F_{[1,29]} = 18.720$, $P < 0.001$.

The sample size was too small to assess the variation in weight and body condition indices during incubation in males. In females, no significant relationship was found between weight and the progress of incubation. Although not significant, muscle score tended to increase (Pearson's correlation: $r = 0.505$, $P = 0.078$, $n = 14$) and lean body weight decrease (Pearson's correlation: $r = -0.484$, $P = 0.098$, $n = 14$) during incubation. The fat score increased significantly during the incubation period in females (Pearson's correlation: $r = 0.565$, $P = 0.044$, $n = 14$).

The female weight declined significantly during the nestling stage, whereas males, for which the sample sizes are smaller, the decline almost reached significance (Figure 2.3a). This decline may be explained partly by a decrease in fat in females, but not in males (Figure 2.3b). Muscle and lean body mass did not vary significantly during the nestling stage.

2.4 Discussion

Male Savi's Warblers captured during the breeding season were significantly larger than females in all body size measurements, but no differences were found in tail roundness. However, the sexual dimorphism is very reduced: males were only 2.4% to 3.8% larger than females, depending on the measurement considered. According to C. S. Roselaar (in Cramp 1992) the differences between the sexes are significant except for tarsus, which is in contrast to this study. Only the extreme birds may be identified in the hand and many remained unidentified even when a discriminant function was used. Among the differences found, the tail length seems to be more divergent than the other features.

As in many other passerines, juveniles had shorter wings and tails (Cramp 1992).

In fact, the adult wing length was 3.2% and tail 11% larger than those of juveniles. These results are similar to those presented by Müller (1981): 3% and 10%, respectively. Cramp (1992) also states that the juvenile wing is on average 1.5 mm and the tail 5.0 mm shorter than those of adults. These results also agree with those obtained in this study. Juvenile Savi’s Warblers also had shorter bills, which probably resulted from the fact that the bill does not reach the full length until at least three or four weeks after fledging (pers. obs.). The tarsus length, however, did not differ between age classes; this was expected because of the importance of the hind limbs in this species (Leisler 1977, Winkler & Leisler 1985) and the fact that they leave the nest before being able to fly (pers. obs.).

Table 2.5 – Biometrics of Savi’s Warblers of the nominate subspecies collected in the Netherlands and Romania (skins, C. S. Roseelar in Cramp 1992), and captured in Hungary (G. L. Lövei in Cramp 1992), East Germany (Müller 1981) and Switzerland (A. Aebischer, pers. comm.). Data presented is: Average (Range;N).

		Netherlands	Romania	Hungary	East Germany	Switzerland
Wing	M	70.5 (69-73;16)	72.4 (70-77;12)	70.6 (68-74;11)	69.5 (64-76;127)	70.9 (65.0-75.5;66)
	F	68.7 (66-71;6)	68.7 (67-71;5)	69.7 (68-72;7)		68.9 (64.5-73.9;23)
Tail	M	57.6 (53-62;26)			56.4 (50-64;109)	56.8 (50.5-62.5;65)
	F	54.4 (50-58;10)				56.0 (52-61;21)
Bill	M	16.1 (15.7-17.9;24)				16.5 (15.5-17.5;63)
	F	15.3 (14.8-15.9;7)				16.6 (15.5-18.0;22)
Tarsus	M	21.5 (20.5-22.7;19)				21.2 (19.0-23.5;64)
	F	21.3 (20.4-22.6;10)				21.0 (19.7-22.0;23)

The published biometrics of Savi’s Warblers belonging to other populations are presented in Table 2.5. These were taken from both museum and live birds. Despite the small samples, the maximum wing length in all countries except the Netherlands and Hungary is 1.5 to 3 mm larger than in Portugal. Although the birds captured in Hungary had on average larger tarsus and bill lengths than in Portugal, the tail length seems to be smaller. However, some of these differences could have been caused by differences in measurement technique. In particular, the tail lengths of Portuguese birds was measured with a ruler inserted between the longest undertail coverts and the central rectrices,

whereas in museum birds (Netherlands and Romania) it was measured with dividers. Although the measurements do suggest that the central and northern European birds are larger than those of southern populations, as in most long-distance migratory passerines, more data are needed particularly to evaluate whether the tail length fits with this cline.

Although females are smaller than males, during the breeding season they had a similar mean and greater variance in weight, and more fat. The variation in weight of male Savi's Warblers depended on their fat, muscle and body size, particularly on the tarsus length, although wing length also affected weight. The same relationships between weight and biometric data were found for juveniles with the exception of muscle, which was not significant (Table 2.5). The variation in weight of females, however, was not explained by these variables, presumably because other factors such as the stage of development of the reproductive system and the production of eggs (which in Savi's Warblers weigh from 1.58 to 2.30 g, per. obs.) affect the weight (Woodburn & Perrins 1997).

It was found that the weight of females, but not of males, depended markedly on the stage of the breeding cycle, females being heavier than males during the egg-laying period, and slightly heavier during incubation. However, although some differences between the sexes were found in fat and muscle through the breeding cycle, the steeper decline in female weight during the breeding cycle seems to be explained mostly by the egg formation and regression of the reproductive system. It was not clear how weight vary during incubation in males. The weight of females seemed to remain constant during the incubation, thus following the incubatory mass constancy strategy described by Moreno (1989a). This is surprising because female Savi's Warblers do most of the incubation and are not fed by the males, and so they were expected to decrease in weight

during this period. However, altricial species seem to maintain body reserves throughout the incubation in order to cope with parental care duties (Moreno 1989a). Despite the weight constancy, females tended to increase in fat and muscle and decrease in lean body mass, probably as a result of the regression of the reproductive organs, which may be metabolised into fat, as was suggested by Woodburn & Perrins (1997). However, these results must be interpreted with caution because of the small sample sizes. During the nestling period, both sexes decreased in weight, which for females was associated with a strong decline in fat. These results are in general agreement with the variation in weight and body reserves described for other species (Freed 1981, Moreno 1989b, Ricklefs & Husel 1984, Woodburn & Perrins 1997).

Both sexes declined in weight, fat, muscle and lean body mass during the breeding season. The decline in male weight seems to be partly caused by their earlier arrival in the breeding quarters because early-arriving males tend to have more fat (pers. obs.), presumably to cope with starvation risk and lower temperatures early in the season. In females, the decrease in muscle score through the season was significantly steeper than in males. It was suggested by Gosler (1991) that the decrease in muscle score indicates stress during the breeding season and its decline may reflect the relative breeding effort of the sexes. The greater decline in muscle score, and presumed greater breeding effort, in females might be partly responsible for the biased sex ratio of the adult population, by causing a higher mortality during the non-breeding season. The same difference between sexes was found by Gosler (1991) for the Great Tit and so this may be a general phenomenon in passerines.

CHAPTER 3

Breeding biology of the Savi's Warbler in Ria de Aveiro, Portugal

Male Savi's Warblers arrived at the Salreu marshlands on the second week of March whereas females arrived 6-10 days later. Early-arriving males bred earlier and had a higher mating and breeding success. Female breeding success also declined with first-egg date. Early-arriving males had smaller tarsus length and more fat, suggesting that birds with lower resource-holding power and higher body condition invested more in getting a good-quality territory. Female laying date was not correlated with any of the body size or condition measures. The return rates of males, females and juveniles were 29.0%, 15% and 2%, respectively. Clutch and egg sizes were smaller than in other populations studied; the first decreased and the latter increased with season. Savi's Warblers seem to start laying before the seasonal peak in clutch size is reached, which is a behaviour typical of resident birds rather than long-distance migrants. Egg size was correlated with female tarsus length, but not with other body size measurements. The differences in egg size between populations seem to be fully explained by differences in female body size. A Mayfield estimate of the probability of nest survival was 40%, and was higher during the incubation period (74%) than during the nestling period (57%). Nest predation was the major cause of nest loss. Differences in clutch size and predation rate explain the differences in breeding success between populations. Females produced on average 5.38 fledglings per season, which seems enough to balance the typical mortality rates of passerines.

3.1 Introduction

It is often assumed that in migratory birds there is a negative relationship between male arrival date in the breeding quarters and male body condition (see for instance Siitari & Huhta 2002). However, Forstmeier (2002) showed that in some circumstances the benefits of early arrival vary between individuals (see also Kokko 1999). In his study on Dusky Warblers, *Phylloscopus fuscatus*, first-year birds arrived later than adults, and among adults, those that had occupied a territory in the previous year arrived earlier than those that had not. In addition, first-time males arrived earlier when they were large whereas the trend was in the opposite direction for returning males. He concluded that arrival dates depend on both: the resource holding power, which seems to be related to the body size, and on value asymmetries, which result from prior ownership. In Savi's Warblers, *Locustella luscinioides*, early-arriving males choose the best territories, have more successful clutches and a higher mating and breeding success (Aebischer *et al.* 1996). They also mate with early-arriving females as measured by the laying date. Although no relationship was found between arrival date and body condition of either males or females (Aebischer *et al.* 1996), Takaki *et al.* (2001) used ptilochronology as a measure of body condition in a closely related species, the Styan's Grasshopper Warbler (*Locustella pleskei*), and found significant positive relationships between the standardized growth bar width of tail feathers and arrival date, breeding success and, for males, survival rate.

Although the breeding parameters of most European species are well known (see for instance Cramp 1992), there is often a large geographical and temporal variation, which, if adequately described, will allow the understanding of the plasticity of breeding strategies and of the factors that influence breeding adaptations. The description of this

variation is important for conservation because it will assess the potential for adaptation to a changing environment. It may also be important for comparative analysis, which often use data based on one or a few published studies carried out in a small part of the species distribution that may not be representative of its breeding parameters and strategy.

Several authors have described aspects of the breeding behaviour and ecology of Savi's Warblers, a long-distance migratory passerine that occurs in reedbeds (Dirkx 1939, Dittberner & Dittberner 1985, 1991, 1992, Mayaud 1930, Mildemberger 1958, Moreau & Moreau 1982, Schiermann 1926, 1928, Suchantke & Schwarz 1961, among others). The most detailed studies on its breeding biology were done by Pikulsky (1986) in Poland and, more recently, by Aebischer & Meyer (1996) and Aebischer *et al.* (1998) in Switzerland. Savi's Warbler males arrive in the breeding quarters and start defending territories from late March to late April in Switzerland (Aebischer & Meyer 1998) and from mid-April in Poland (Pikulski 1986). Females arrive one week later than males and start laying on 20 April in Switzerland and 30 April in Poland (Aebischer & Meyer 1998, Pikulski 1986). The incubation and nestling periods last 12-13 and 11-15 days, respectively, depending on air temperature and number of nestlings, and the eggs hatch within 1-3 days of each other, depending on the clutch size (Pikulsky 1986). Aebischer & Meyer (1998) estimated incubation periods of 11-14 days and that the nestlings fledged when 11-14 days old. Females undertake most of the incubation and both sexes feed the nestlings.

The breeding system seems to be 'facultative polygamous'. In Switzerland, one out of 18 males mated with two females even though a moderately high proportion of the males (21 to 41%) remained unmated during the whole season (Aebischer *et al.* 1996). In Poland, the 48% of males that remained unmated occupied sub-optimal habitats, mainly

reeds in deep water and *Caricetum* on unflooded ground (Pikulski 1986). However, the females do not seem to actively select any measured feature of the male or its territory. Aebischer *et al.* (1996) argue that a simple lottery model assuming habitat preferences of males and non-selection by the females is sufficient to explain the variation in mating success of the males and the correlation between arrival of males and laying dates. Early-arriving males simply had a higher chance of meeting a female due to the overlap between the arrival of females and males and the slightly biased sex ratio during that period (Aebischer *et al.* 1996).

All these studies were carried out in central and eastern Europe and none was undertaken in the southern part of its distribution. In contrast to other *Sylviidae* species, Savi's Warblers, as well as other *Locustella*, are relatively little known probably because of their skulking behaviour and insect-like song, which, associated with a dense habitat, make them and their nests very inconspicuous. Their populations have declined in western Europe (Snow & Perrins 1998), and in Portugal Savi's Warblers are considered vulnerable (Cabral *et al.* 1990) because they have a very localised distribution, being confined to large reedbeds, a habitat which has suffered a marked decline and continues to be threatened. The study of its breeding ecology is important for clarifying their current status and, if necessary, for implementing conservation measures. In addition, Savi's Warblers, and *Locustella* species in general, are quite unique in many aspects of their biology and so its study may provide interesting data for comparison with the other birds.

The aims of this chapter are: (1) to determine the relationships between arrival date, breeding success and body condition, (2) to describe the breeding parameters of a Portuguese population of Savi's Warblers and compare with published data from other parts of its distribution, (3) to analyse the factors that influence egg size, clutch size and

breeding success and (4) to clarify the conservation status of this species.

3.2 Material and methods

3.2.1 Study area

The fieldwork was carried out in the Salreu marshlands (40°43'N, 08°35'W), Ria de Aveiro, western Portugal. The habitat is a mosaic of reeds (*Phragmites australis*), rushes (*Juncus* sp.), reed maces (*Typha latifolia*), club-rushes (*Scirpus maritimus*), all of which can be dominant in particular sites, and scattered willows (mainly *Salix atrocinerea*) and alders (*Alnus glutinosa*). The vegetation is frequently cut for animal bedding by the local community, mainly from July to October. A dyke separates the marshes from the Aveiro lagoon, allowing the control of the water level and preventing the entrance of saltwater (for more details see §Chapter 1).

In 2000, an area of 21.7 ha located immediately north of the Salreu Canal was selected as a study site. In order to increase the sample size, this area was extended to the southern part of the canal in May 2001, then becoming 31.8 ha. This new area is somewhat influenced by the tides because the dyke cannot completely prevent the entrance of salt water. Here, the proportion of rushes is higher and the reeds only occur in the deeper parts, usually along the ditches.

3.2.2 Methodology

The study area was visited almost daily from early March (mid-March in 2000) to the end of August from 2000 to 2002. In 2003, fieldwork was carried out until the end of May only. The arrival date of males, i.e., when they started singing in their territories was determined by daily visits to the territories from early March to late April. Because of

their skulking behaviour, it was not possible to assess the date of arrival of females. Males were trapped generally by mist-net and a tape-recording made of the song during the first week after arrival, but the females were captured only after their first nest was found. They were marked with a metal ring, issued by the Portuguese Ringing Centre (CEMPA/ICN), and three colour-rings in a unique combination. The following measurements were taken: wing length (maximum chord, Svensson 1992), 3rd primary length (numbered ascendantly) following the method described by Jenni & Winkler (1989), tail length, tail roundness, which is the difference between the longest and the shortest tail feather, tarsus length, bill length to skull and weight. A 0 to 8 fat score with half points (Kaiser 1993) and a 0 to 2 muscle score with half points (Gosler 1991) were also recorded. For females, a brood patch score was used to assess their breeding status (Boddy 1992).

In order to determine if there is a relationship between arrival date and body condition, the ptilochronology method was used (Grubb 1986, Takaki *et al.* 2001). Many of the birds retrapped later in the season did not replace the missing tail feather before the post-breeding moult, therefore the average growth bar width of the induced tail feather could not be used as a measure of body condition. Hence, from 2001 to 2003, the 3rd tail feather on the right side of the tail, or the left if the one on the right was missing, was collected. The average width of the growth bars, which are very conspicuous in Savi's Warblers, was determined following the methodology described by Grubb (1986) and modified by Takaki *et al.* (2001). The average growth bar width of the tail feather was not standardized, as it was not significantly related to the tail feather length nor year. The other measure of body condition used was the weight divided by the tarsus length, which was not corrected for time of day because all birds were captured during the morning.

Two estimates of return rates were made. One was based on the number of recaptures in one year in relation to the number of marked birds in the previous year. The other estimate excluded the males that defended a territory for a short period of time (which could have been passage migrants or may have bred successfully elsewhere) and five females that were most probably predated during the breeding season. Because all birds that returned to the study area were seen in the previous year, the recapture probability was assumed to be 100%.

The positions of singing adults were mapped. Nests were searched for by observing the behaviour of adults, namely, regular movements to and from nests, transport of nest material or food, and intensity of alarm calls. The nests were visited regularly in order to determine laying, hatching and fledging dates. Savi's Warblers seem to be sensitive to disturbance during nest building and egg laying only, when they were visited with caution, although many nests were found and visited during these periods without apparently causing any harm. This is because two nests that were found at the building stage were apparently abandoned due to human interference, but the females started building another nest close by in which they laid eggs. In addition, two nests were abandoned during egg-laying on the day the females were trapped (pers. obs.).

The habitat type at the nest site was classified as follows: (1) *Scirpus maritimus* beds with less than 10% cover of *Typha latifolia*, (2) mixture of *Scirpus maritimus* and *Typha latifolia*, (3) pure *Typha latifolia* beds, (4) mixture of *Typha* and *Phragmites*, occasionally with some *Scirpus maritimus*, (5) pure *Phragmites* beds (with less than 10% cover of *Typha latifolia*, but occasionally with *Iris pseudacorus*), (6) mixture of *Juncus* and *Phragmites*, largely dominated by *Juncus* but with more than 10% cover of *Phragmites*, (7) *Juncus* beds, locally co-dominant with some *Graminae* (mainly *Spartina*

versicolor and *Agrostis stolonifera*), where *Lysimachia*, *Calistegia*, *Hydrocotyle* and *Vicia* were frequently found, and (8) *Rubus* sp. frequently with some *Juncus* and occasionally *Phragmites*.

The length and width of eggs were measured to the nearest 0.1mm. Volume was estimated according to the equation: $\text{Volume} = 0.51 \times \text{length} \times (\text{width})^2$ (Hoyt 1979). The nestlings were colour-ringed as for the adults and measured when they were 7 to 12 days old. For nests found during the incubation or nestling stages, the date of laying of the first egg was estimated assuming an incubation period of 14 days, starting when the penultimate egg was laid, and a nestling period of 13 days (pers. obs.). When the laying, hatching and fledging dates were unknown, the laying date was calculated after estimating the age of nestlings by observing the opening of the eyes, feather growth and by biometry.

The indices of breeding success estimated were: hatching, fledging, total breeding (number of young fledged per egg) and overall (number of fledglings per nest) success and percentage of successful nests. However, as the nests were found at different stages of the breeding cycle, not all breeding parameters were determined for all nests, which caused variations in sample sizes. Since a considerable proportion of nests were found after egg laying, the Mayfield method modified by Johnson was used to estimate nest success (Johnson 1979, Mayfield 1961, 1975). Predation was assumed when all eggs and young nestlings disappeared from the nest because at this stages the nests were often left intact. When the young were older, the nests were invariably partially or totally destroyed and there were remains of feathers and sometimes partially devoured nestlings (pers. obs.).

Seasonal breeding success, that is, the total number of fledglings produced per

season, was determined for both males and females, assuming that there were no instances of intraspecific brood parasitism nor extra-pair paternity. The latter assumption, however, is unlikely to be correct so the variation in seasonal breeding success of males must be interpreted with caution. Although some nests built in the study site were not found at all, particularly in the first year of study, its existence could be determined by the behaviour of the adults. This is because males hardly ever sang during the incubation period, despite their share in incubation being very low, and both parents were very inconspicuous during this period (Pikulski 1986, pers. obs.). The only exceptions were the males that tried to attract a second female during the incubation period of their first nest, but these tended to sing some distance away from their nest and not at the primary song posts (pers. obs.). However, the males changed their behaviour dramatically when the nest was predated, starting to sing from the first day after predation. The presence of a brood patch in females and the observation of adults feeding fledglings also allowed the detection of nesting attempts for which no nest was found, but the vast majority of these attempts were unsuccessful.

Although the annual difference in the arrival dates of males was small, these dates were standardized by converting to the number of days after the arrival of the first male on each year. In order to determine if the standardized arrival date was associated with any of the biometric and condition measures, all possible subsets of these factors were tested with GLM mixed models in which the individual was included as a random factor, and the models were compared with the Akaike information criterion. Year was initially used as a covariate, but was excluded from the final model as it had no significant effect. Sequential Bonferroni tests were applied for large sets of correlations (Rice 1989). Statistical analysis followed Sokal and Rohlf (1995) and were undertaken with the statistical packages SPSS 10.0 and GenStat 7.1. Unless otherwise stated, the results are

presented as mean $\pm$ s.e., and the statistical tests are two-tailed.

3.3 Results

3.3.1 Arrival and territory establishment

The first males started singing in the study site on the 9, 10 and 12 March 2001, 2002 and 2003, respectively, although they were detected in Salreu marshlands as early as 5 March. Although it was not possible to determine the arrival date for individual females due to their skulking behaviour, females seemed to arrive approximately 10 days after the males: the first female was captured on the 22 March 2002. Males established new territories in the study area until the second week of April. There is no significant difference between the date of arrival of males ringed in previous years and unringed males (ANOVA $F_{[1,66]} = 0.161$, $P > 0.10$).

Laying date (Pearson's correlation: $r = 0.557$, $P = 0.004$, $n = 25$) and mating success (Spearman rank correlation: $r_{sp} = -0.396$, $P = 0.013$, $n = 39$) were significantly correlated with standardized male arrival date. In addition, seasonal breeding success tended to be higher in early-arriving males (Pearson's correlation: $r = -0.560$, $P = 0.058$, $n = 12$). Tarsus and bill lengths are positively correlated with standardized male arrival date and there is a tendency for a negative relationship with fat score (see Table 3.1), but all other biometric and condition variables, including ptilochronology, are not significant. The best GLM model that predicted arrival date included only tarsus length and fat score (Tarsus: Wald = 5.31, $P = 0.021$, Effect size = 4.576 ± 1.8727 ; Fat: Wald = 5.69, $P = 0.017$, Effect size = -2.848 ± 1.1941). According to Akaike information criterion (AIC), this model was not different from models that included bill length in addition to or instead of tarsus (difference in AIC < 2), although the variables became non-significant, because

of collinearity between bill and tarsus lengths. Therefore, early-arriving males tended to have shorter tarsus and bill and more fat.

Table 3.1 – Pearson correlation coefficients, probability and n between biometric and condition measurements and standardized male arrival date and female laying date. Ptiloc refers to ptilochronology or the width of the growth bars of the third tail feather, and condition is the weight divided by the tarsus length. These correlations are not significant after sequential Bonferroni corrections.

	Wing length	Primary length	Tail length	Tail round.	Tarsus length	Bill length	Weight	Fat score	Muscle score	Condition	Ptilochr.
Male arrival date	.084 .462 78	.086 .458 77	-0.045 .697 76	.097 .413 74	.245 .031 78	.248 .029 78	-.013 .909 78	-.189 .098 78	-.090 .436 78	-.143 .213 78	-.016 .889 77
Female laying date	.310 .095 30	.236 .209 30	-.054 .785 28	-.109 .580 28	.128 .500 30	.127 .504 30	.172 .373 29	.041 .831 30	-.023 .903 30	.123 .525 29	.035 .866 26

Regarding females, there was a significant correlation between laying date and seasonal breeding success (Pearson’s correlation: $r = -0.738$, $P = 0.01$, $n = 11$), but not with any of the biometric or body condition measurements (Table 3.1).

3.3.2 Site fidelity

The numbers of birds captured and recaptured during the breeding season are presented in Table 3.2. The return rates of males in 2001, 2002 and 2003 were 28.6%, 31.3% and 22.5%, respectively. If the presumed transient males are excluded, these values became 29.6%, 33.3% and 25.6%. The equivalent numbers for females were 7.7%, 10.0% and 21.1%; or, when the predated females were excluded, 8.3%, 11.1% and 23.5%. The returning rate of first-year birds was extremely low. Only three out of 156 nestlings (1.9%) returned to the study site, all of which were males produced in the same territory and year. Both males and females returned to the same territory in subsequent years, or, if the vegetation was cut, to a neighbouring territory. One male, however, arrived when its neighbouring territories were occupied and moved 420 m from its previous territory. Only one returning female out of seven moved to a neighbouring

territory apparently because when it arrived its previous territory was accupied by a pair.

Table 3.2 – Number of males, females and nestlings first captured (in diagonal) and recaptured at the study site during the breeding seasons from 2000 to 2003. The values excluding predated females and transient males are indicated between parenthesis. The average return rate for males, females and nestlings, was 26.6-29.0%, 13.5-14.9% and 1.9%, respectively. The range of these return rates depend on weather trasient males and predated females were included (see above).

Year		2000	2001	2002	2003
2000	Males	28 (27)	8	1	0
	Females	13 (12)	1	1	0
	Nestlings	22	0	0	0
2001	Males		26 (22)	9	2
	Females		19 (17)	1 (0)	0
	Nestlings		58	3	2
2002	Males			36 (31)	7
	Females			17 (16)	4
	Nestlings			76	0
2003	Males				30
	Females				13
	Nestlings				50

3.3.3 Nest building and location

In total, 93 active nests were found in the study area. These were built with old reed leaves, except two which had *Typha* leaves, and had no lining. They were located close to the soil or water except those located in tall bramble, which were built just beneath the leaves (distance from soil to the upper rim of the nest: mean = 36.9 cm, range 14–145, n = 89).

Table 3.3 – Number of nests found on each habitat type available at the study site. For more information about nest-site selection see §Chapter 4.

Habitat type	Number of nests
<i>Scirpus maritimus</i>	0 (0%)
<i>Scirpus</i> & <i>Typha</i>	2 (2.1%)
<i>Typha</i>	0 (0%)
<i>Typha</i> & <i>Phragmites</i>	0 (0%)
<i>Phragmites</i>	3 (3.2%)
<i>Phragmites</i> & <i>Juncus</i>	48 (51.6%)
<i>Juncus</i>	29 (31.2%)
<i>Rubus</i>	11 (11.8%)

Nests were located in dense vegetation, usually in rushes or rushes with reeds and occasionally in *Scirpus maritimus* mixed with *Typha*, and bramble (Table 3.3). Pure reedbed was rarely used for nesting; the only three instances were in dense tufts of broken old reeds in a dry area (Table 3.3).

3.3.4 Breeding phenology

The earliest first-egg date was on 13 April and the last nestling fledged on 23 August. Therefore the breeding season may last 132 days. The distribution of the first-egg date of each nest is presented in Figure 3.1. The first-egg date of the first nesting attempt ranged from 13 April to 23 May and was later in 2001 than in 2002 and 2003 (ANOVA: $F_{[3,37]} = 7.57, P < 0.001$; Tukey test: 2001 vs. 2002 $P = 0.009$; 2001 vs. 2003 $P < 0.001$).

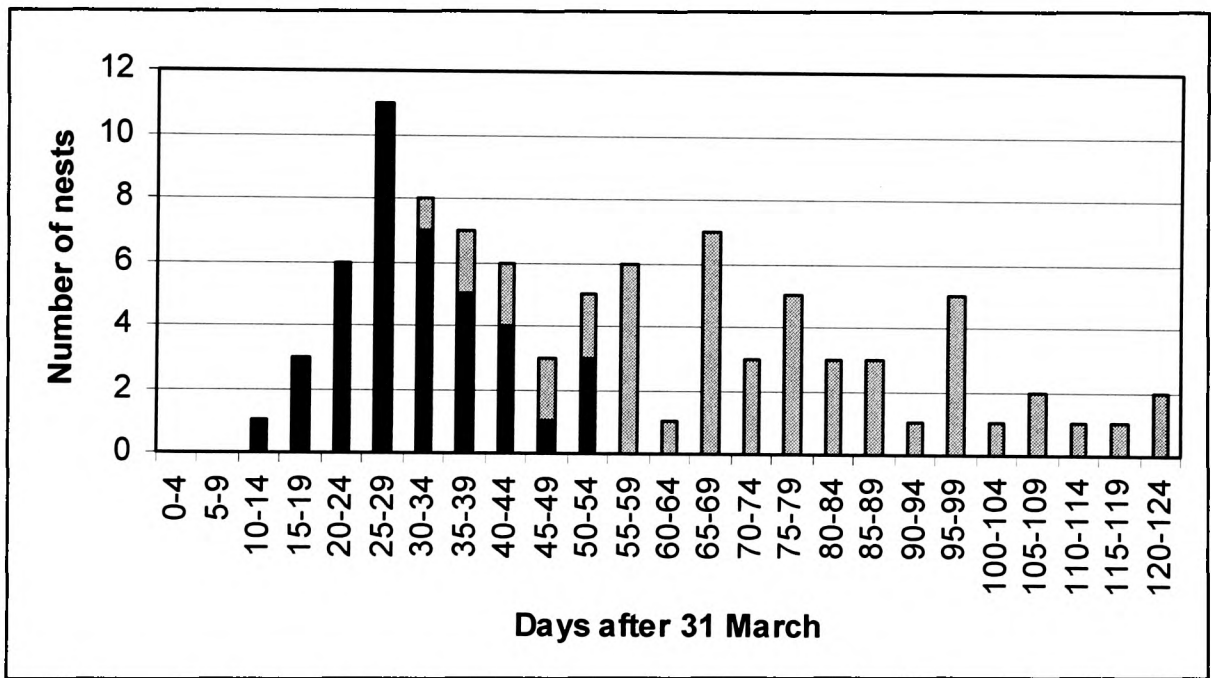


Figure 3.1 – Distribution of first-egg dates per five-day periods in 2000 to 2003 (n = 90 nests). Black bars represent first nests.

In 2001, three polygynous (secondary) females bred within the territory of a paired male (which contributed with food for the nestlings). This may have caused the inter-annual difference in laying dates since the average difference in first-egg date between secondary females and the corresponding primary females was 14 days

(Paired t -test: $t_2 = 3.729$, $P = 0.065$). One secondary female was also found in 2002, but the first-egg date was not determined. The first replacement clutch was initiated on 8 May thus overlapping considerably in time with the first-egg dates of first clutches.

The hatching period took less than one day in two nests, less than two days in six nests and less than three days in five nests (2.23 ± 0.201 , range 1-3, $n = 13$). Hence, although there was some variation in the start of the incubation period, which generally began with the laying of the penultimate egg, incubation seemed to last invariably 14 days. The nestling period varied from 9-15 days (mode 13 days). Hence, the total nest period for a four-egg clutch was 30 days. The time between fledging and the laying date for a subsequent nest was on average 10 days (range 5-14, $n = 10$) and in the case of predated nests, eight days (range 5-15 days, $n = 6$; ANOVA: $F_{[1,14]} = 0.92$, $P > 0.05$). Therefore, in a 132 day long breeding season it is possible to produce three clutches of four eggs, which would take on average 110 days. In this study, only one such case was detected; and one female laid four clutches of eggs and was successful with two of them.

3.3.5 Clutch size

The average clutch size was 4.3 eggs per nest (range 2-5, $n = 72$). The mode was four eggs in all years except in 2003 when more nests with five eggs were found (Table 3.4). This difference probably resulted from the fact that in 2003 nests were searched for only before the end of May, and, as shown in Figure 3.2, clutch size decreases during the season. Despite this bias, it is obvious from Table 3.4 that the mean clutch size of the Portuguese population is smaller than those of the Polish and German populations (Pikulsky 1986, Dittberner & Dittberner 1991) and possibly smaller than the one reported for Switzerland (4.7 eggs per nest) by Aebischer & Meyer (1998).

Clutch size decreased significantly during the season (Spearman rank correlation:

$r_{sp} = -3.31$, $P = 0.003$, $n = 80$). However, the best model seems to be a quadratic rather than a linear one because, although the probability of the F test is the same (< 0.001), the R^2 is higher in the quadratic relationship (see Figure 3.2).

Table 3.4 – Frequency of clutch sizes in Portugal (this study), Poland (Pikulsky 1986) and Germany (Dittberner & Dittberner (1991). Note that in 2003 nest were only found before the end of May. Two nests with one and two eggs were not included because they were abandoned apparently as a consequence of human interference.

Clutch size	2000	2001	2002	2003	Portugal	Germany	Poland
2	0 (0%)	1 (3.4%)	0 (0%)	0 (0%)	1 (1.4%)	0 (0%)	0 (0%)
3	1 (10%)	1 (3.4%)	2 (6.5%)	1 (8.3%)	5 (6.9%)	1 (2.2%)	1 (1.4%)
4	8 (80%)	17 (58.6%)	16 (51.6%)	2 (16.7%)	33 (45.8%)	10 (22%)	13 (18.6%)
5	1 (10%)	10 (34.5%)	13 (41.9%)	9 (75%)	33 (45.8%)	31 (67%)	42 (60.0%)
6	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (8.8%)	14 (20.0%)

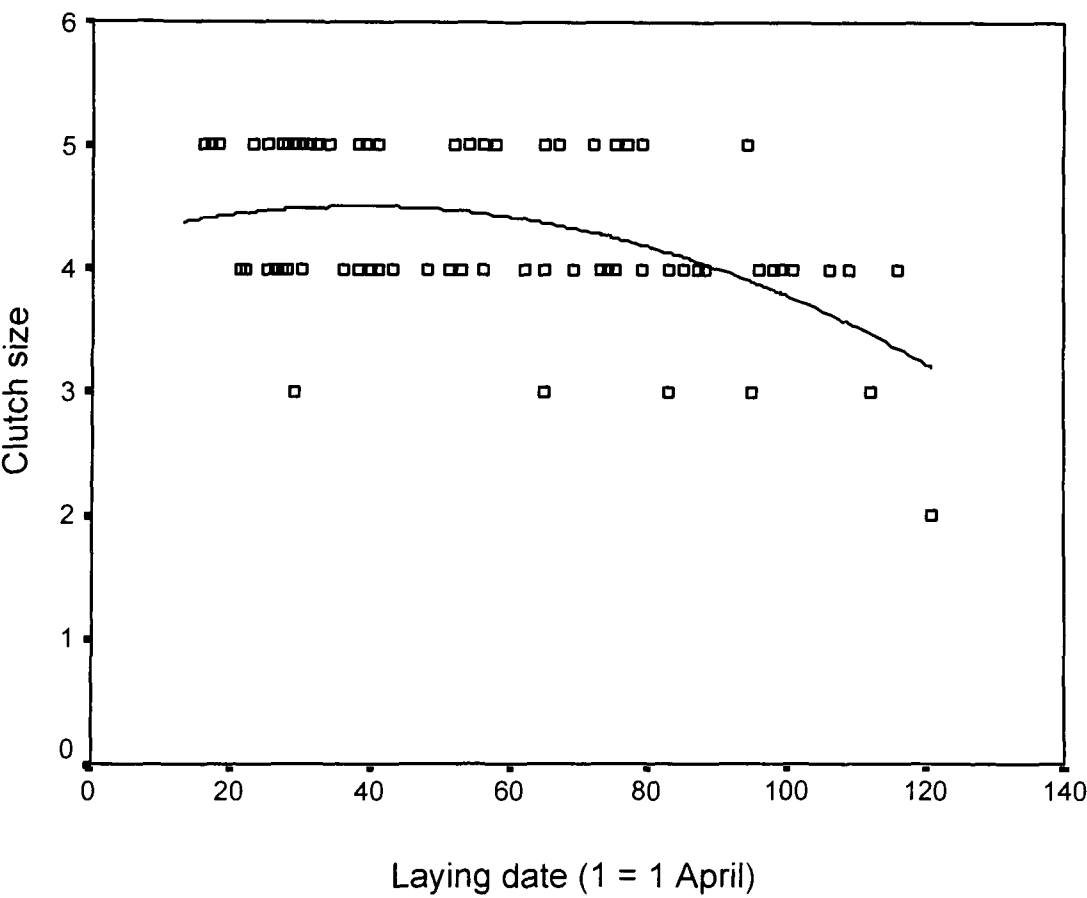


Figure 3.2 – Seasonal variation in clutch size. The line represents the quadratic regression (Clutch size = $0.014913 \text{ (Laying date)} - 0.000192 \text{ (laying date)}^2 + 4.2157$, $R^2 = 0.213$, $F_{[1,78]} = 10.40$, $P < 0.001$). The linear regression would have $R^2 = 0.163$ and $P < 0.001$.

3.3.6 Egg dimensions

A total of 183 eggs belonging to 47 clutches of 32 females was measured (Table 3.5). Both length and width were significantly smaller (width $t_{308} = 2.31$, $P < 0.05$; length $t_{309} = 4.25$, $P < 0.01$) than the corresponding sizes published by Aebischer & Meyer (1998) for Switzerland (length x width: average 19.2 x 14.5, S.D. 1.0 x 0.5, range 17.5-22.0 x 13.0-16.0, n = 127). They also seem to be smaller than the measurements presented by Pikulski (1986) for Poland (average 19.7 x 14.4, range 17.5-22.4 x 13.2-15.9, n = 317).

Table 3.5 – Egg dimensions (in mm and mm³) of Savi’s Warblers breeding in Ria de Aveiro.

	Average	S.E.	Minimum	Maximum	N
Width	14.38	0.029	13.4	15.5	183
Length	18.75	0.058	16.8	21.1	184
Volume	1980.10	12.178	1556.79	2548.57	183

The average dimensions of eggs laid by different females are significantly correlated with the tarsus length of the female, but not with the other body size measurements (Table 3.6, Figure 3.3). In Figure 3.3, the average egg volume and tarsus length for the Swiss population is presented, in addition to the data for the Portuguese population. Its proximity to the regression line strongly suggests that the difference in the size of the females fully explains the difference in egg size between populations.

Table 3.6 – Pearson correlation coefficients between female biometrics and average egg size. Females for which less than three eggs were measured were excluded. Significant correlations are indicated in bold, however, after sequential Bonferroni tests, only tarsus vs egg volume is significant.

		Wing length	Tail length	Tarsus length	Bill length
Average egg length	Pearson corr.	0.366	0.218	0.525	0.192
	Sig. (2-tailed)	0.078	0.317	0.008	0.381
	N	24	23	24	23
Average egg width	Pearson corr.	-0.176	-0.105	0.555	-0.083
	Sig. (2-tailed)	0.411	0.632	0.005	0.708
	N	24	23	24	23
Average egg volume	Pearson corr.	0.093	0.038	0.605	0.044
	Sig. (2-tailed)	0.666	0.864	0.002	0.842
	N	24	23	24	23

The average volume and width of eggs per clutch significantly increased with the date of laying of the first egg whereas egg length almost reached significance (Pearson's correlation: volume vs. date $r = 0.324$, $P = 0.024$, $n = 38$ clutches; width vs. date $r = 0.361$, $P = 0.013$, $n = 38$; length vs. date $r = 0.239$, $P = 0.074$, $n = 38$). Total egg volume per clutch was not correlated with date ($r = -0.170$, $P = 0.154$, $n = 38$) apparently because the number of eggs per clutch decreased during the season. Due to the small samples, it was not possible to compare the egg dimensions between different clutch sizes.

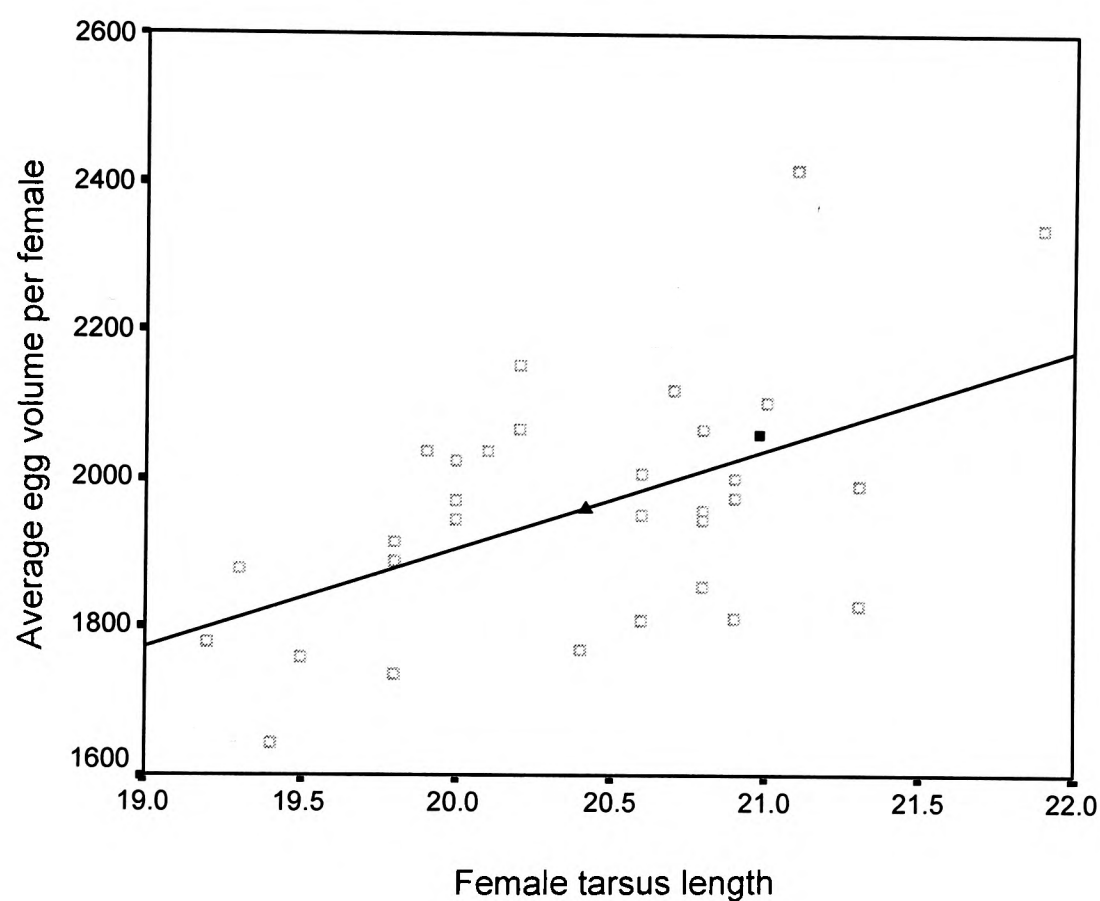


Figure 3.3 – Relationship between female tarsus length and average egg volume per female (Regression analysis: Average egg volume = 134.89 (Female tarsus length) – 792.396, $R^2 = 0.288$, $F_{[1,32]} = 12.97$, $P = 0.001$). Open grey squares are individual females, the black triangle is the average for the Portuguese population and the black square is the average for the Swiss population (Aebischer & Meyer 1998, A. Aebischer, pers. comm.).

3.3.7 Breeding success

The Mayfield estimate of the probability of survival during incubation for the

whole study period was 0.74 (confidence limits: 0.665 – 0.823). The probability of nest survival during the nestling period was 0.57 (confidence limits: 0.503 – 0.635). Considering that the probability that an egg will hatch was 0.95, and that there were five instances of nest loss during the egg laying period, the probability of nest survival was 0.40. No cases of partial loss were detected during the egg stage. One case was detected during the hatching period and five cases during the nestling period. If partial loss is taken into account, the probability that an egg would produce a fledgling was 0.36.

The traditional indices of breeding success are presented in Table 3.7. These were calculated for comparative purposes since they overestimate the breeding success. The Portuguese population seems to have had an intermediate success in relation to the other populations studied. Although fledging success seems to be slightly higher in Portugal, it may have been caused by the fact that in 2003 only the first nests were found. If 2003 is not taken into account, the fledging success becomes remarkably consistent among the populations (Table 3.7).

Table 3.7 – Indices of breeding success for the Portuguese population (this study) and comparison with other populations, namely Switzerland (Aebischer & Meyer (1998), Poland (Pikulski 1986) and East Germany (Dittberner & Dittberner 1991).

Indices of success	2000	2001	2002	2003	Portugal	Switzerland	Germany	Poland
Clutch size	4.0	4.1	4.4	4.6	4.3	4.7	4.8	5.0
Hatching success	83%	84%	84%	89%	84.9%	91.8%	72.4%	73.9%
Fledging success	84%	70%	69%	95%	76.2%	69.9%	69.7%	68.6%
Total breeding success	62%	48%	54%	86%	58.3%	64.1%	50.5%	50.7%
Overall success	2.36	2.0	2.05	3.43	2.31	3.2	1.8	2.5
% of successful nests	73%	52%	60%	81%	62.4%	80.7%	41.2%	56.2%
Number of nests found	11	31	35	16	93	132	46	73

The major cause of total nest loss was predation, being responsible for the loss of 34.4% of all nests and 91.4% of lost nests. In addition, two nests (2.2%) were probably abandoned due to human interference and one (1.1%) was flooded during a spring tide. As mentioned earlier, partial loss, that is, the disappearance of part of the eggs

or nestlings of a nest, was rare and always occurred during hatching or nestling periods. Three out of five cases were caused by starvation, in which the youngest nestling of three five-nestling broods died shortly before fledging even though they were being fed by both parents. The other cases occurred for unknown reasons. In addition, 21 eggs out of the 304 eggs (6.9%) that reached the hatching period failed to hatch. Of these, four had dead embryos whereas the others seemed to be infertile. At least two females were killed by predators when brooding or feeding their nestlings because their remains including the metal ring remained at the nest. It is probable that up to three more females which disappeared rather early in the season were also predated. Their nests were found partially destroyed with many adult feathers nearby.

The habitat type did not influence predation (Chi-square = 2.78, $P > 0.05$, d.f. = 4), clutch size (Chi-square = 6.69, $P > 0.05$, d.f. = 12) or number of fledglings produced (Chi-square = 15.26, $P > 0.05$, d.f. = 16). The occurrence of predation did not vary with the number of eggs at the nest (Chi-square = 2.21, $P > 0.05$, d.f. = 3), but was dependent on the number of nestlings (Chi-square = 10.18, $P = 0.006$, d.f. = 2). In fact, 57% of the nests with three nestlings were predated ($n = 14$), whereas only 16.1% of the nests with four nestlings ($n = 31$) and 15.0% of the nests with five nestlings ($n = 20$) were predated. There was no difference in the laying date of predated and successful nests (ANCOVA: $F_{[1,24]} = 0.8$, $P > 0.05$, with year and number of nestlings as covariates), that is, predation rate was constant through the season.

Considering only the successful nests, there was a significant decline in the number of fledglings per nest in relation to laying date (Spearman rank correlation: $r_{sp} = -0.531$, $P < 0.001$, $n = 55$). This decline was not just caused by the decline in clutch size (Spearman rank correlation: $r_{sp} = -0.364$, $P < 0.007$, $n = 54$, see Figure 3.2), but also by a

decline in the total breeding success (Spearman rank correlation: $r_{sp} = -0.364$, $P = 0.009$, $n = 51$), which is independent of clutch size (Figure 3.4). Both hatching (Spearman rank correlation: $r_{sp} = -0.269$, $P = 0.053$, $n = 52$) and fledging success (Spearman rank correlation: $r_{sp} = -0.380$, $P = 0.008$, $n = 53$) contributed to the decline in total breeding success.

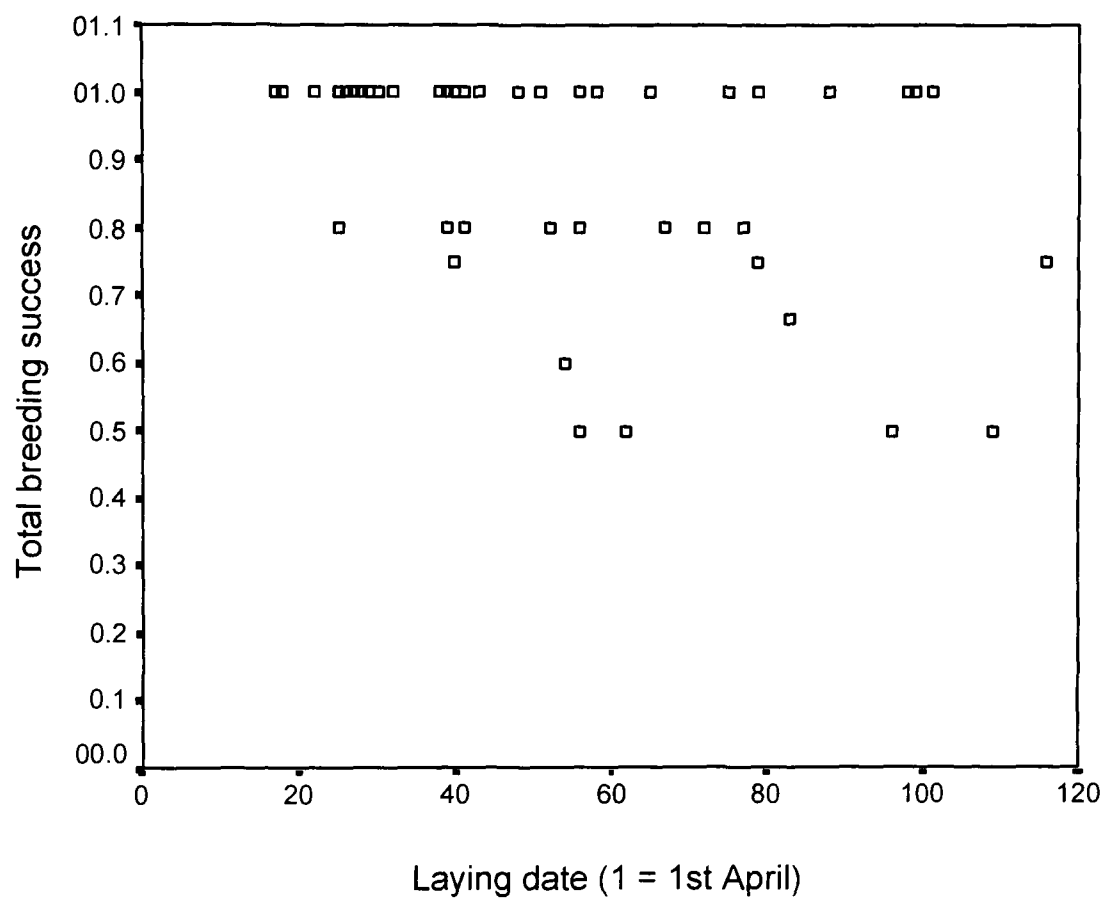


Figure 3.4 – Seasonal variation of the total breeding success (number of fledglings produced per egg) of successful nests.

The average weight of the nestlings per clutch was analysed to determine if the decline in total breeding success was associated with their body condition. The residuals of a multiple regression in which the average weight of nestlings per clutch was the dependent variable and time, brood size and age were independent variables ($\text{Weight} = 0.207(\text{Time}) - 0.409(\text{Brood size}) + 0.501(\text{age}) + 7.918$, $F_{[3,40]} = 10.714$, $P < 0.001$, $R^2 = 0.446$, all variables significant $P < 0.01$), were used as a measure of the average body

condition of the nestlings per clutch. There was no significant relationship between these residuals and date of hatching (Linear regression: $F_{[1,42]} = 0.091$, $P = 0.351$, $R^2 = 0.021$). Therefore, the average body condition of the nestlings was constant throughout the season.

The number of nesting attempts per female per season was 2.56 ± 0.18 nests (range 1-4, $n = 16$) and the seasonal breeding success was 5.38 ± 0.89 nestlings (range 0-11, $n = 16$). If the predated females are excluded, the number of nesting attempts becomes 2.71 ± 0.16 (range 2-4, $n = 14$), and the average seasonal breeding success 6.14 ± 0.82 (range 0-11, $n = 14$ females).

3.4 Discussion

Male Savi's Warblers benefit greatly from arriving early by choosing the best territories (Aebischer *et al.* 1996), attracting more mates and having higher breeding success (Aebischer *et al.* 1996, this study). Philopatric males generally occupied the same territory, except if the vegetation was cut, and even evicted males that arrived earlier (pers. obs.), suggesting that occasionally early-arriving males may not get the best territories. In this study, there was no difference in the arrival date between unringed and ringed (returning) males. Furthermore, early-arriving males tended to have smaller tarsus and bill lengths independently of their fidelity to the study area, suggesting that smaller birds invested more in arriving early due to their low resource holding power (see Forstmeier 2002). Aebischer *et al.* (1996) found the opposite trend between male arrival date and tarsus length in Savi's Warbler, which raises doubts about the validity of these relationships. Early-arriving males also tended to have more fat, which suggests that the body condition of these males, and perhaps their quality, was better. However, this must

be interpreted with caution, as the body condition could have changed between the arrival date and time of capture, which was variable between males, and the greater fat of early-arriving males might be associated with an effect of starvation risk early in the season rather than male quality.

In contrast to the results presented by Takaki *et al.* (2001), the average growth bar width of the 3rd tail feather, which may be a good measure of body condition during the previous post-breeding moult in adults, was not correlated with arrival date. A possible reason for this may be that some, but not all, adults replace the tail feathers in the winter quarters (see §Chapter 6). Therefore, the width of the growth bars may not be comparable across all individuals.

In total, 120 different males and 62 females were captured in the study area. Although the sex ratio of the adult population seems to be biased towards males, it may be overestimated because males are more conspicuous and easier to capture than females and tend to move more (pers. obs.). This is particularly true of the unmated males. It was obvious, however, that the number of males exceeded the number of females and a few remained unmated throughout the breeding season. One of the reasons for the biased sex ratio of the adult population might be the higher mortality rate of females during the breeding season. At least two to five females out of 62 may have been predated at the nest whereas no male mortality was detected. It is not clear if the return rates of males are higher than those of females because in 2003 the percentage of retraps of males decreased substantially whereas females went in the opposite direction reaching similar values to males. Moreover, differences in return rate may be caused by differences in dispersal rather than in mortality.

Despite the biased sex ratio, three instances of polygyny were detected in 2001

and one in 2002, that is, in 2001 17.6% of known mated males were polygynous and 15% of the females had secondary status, whereas the equivalent numbers for 2002 are 5.5% of males and 5.3% of females. However, some secondary females may have been overlooked, since they are the most difficult to detect, therefore the frequency of polygyny was probably underestimated. The existence of polygyny despite the availability of unmated males, and the pattern of arrival in which the majority of the population arrive within a fairly short interval followed by a much later floating fraction (see Kokko 1999), suggests that at least the higher quality habitat is saturated. In fact, the long time interval between male arrival and laying (about 5 weeks) suggests a very strong competition for high quality territories.

The breeding season in Savi's Warblers is very protracted in Portugal as it is in other parts of its distribution (Aebischer & Meyer 1998, Pikulsky 1986). This has also been reported for Grasshopper Warblers, *Locustella naevia* (Glue 1990) and seems to be a feature of many, if not all, *Locustella* warblers, apparently resulting from an early start rather than a late end to the breeding season. Early breeding females are able to produce three successful clutches even though this rarely happens due to the high predation rate. In this study, only one such case was detected, but in Switzerland, where the predation rate was lower, three out of 15 females successfully reared three broods (Aebischer *et al.* 1996).

The early start of breeding of Savi's Warblers in comparison to other species that use the same habitat (for instance, Reed and Great Reed Warblers, *Acrocephalus scirpaceus* and *A. arundinaceus*, arrive and start breeding three to four weeks later in the same site; Neto 1993, pers. obs.) agrees with the seasonal variation in clutch size. In fact, although the sample size is small, Savi's Warblers seem to start breeding before the date

when their optimal clutch size is greatest. This is typical of resident multi-brooded species, whereas migrants usually start laying later than residents do with respect to the peak clutch size (Crick *et al.* 1993).

Egg size is smaller than in the other populations studied. It was shown that the tarsus length of the females strongly influences the size of the eggs, and so differences in egg size between populations could have resulted from differences in the size of the females. Measurements of tarsus length and egg size from Switzerland seem to confirm this (Figure 3.3). The average egg size per clutch increased with laying date, but it is not clear whether this resulted from better feeding conditions later in the season or from a trade-off between egg and clutch size, which decreased during the season.

The Mayfield estimate of nest success (40%) seems to be lower than in other open-nesting passerines. However, it is possible that the actual percentage of nest loss is lower than the one reported because the frequent visits to the nests may have made them more prone to predation. If the probability of predation varies between sites and years, the influence of human interference may increase the difference in breeding success estimates. That is, human interference may be more important in sites where the probability of predation is high, underestimating the productivity at those sites.

The four populations of Savi's Warblers for which breeding success estimates were presented (Table 3.7) seem to differ in two main aspects: clutch size and percentage of successful nests. The clutch size is smaller in Portugal than in the other countries and may follow a latitudinal gradient although the number of populations studied is too small to confirm this. The percentage of successful nests seems to be a good measurement of the predation rate, which is the main cause of nest loss in all sites except in Switzerland, where the predation rate is much lower than in the other populations and the main cause

of nest loss was flooding (Aebischer *et al.* 1998). Interestingly, the difference in predation rate between populations seems to occur at the egg stage only, because, in contrast to the fledging success, which is very similar in all populations, the hatching success is quite variable and consistent with the percentage of successful nests. As the percentage of unhatched eggs is quite low and seems to be similar between populations (Pikulski (1986) reports an average hatchability of 95.4%), the difference in hatching success may be attributed to predation only. The reasons for this are not clear, but may be related to differences in abundance of egg predators between the sites.

The predation rate was constant during the season, which is consistent with Aebischer *et al.* (1996), who did not find differences in predation rate between first and second broods. However, the breeding success of successful nests declines during the season independently of clutch size. This seems to be associated with a decline in hatching success and an increase in partial loss. Three instances of partial loss were caused by starvation, therefore variation in food availability could potentially explain the trend. However, the body condition of the nestlings did not decline with season, presumably because of the decline in clutch and brood size which were negatively correlated with the average body condition of nestlings per clutch. The increase in infertility or hatching failure could be due to a different investment in breeding effort between the sexes or individuals of a pair later in the season.

An important breeding success measurement that is difficult to obtain in multi-brooded species is the total breeding success per season. This is particularly important in species which have a long breeding season because the productivity of the population is seriously underestimated. In this study, despite the difficulty in finding all the nests, it was shown that the number of fledglings produced by each female during one breeding

season was at least 5.38. This exceeds the productivity necessary to balance adult and juvenile mortalities of 60 and 70%, respectively, (4.1 fledglings, Aebisher & Meyer 1998) suggesting that this is a source population. This means that the population size is not constrained by its productivity, and is more likely to depend on habitat availability, which seems to be saturated (see above), and/or on the higher mortality of females during both the breeding and non-breeding season.

CHAPTER 4

Nest-site selection and predation in Savi's Warblers, *Locustella luscinioides*

Comparisons were made between habitat characteristics measured at nest-sites and random points and between predated and successful nests. Savi's Warblers preferred sites dominated by high and dense *Juncus* and *Rubus* with thick litter and avoided pure reeds. The significant habitat features entered in a stepwise logistic regression using nest-sites vs. random points as the dependent variable were: total vegetation cover, litter thickness, habitat type, undergrowth height and undergrowth cover, which were higher or denser at nest-sites. It is suggested that the use of perennial plants for nesting by Savi's Warblers might explain their earlier arrival to the breeding quarters, in comparison with *Acrocephalus* species, which nest in reeds. Litter thickness was the only variable distinguishing predated and successful nests; predation was higher in areas with less dense litter. Although this species feed in pure reed habitat, it is dependent on the presence of dense low vegetation and litter for nesting, which should be maintained close to or within reedbeds for its benefit.

4.1 Introduction

Habitat selection by Savi's Warblers, *Locustella luscinioides*, during the breeding season was studied by Aebischer & Antoniazza (1995), Aebischer *et al.* (1996), Catry (1997), van der Hut (1986), Moskát *et al.* (1992) and Rolando & Palestini (1989), who

described the characteristics of the territories or the sites where individual birds were detected. In addition, nest-site descriptions were provided by Aebischer & Meyer (1998), Dirkx (1939), Dittberner & Dittberner (1985), Mayaud (1930), Mildenberger (1958), Moreau (1982), Pikulski (1986), Schiermann (1928) and Suchantke & Schwarz (1961) in studies or simple observations of Savi's Warbler breeding biology. However, with the exception of Aebischer & Meyer (1998) who state that straw litter was more extensive at nest-sites than elsewhere in the territories, no comparisons were made with the available habitat at their study sites.

Savi's Warblers occur in large wet reedbeds and are generally considered a reedbed species (Cramp 1992). Bibby & Lunn (1982) found a positive association between the occurrence of Savi's Warblers and size of the reedbed, presence of water and of mixed herbaceous fen vegetation. Catry (1997), however, found higher densities of Savi's Warblers in *Scirpus maritimus* than in pure reedbeds, which seem to be used mostly by unmated males (Pikulsky 1986, pers. observ.). According to van der Hut (1986), its territories are located in wet areas with a dense ground layer and medium vegetation height. The physiognomy of the habitat and in particular the presence of dense vegetation, broken stems and leaves near the ground as well as of water seem to be important for this species (Aebischer & Antoniazza 1995, Aebischer *et al.* 1996, van der Hut 1986, Pikulsky 1986). Aebischer *et al.* (1996) stated that the availability of nest-sites rather than food availability appeared to be the important choice criterion for territories. Savi's Warbler nests are supported by a variety of low dense vegetation, for instance, *Carex* sp., *Scirpus* sp., *Cladium mariscus*, *Juncus* sp., and occasionally are built on broken reed stems, *Phragmites australis*, and *Typha* sp., but always next to or within large reedbeds (Aebischer & Meyer 1998, Dirkx 1939, Dittberner & Dittberner 1985, Mayaud 1930,

Mildenberger 1958, Moreau 1982, Schiermann 1928, Suchantke & Schwarz 1961).

Nest-site selection is an important aspect of the breeding biology particularly for open-cup and ground nesting passerines, and is important for conservation and habitat management. This study aims to describe the habitat characteristics of the nest-sites of Savi's Warblers found in Ria de Aveiro, Portugal, to compare them with random points, and to determine which features of the habitat and nests are important for predation and breeding success in this species.

4.2 Material and methods

The study was conducted at Salreu (40°43'N, 08°35'W), Ria de Aveiro, a large coastal lagoon located in west Portugal. The whole area was occupied by rice fields but most of these have been abandoned in recent decades and colonized by marsh plants. At present, there is a mosaic of reeds (*Phragmites australis*), rushes (*Juncus* sp.), reed maces (*Typha latifolia*), club-rushes (*Scirpus maritimus*), all of which can be dominant in particular sites, and scattered willows (mainly *Salix atrocinerea*) and alders (*Alnus glutinosa*). The reeds, and other plants, are commonly cut for animal bedding by the local community, mainly from July to October, although in some areas they are cut earlier in the year. A dyke separates the marshes from the lagoon, allowing the control of the water level and preventing the entrance of saltwater. The water level depends on human activities in the surrounding rice fields. During March and April and from mid-August until October, the water level is lowered in order to plough the rice fields and harvest the rice. In May, fresh water from the River Antuã is poured into the rice fields and after October the level rises again through rainfall (Neto 2003).

A study site was selected within the Salreu marshlands. In 2000, an area of 21.7

ha located immediately north of the Salreu Canal was surveyed. In order to increase the sample size, this area was extended to the southern part of the canal in May 2001, then becoming 31.8 ha. This new area is somewhat influenced by the tides because the dyke cannot completely prevent the entrance of salt water. Here, the proportion of rushes is higher and the reeds only occur in the deeper parts, usually along the ditches.

Savi's Warblers arrive in the first week of March and lay their first eggs in mid-April. The breeding season is very long; the last nests are active until the last week of August (see §Chapter 3). During this period, the vegetation structure changes dramatically due to the growth of the aerial part of the reeds and some other annual species. Seasonal changes in habitat are known to affect nest-site location in Savi's Warblers (Dittberner & Dittberner 1985) at least in some circumstances, so that this variation had to be taken into account.

Nests were searched for during the breeding seasons of 2000 to 2003 by observing the behaviour of adults. Nest predation was identified either by the disappearance of the whole clutch, in which cases the nest was frequently left intact, or of nestlings, in which cases remains of feathers and nest damage were common. Predation caused the total loss of 33 out of the 91 nests (36.3%, pers. observ.). Only three other nests failed completely: two because the female abandoned the nest or was predated, since it was not seen subsequently, and the other because the nest was flooded during a spring tide. Therefore, predation is the major cause of total nest failure at this site (pers. obs.).

Several habitat features were measured within a 1 m radius of the nests as soon as they were abandoned or predated. In 2002, 100 random points were selected by using a table of random numbers to determine the latitude and longitude, and located within the study area with a GPS. The habitat features were measured at the random points from

April to August 2002 to take into account seasonal variation.

The habitat features measured were: total vegetation cover, water depth, maximum height and cover of the upper layer of the vegetation (i.e. *Typha* and *Phragmites*; referred to as “uppergrowth”), maximum height and cover of the undergrowth (i.e. *Juncus*, *Scirpus* and *Rubus*) and litter thickness, that is the maximum height of broken dead vegetation. The cover was estimated visually in classes of 10%. I measured the maximum height of the stems of *Phragmites* and *Typha* grown up in the previous year (dead) rather than the green ones to account for the seasonal variation. In addition, the habitat type was classified as follows (from deep water to dry areas): (0) open water, (1) *Scirpus maritimus* beds with less than 10% cover of *Typha latifolia*, (2) mixture of *Scirpus maritimus* and *Typha latifolia*, (3) pure *Typha latifolia* beds, (4) mixture of *Typha* and *Phragmites*, occasionally with some *Scirpus maritimus*, (5) pure *Phragmites* beds (with less than 10% cover of *Typha latifolia*, but occasionally with *Iris pseudacorus*), (6) mixture of *Juncus* and *Phragmites*, largely dominated by *Juncus* but with more than 10% cover of *Phragmites*, (7) *Juncus* beds, locally co-dominant with some *Graminae* (mainly *Spartina versicolor* and *Agrostis stolonifera*), where *Lysimachia*, *Calistegia*, *Hydrocotyle* and *Vicia* were frequently found, and (8) *Rubus* sp. frequently with some *Juncus* and occasionally *Phragmites*.

The nest measurements taken were: inner and outer diameter, outer depth, depth of the cup and nest height, i.e., the vertical distance from the upper rim of the nest to the ground, from which bottom thickness and wall thickness were calculated. The effect of date (day from 1 April), clutch size and distance to the nearest tree or bush on predation was also determined.

The fact that at random points the measurements were only taken during 2002

does not seem to influence the results because the annual variation in vegetation structure is very limited. In six years of study at this site, changes in vegetation were only detected at the fringes of open water, where *Typha* and *Scirpus* colonised about five metres of the latter habitat, and in areas where the vegetation was cut for bedding (pers. obs.). The cut area differed little between years. Furthermore, the vast majority of the nests were found in 2001, 2002 and 2003 (31, 35 and 16 respectively); only 11 were found in 2000.

Pseudoreplication could be a problem since up to four nests of each pair were found in the same year, and although the return rate is low, there are three cases of re-nesting by the same female in subsequent years. However, because the distance between the nests of any given pair in a season varied substantially (from five to 70 m, pers. obs.) and all available nesting habitat was occupied, the nesting attempts were considered independent. Moreover, female identity was not found to be a significant factor when entered into the predation statistical model and the nest-site selection analysis produced similar results when only one nest per female was used.

The discrete and continuous variables were compared between nest-sites and random points by using Chi-square and Mann-Whitney U tests, respectively (Sokal & Rohlf 1995). Sequential Bonferroni tests were applied to tables of statistical comparisons (Rice 1989). A stepwise logistic regression analysis was used to identify the most important features that distinguish nests-sites from random points. The log-likelihood of the final model was compared with the log-likelihood of the null model, that is, the model that only included the constant, with a Chi-square test. The same statistical procedure was carried out for predated and unpredated nests in order to identify which habitat features are important for breeding success. All analyses were performed with the statistical package SPSS 10.0.

4.3 Results

In total, 78 nests were found from 2000 to 2002. With the exception of two nests, these were built with old reed leaves. In the two exceptions old *Typha* leaves were used, apparently because of its availability at their site, which was next to old *Typha latifolia* stems. As described by other authors, the nests had no lining, and were invariably supported by dense vegetation; simply resting on the vegetation rather than attached to it and hanging like a Reed Warbler's (*Acrocephalus scirpaceus*) nest. Most nests were located very near the ground or water surface. There were two exceptions, which were supported by tall *Rubus* sp. and were located near its top, just below the leaves.

Most nests were found in *Juncus*, frequently mixed with *Phragmites* in relatively high and dry areas (Table 4.1). Savi's Warblers had a clear preference for *Juncus*-dominated habitats particularly those with reeds and *Rubus* sp., but nests also occurred in *Scirpus* mixed with *Typha* (Table 4.1). However, pure reedbeds were avoided, and the only nests found in this habitat were located in dense tufts of broken stems that grew up in the previous year. Areas that were cut in the two previous years either with rushes or reeds were neither used for nesting nor for feeding (pers. obs., see also Neto 1998).

Table 4.1 - Comparison between the habitat types of Savi's Warbler nest-sites (n = 77) and random points (n = 100). The difference in the frequencies of habitats between nest-sites and random points is highly significant (Chi-square = 84.621, P < 0.001, d.f. = 8).

Habitat type	Random points (n = 100)	Nest sites (n = 92)
Open water	7 (7%)	0 (0%)
Scirpus	2 (2%)	0 (0%)
Scirpus & Typha	4 (4%)	2 (2.6%)
Typha	2 (2%)	0 (0%)
Phragmites & Typha	3 (3%)	0 (0%)
Phragmites	44 (44%)	2 (2.6%)
Juncus & Phragmites	7 (7%)	42 (54.5%)
Juncus	29 (29%)	35 (45.5%)
Rubus	2 (2%)	11 (14.3%)

The habitat measurements taken from nest-sites and random points are presented in Table 4.2. Dense and high undergrowth with thick litter was clearly selected for nesting whereas the uppergrowth height and density as well as the water depth were not important. Nests occurred significantly more often in the absence of water, in the absence of uppergrowth and in the presence of undergrowth (Table 4.2). This might be a consequence of their preference for rushes, which occur primarily at higher, dry sites whereas reeds and *Typha* occur primarily at wetter sites.

Table 4.2 - Descriptive statistics of the habitat measurements (mean $\pm$ s.e., range; n) taken from nest-sites and random points and comparison with Mann-Whitney U test for continuous variables and Chi-Square for discrete variables. NS-non-significant, *P < 0.05, ** P < 0.01, ***P < 0.001 Uppergrowth presence and water depth are not significant after sequential Bonferroni tests.

Habitat features	Nest sites (n = 77)	Random points (n = 100)	Statistics
Water depth (cm)	3.34 $\pm$ 0.70 (0-30; 90)	4.84 $\pm$ 0.90 (0-60; 93)	2949.0*
Water presence ¹	26 out of 90 (28.9%)	54 out of 100 (54%)	12.25***
Total vegetation cover (%)	83.33 $\pm$ 2.01 (30-100; 84)	38.97 $\pm$ 2.77 (0-100; 87)	653.0***
Uppergrowth height (cm)	159.49 $\pm$ 9.17 (0-300; 90)	137.15 $\pm$ 10.92 (0-332; 89)	3130.0 ^{NS}
Uppergrowth cover (%)	14.40 $\pm$ 1.33 (0-50; 91)	17.64 $\pm$ 1.64 (0-50; 89)	3073.0 ^{NS}
Undergrowth height (cm)	104.54 $\pm$ 3.02 (0-160; 90)	42.76 $\pm$ 4.89 (0-145; 87)	895.0***
Undergrowth cover (%)	74.95 $\pm$ 2.56 (0-100; 91)	22.33 $\pm$ 3.29 (0-100; 86)	858.5***
Litter thickness (cm)	51.89 $\pm$ 2.68 (0-90; 75)	11.69 $\pm$ 1.87 (0-60; 86)	671.0***
Uppergrowth presence ¹	38 out of 92 (41.3%)	57 out of 99 (57.6%)	5.05*
Undergrowth presence ¹	89 out of 92 (96.7%)	50 out of 100 (50%)	52.37***

¹-Chi-Square with continuity correction

A stepwise logistic regression was performed with habitat type and all continuous variables presented in Table 4.2 to determine which are the most important factors that distinguish nest-sites from random points. Water, uppergrowth and undergrowth presence were not used to avoid multicollinearity. The variables that significantly improved the null model were, in order of entering: total vegetation cover, litter thickness, habitat type, undergrowth height and undergrowth cover (Chi-square = 153.572, P < 0.001, d.f. = 11). The model correctly classified 92.8% of the cases.

The analysis was also performed considering only one nest per female (n = 59 nests). The results of the Mann-Whitney and Chi-square comparison were qualitatively similar with the exception of water depth that was not significantly different between nest-sites and random points. The only variables that significantly improved the stepwise logistic model were litter thickness, total vegetation cover and habitat (Chi-square = 112.203, P < 0.001, d.f. = 9), correctly classifying 93.5% of the cases.

Table 4.3 - Comparison between the habitat and nest characteristics of predated and unpredated nests. Data are presented as follows: mean ± s.e. (range; n). Continuous variables were compared with the Mann-Whitney U test whereas discrete variables were compared with Chi-square test. Date of laying is number of days from 1 April. NS - non-significant, ** - P < 0.01. The statistical comparisons remain the same after sequential Bonferroni tests.

Habitat and nest features	Successful nests (n = 58)	Predated nests (n = 33)	Statistics
Date of laying (1 March = 1)	56.5 ± 3.80 (13-131; 58)	56.3 ± 5.52 (16-130; 32)	907.5 ^{NS}
Clutch size	4.35 ± 0.09 (2-5; 51)	4.29 ± 0.12 (3-5; 28)	674.0 ^{NS}
Outer diameter (cm)	11.8 ± 0.21 (9-16; 45)	11.8 ± 0.26 89-14; 28)	615.0 ^{NS}
Inner diameter (cm)	7.2 ± 0.11 (5.5-9.0; 45)	6.6 ± 0.14 (5-8; 28)	376.5**
Wall thickness (cm)	4.6 ± 0.19 (3-8; 45)	5.1 ± 0.23 (3.5-8; 28)	470.5 ^{NS}
Outer depth (cm)	10.7 ± 0.24 (7.5-14; 45)	10.6 ± 0.34 (7-15; 28)	601.0 ^{NS}
Depth of the cup (cm)	6.6 ± 0.13 (4.5-8.5; 45)	6.4 ± 0.14 (5-8; 28)	568.5 ^{NS}
Bottom thickness (cm)	4.19 ± 0.22 (1.5-7; 45)	4.2 ± 0.37 (1-9; 27)	592.0 ^{NS}
Nest height (cm)	38.3 ± 2.65 (14-145; 56)	34.6 ± 2.66 (14-95; 33)	825.0 ^{NS}
Water depth (cm)	3.27 ± 0.94 (0-30; 56)	2.9 ± 0.93 (0-20; 33)	868.0 ^{NS}
Water presence ¹	14 out of 56	11 out of 33	0.714 ^{NS}
Total vegetation cover (%)	91.3 ± 2.31 (40-100; 31)	85.3 ± 4.38 (30-100; 17)	202.0 ^{NS}
Uppergrowth height (cm)	163.4 ± 11.37 (0-291; 56)	149.2 ± 15.59 (0-300; 33)	808.0 ^{NS}
Uppergrowth cover (%)	15.54 ± 2.15 (0-80; 56)	15.3 ± 2.98 (0-80; 32)	865.0 ^{NS}
Undergrowth height (cm)	104.5 ± 4.40 (0-160; 56)	103.5 ± 3.33 (74-160; 33)	757.0 ^{NS}
Undergrowth cover (%)	76.4 ± 3.15 (0-100; 56)	72.4 ± 4.56 (10-100; 33)	865.0 ^{NS}
Litter thickness (cm)	61.83 ± 2.30 (30-90; 41)	45.43 ± 4.37 (0-90; 28)	325.5**
Distance to the nearest Tree (m)	42.5 ± 8.64 (1-200; 42)	31.1 ± 8.94 (1-180; 30)	553.0 ^{NS}

1-Chi-square with continuity correction

The comparison between the date of laying of the first egg, clutch size and the habitat and nest features of predated and unpredated nests is presented in Table 4.3. The difference in litter thickness between predated and successful nests is highly significant, the latter having thicker litter. In addition, the inner diameter of the nests is significantly

larger in predated nests; this may have resulted from damage of the nest caused by the predation itself. There was no difference in habitat type between predated and successful nests (Chi-square = 4.086, $P = 0.395$, d.f. = 4).

A stepwise logistic regression was performed using all variables presented in Table 4.3 in order to determine which are important in distinguishing predated from successful nests. Only litter thickness significantly improved the null model (Chi-square = 5.07, $P = 0.024$, d.f. = 1) classifying 68.2% of the cases correctly.

4.4 Discussion

This study shows that although Savi's Warblers have a general preference for large reedbeds, they require a different habitat for nesting. The structure of the nests and the way they are attached to the vegetation seem to constrain its construction to places in dense vegetation with rather stiff stems. In Salreu, Savi's Warblers showed an obvious preference for *Juncus*-dominated habitats particularly with reeds and *Rubus*, but they also nested in *Scirpus* mixed with *Typha*, avoiding pure reeds. Nests in *Scirpus* and *Typha* might have been underestimated because the nests were more difficult to find in low, deep areas due to the generally higher vegetation and consequent lower visibility. Besides habitat type, the most important features of the vegetation structure chosen by Savi's Warblers were: total vegetation cover, undergrowth height and cover and litter thickness. Most interesting is the fact that the uppergrowth was not selected for nesting although it may be important for other aspects of its biology, namely feeding (see below). *Juncus* are perennial plants with quite strong stems, especially the species that occur in the study area (*J. maritimus* and *J. effusus*), whereas *Scirpus maritimus* are annual plants, and are less dense and stiff than *Juncus*, which could explain their being used less for nesting.

The nesting habitat used by Savi's Warblers might have important consequences for their breeding biology. Schulze-Hagen *et al.* (1996) suggested that Marsh Warblers, *Acrocephalus palustris*, arrive later than Reed Warblers and have a shorter breeding season because they use an ephemeral nesting habitat that is only available later in the season. The same reasoning can be used to explain the difference in arrival dates between Savi's and Reed Warblers at Salreu. Savi's Warblers arrive in early March, about three weeks before Reed Warblers (Neto 2003). This could be due to differences in nest-site availability because Savi's Warblers mainly use perennial plants (*Juncus*, *Rubus*) available all year, whereas Reed Warblers are very dependent on reeds, which grow rather late (from the middle of spring to summer). However, the true effect of nesting habitat on the start and length of the breeding season can only be assessed by use of a comparative analysis.

Only litter thickness was important in distinguishing predated from successful nests. This is hardly surprising since the variety of potential predators is very high, and they presumably approach the nest in different ways. The most important predators seem to be Brown Rats, *Rattus norvegicus*, Southern Water Voles, *Arvicola sapidus*, Water Rails, *Rallus aquaticus*, and Marsh Harriers, *Circus aeruginosus*, all of which are common in the study area. Others could also predate some nests, namely: Little Bitterns, *Ixobrychus minutus*, Foxes, *Vulpes vulpes*, and even Moorhens, *Galinilla chloropus*. Nests located in sites with thick litter may be better protected from predators because the habitat structure is particularly dense at these sites, presumably limiting predator access to the nest.

Although water presence has been described as an important feature of Savi's Warbler territories (van der Hut 1986), no relationship was found with nest-site selection

or predation. Pikulsky (1986) states that lower nests and nests built over shallower water were predated more frequently; but no such relation was found in Salreu. It is possible that water is important for nesting, and the fact that in this study area the majority of potential nest-sites are located in relatively high and dry areas prevented this from being detected. However, the most likely predators do not seem to be constrained by water, being good swimmers or fliers, and the predators themselves might be different from those in Pikulsky's study area. Marsh Harriers almost certainly were responsible for the loss of at least two nests that were located very high in the vegetation, thus, the distance from the nest to the top of the vegetation or the cover of the vegetation over the nest might also be important.

In this study area, Savi's Warblers were seen feeding in wet reedbeds, which were located from zero to 35 m from the nests (pers. obs.), even if they were nesting in pure *Juncus* or in any other habitat. It was not possible to determine whether the distance to the reedbed plays a role in nest-site selection because at this study site, the rush beds are quite small and always near reedbeds. However, in other parts of Ria de Aveiro, large rush beds do exist, but no Savi's Warblers were seen in such areas; they were apparently confined to the edge of large reedbeds (pers. obs.). The presence of water as well as reeds in or near Savi's Warbler territories may be important for feeding rather than for nesting ecology. In fact, this species is more prone to hop and cling than other *Locustella* species, a behaviour which has been interpreted as an adaptation to the use of reedbeds (Leisler 1977, Winkler & Leisler 1985). Due to the cryptic behaviour of this species, a radio-tracking study is needed to assess the importance of reedbeds for feeding.

The facts that Savi's Warblers only occur in places with both pure reedbeds and marsh fens, and that they are very dependent on the amount of litter and on the presence

of old vegetation, have important implications for habitat management and conservation. The cutting of the vegetation reduces the area available for nesting and feeding for at least two years (pers. obs., see also Neto 1998). The presence of dense low vegetation scattered throughout reedbeds needs to be taken into account when restoring marshlands where Savi's Warblers are expected to breed. The type of undergrowth, though, is not important since they nest in a large variety of vegetation types. In Salreu, flooding is not an important cause of nest loss because the water level is strictly controlled for the production of rice. However, they nest very low, in a narrow belt between water level and just below the undergrowth cover, which in areas without control of water level may cause severe losses (Aebischer & Antoniazza 1995).

CHAPTER 5

Post-juvenile and post-breeding moult of Savi's Warblers in
Portugal

In order to clarify the complex moult strategy of Savi's Warblers, the post-juvenile and post-breeding moult phenology, sequence and extent are described. The post-juvenile moult overlapped considerably with the 'filling moult', and occurred in at least 44% of the birds. Although at least 5% of the juveniles moulted some or all tertials and greater coverts, later-moulting birds seem to have had no post-juvenile moult in the breeding quarters. Data from retraps showed that the post-breeding moult took on average 59 days to complete. From July onwards, all males overlapped breeding and moult whereas only 11% of the females did so. The start of moult varied from 6 June to 25 August, and was significantly earlier in males. Only 21% of the birds completed the moult whereas the remaining individuals retained a variable number of inner primaries and/or secondaries. The number of retained primaries and the primary number in which the moult started were positively associated with the date of moult. This adaptation allows the replacement of the most important feathers for flight when the time available for moult is short. Body condition did not vary with the progress of moult when date was taken into account, but fat still tended to decrease and then increase. The body condition was positively related with the wing raggedness, and so Savi's Warblers do not compensate for an increasing wing load during moult.

5.1 Introduction

The vast majority of birds, and in particular the passerines, replace (moult) their entire plumage at least once a year. This allows them to adjust the plumage characteristics to the changing needs during their lifetime, namely regarding thermal insulation, flight, visual communication and camouflage (Jenni & Winkler 1994). Moulting, however, is costly in terms of time and energy because of the production of new feathers, increased thermoregulatory cost caused by impaired feather insulation and increased flight cost due to the reduced wing area (Ginn & Melville 1983, Klaassen 1995, Lindström *et al.* 1993, Pennycuik 1975, Swaddle & Witter 1997). Consequently, breeding, moulting (especially the moulting of flight feathers) and migration, the most nutrient-demanding periods of a bird's annual cycle, generally do not overlap in time (Hemborg 1999, Herremans 1990, Jenni & Winkler 1994).

In most passerines, the annual complete moulting often takes place shortly after the breeding season, particularly in resident birds, but this is not always the case for migratory species (Jenni & Winkler 1994). Migratory species have a large variety of moulting strategies that cope in different ways with breeding and migration. These strategies include complete winter moulting, suspended or arrested moulting and split-moulting (reviewed by Jenni & Winkler 1994). Moulting seems to be the most plastic of the three nutrient-demanding periods, both at individual and species level. Due to this variability and the lack of information about the complementarities of the different moultings that may take place during the annual cycle (particularly in migratory birds which are rarely retrapped between breeding and wintering quarters), a natural classification of moulting types is still lacking (but see Jenni & Winkler 1994), and hence, little is known about the evolution of moulting strategies (Hall 2003, Svensson & Hedenström 1999). Furthermore, very little is

known about the factors that influence the variability in moult phenology and extent. Studies involving several aspects of bird ecology are needed to understand the control of moult (eg. Bensch *et al.* 1985, Bojarinova *et al.* 1999).

One of the most interesting passerine species with regard to its moult is the Savi's Warbler, *Locustella luscinioides*. It has been shown that this long-distance migrant has a very variable moult strategy and an almost unique moult sequence (Márton *et al.* 2000, Mead & Wartmough 1976, Müller 1981, Steiner 1970, Stresemann & Stresemann 1970, Thomas 1977, Trias *et al.* 1982), although the sample sizes were rather small in these studies.

According to Heinroth & Heinroth (1924-26 cited by Cramp 1992) and Müller (1981), Savi's Warblers do not undertake a post-juvenile moult, but Trias *et al.* (1982) captured one juvenile Savi's Warbler in Spain, which was moulting the tertials. At least some adult Savi's Warblers have a complete or an interrupted moult before the autumn migration in Europe, or at a stopover site (Galea 2002), in which the primaries are often moulted divergently from the middle of the tract (Mead & Wartmough 1976, Steiner 1970, Thomas 1977, Trias *et al.* 1982) or in the normal sequence, i.e., descendant from the inner primary (Márton *et al.* 2000, Stresemann & Stresemann 1970). The winter moult seems to be as variable as the summer moult in terms of both sequence and extent (Aidley & Wilkinson 1987, Bensch *et al.* 1991, Nikolaus & Pearson 1991), but the age of the individuals studied in the winter quarters is unknown except for one or two birds reported in Cramp (1992).

Savi's Warblers are able to live flightless in late summer in reedbeds, and so can afford considerable individual variability in the speed of moult in various parts of the wing, asymmetry in the pattern of moult and the renewal of a large number of wing

feathers at the same time (Steiner 1970). Most of the body feathers, up to seven wing feathers and the whole tail grow simultaneously during the period of flightlessness. In addition, the post-breeding moult starts shortly after the last brood hatches, when the growing juveniles are still being fed (Steiner 1970). Mead & Watmough (1976) considered that the complexity and variability of this species, with adults migrating with old, new and partly new primaries, was astonishing. They thought that this could illustrate the ability of individuals with different strategies to maximise their use of potentially superabundant late summer feeding resources, and that the primary moult progressing both ways from the vicinity of primaries three, four or five could enable primary moult to be completed more quickly than in the normal sequence. Thomas (1977) stated that at least 79% of the birds start moulting in the middle of the primary tract, and at least 25% suspend moult. All those that suspended moult also moulted eccentrically, i.e., started moulting in the middle of the primary tract, suggesting that this strategy enables them to moult the most important feathers for flight more quickly (Thomas 1977). On the basis of a few retraps presented by Thomas (1977), Cramp (1992) estimated a moult duration of 55 days, whereas Muller (1981) estimated a duration of six weeks and Stresemann & Stresemann (1970) of five weeks. Despite his sample sizes being small and taken from widely separated populations in different years, Thomas (1977) suggested that birds suspending moult were not late starters. However, Márton *et al.* (2000) showed that the number of primaries involved in moult decreased and the speed of moult increased with the date of moult initiation. Those that retained more feathers started to moult later in the season but finished moulting comparatively early, i.e., the difference in time of moult initiation between the individuals is larger than the difference in time at the end of moult, suggesting that season constrains the extent of post-breeding moult.

On the basis of published studies, Jenni & Winkler (1994), in their review of the

moult strategies of European passerines, classified the moult strategy of adult Savi's Warblers into three groups: one with no post-breeding moult and complete pre-breeding moult (group 10), and two with post-breeding moult complete or involving just part of the primaries and with unknown moult in the winter quarters (groups 11 and 13). Regarding the moult strategies of migratory species, they state: "More detailed studies, especially of the species mentioned in groups 11-14, will certainly add more species to the groups mentioned here, modify and clarify the classification of certain species and perhaps reveal additional moult strategies. In this respect, *Locustella luscinioides* with its variety of recorded moult patterns might be a promising species for further studies...". Clearly the peculiar moult of Savi's Warblers remains puzzling in many respects and deserves further research.

Here, the post-juvenile and post-breeding moult phenology, sequence and extent of a Portuguese population of Savi's Warblers are described in order to clarify its moult strategy, as well as the variation in body condition during moult. As this study was part of a larger autecological study of this species, it was also possible to determine the influence of breeding phenology on moult, and to assess the complementarities of the pre-breeding and post-breeding moults.

5.2 Material and methods

From 2000 to 2002, Savi's Warblers were captured by mist-net at Salreu marshlands, Ria de Aveiro, Portugal, and ringed with a metal ring, issued by the Portuguese Ringing Centre, and three colour-rings in a unique combination. Many adults were captured in March and April, when they returned from the wintering quarters (see §Chapter 2), but, after breeding, standardised mist-netting (see Neto 2003) was performed

at four different sites within the study area in order to capture moulting birds and recapture local breeding birds.

Ageing was based on the wear of the feathers, particularly the remiges, rectrices, primary coverts and alula, iris colour, mouth colour and presence of tongue spots. Recently-fledged juveniles were readily identified as they had obviously fresher plumage than adults, a yellow mouth, strongly marked tongue spots and a dark grey iris. However, shortly after fledging, the mouth becomes more pinkish and the tongue spots may disappear completely, particularly in early-fledging juveniles, or become faded as in many adults. Because of this, these characters could not be used for ageing (pers. obs.). Most adults had very worn plumage in summer and autumn, the iris colour was grey-brown, brown or light brown, the mouth colour pinkish, although the gape varied from grey to yellow, and sometimes had faded tongue spots. Birds undertaking a wing moult were aged by the wear of the retained feathers and iris colour. The few birds that had completed their moult and did not retain any old feathers were aged only by the iris colour and were all adults. However, some individuals were quite difficult to age, particularly those undertaking an interrupted wing moult which had relatively fresh older primaries and secondaries and a greyish iris. On the basis of retraps, these birds were considered to be adults (probably second-year birds or floaters) that may have had a complete pre-breeding moult in Africa.

The birds were measured as described in Chapter 4 and the moult recorded by attributing a score from zero (old feather) to five (completely grown feather) to all flight feathers (Ginn & Melville 1983), greater and primary coverts and alula of both wings, and tail. From 2001 onwards, the moult intensity of the body feathers was also recorded by attributing a score of zero (no feathers growing), one (1 to 5), two (5 to 10) or three (more

than 10 feathers growing) to each body feather tract (capital, dorsal, pectoral, abdominal, femoral and crural; Ginn & Melville 1983, Redfern & Alker 1996). This was done for both adults and juveniles. Some data gathered from 1997 to 1999, in which only the moult of the primaries of the right wing was recorded, were also used.

As some nests were found during the study period (see Chapter 3), the overlap between breeding and moult was determined by recapturing the adults and measuring the moult score when their nestlings were about to fledge. When the parents were captured earlier or later than the fledging date, the moult score at fledging date was calculated assuming a moult progress of 0.773 scores per day (see §Results).

The total moult score was determined for each bird by adding the moult scores of individual primaries of the right wing, but excluding the outer primary because of its very small size, to give a maximum of 45. For the analysis of recaptures and to estimate the start of moult, it was assumed that the primary moult score was linearly related to time even though it was shown by Dawson (2003) that it is the feather mass rather than the moult score that increases linearly with date (see also Underhill & Zucchini 1988 and Underhill *et al.* 1990). This approximation, however, is unlikely to influence the comparison between the sexes, and allows comparisons to be made with data presented in other studies. The wing raggedness was calculated by adding the difference between five and the score of each growing primary and secondary thus measuring the gap in the wing in terms of moult scores. The moult asymmetry was measured by adding the difference in moult score between the individual remiges of the left and right wing.

As all birds were captured during the morning, time of day did not affect the weight of individuals. Therefore a body condition index was calculated simply by dividing the weight by the tarsus length. The variation in body condition, fat and muscle

scores during moult could not be assessed directly using the primary moult scores because of differences in moult extent between individuals. Hence, the progress of moult was determined by calculating the moult scores necessary to finish the moult for each individual.

Here, the abbreviation 'P' means primary, 'S' secondary, 'T' tertiary, 'PC' primary coverts and 'GC' greater covert. Primaries are numbered descendantly, secondaries and tertials ascendantly and the coverts follow the number of the corresponding remiges.

5.3 Results

5.3.1 Post-juvenile moult

A total of 65 juveniles was captured from early June to 1 October in 2000 to 2002. Because only four of the 156 nestlings ringed from 2000 to 2002 were retrapped, the exact age of most birds was unknown.

As in most passerines, the young Savi's Warblers left the nest when the wing and tail feathers were still growing; the tail was less than one third of its full size (score 1) and the skin was bare between the feather tracts (pers. obs.). The wings and tail were not fully grown two weeks after fledging, as was shown by a juvenile which was retrapped when it was 28 days old and still had the outer primaries and tail growing (score 4). A 'filling moult' similar to the one described by Redfern & Alker (1996) for Sedge Warblers (*Acrocephalus schoenobaenus*) was observed in individuals up to the age of 38 days. They had plumes growing between the feather tracts, particularly in the ventral apterium, as well as growing underwing coverts and one or two rows of feathers in the lateral parts

of the dorsal, ventral and abdominal tracts. The occurrence of the post-juvenile moult was identified by the location of the growing feathers, which were at the centre of the feather tracts. This moult started in the middle of the dorsal tract, extending to the abdominal and femoral tracts and then to the capital, pectoral and lastly to the crural tract. The progress of moult from one feather tract to the others seemed to occur very rapidly.

The post-juvenile moult started while the filling moult was still being undertaken, when the simultaneous growth of feathers in the centre and at the edge of the dorsal tract as well as in the ventral apterium could be seen. Since the 28-day-old bird mentioned earlier, which was captured on 24 August, had 1-5 feathers growing in the centre of the dorsal tract, it appears that at least in some birds the post-breeding moult starts before the wings and tail are fully grown. This is not always the case, however, because two birds, which were 37 and 38 days old and were captured on 18 and 17 September, respectively, were still undertaking a filling moult and, apparently, did not have a post-juvenile moult. These dates suggest that the post-juvenile moult of later-fledging birds may be less extensive. At least 44% of the individuals captured undertook a post-juvenile moult. This percentage may be an under-estimate since some of the birds that were not actively moulting may have replaced some of the juvenile feathers (which were very difficult to distinguish from the older feathers), and those which were having a filling moult may have had a post-juvenile moult later on. Five juveniles (5.2%) replaced the median coverts, 3-10 greater coverts and 1-3 tertials. One of these individuals was moulting the 6th primary of the right wing, but this could have been caused by an accidental loss of a feather.

The variation in moult intensity with time is presented in Figure 5.1. Individuals undertaking a filling moult were captured throughout the season until 19 September,

which was expected given that Savi’s Warblers fledge until late August (see §Chapter 3) and the filling moult may take three weeks. In contrast, as birds undertaking a post-juvenile moult were only detected until 5 September, some individuals, particularly those that fledged later in the season, may not have moulted at all. The overlap between filling and post-juvenile moult was detected from early August to 5 September, whereas individuals which were moulting very intensively and showed the most extensive moult, were only captured until mid-August.

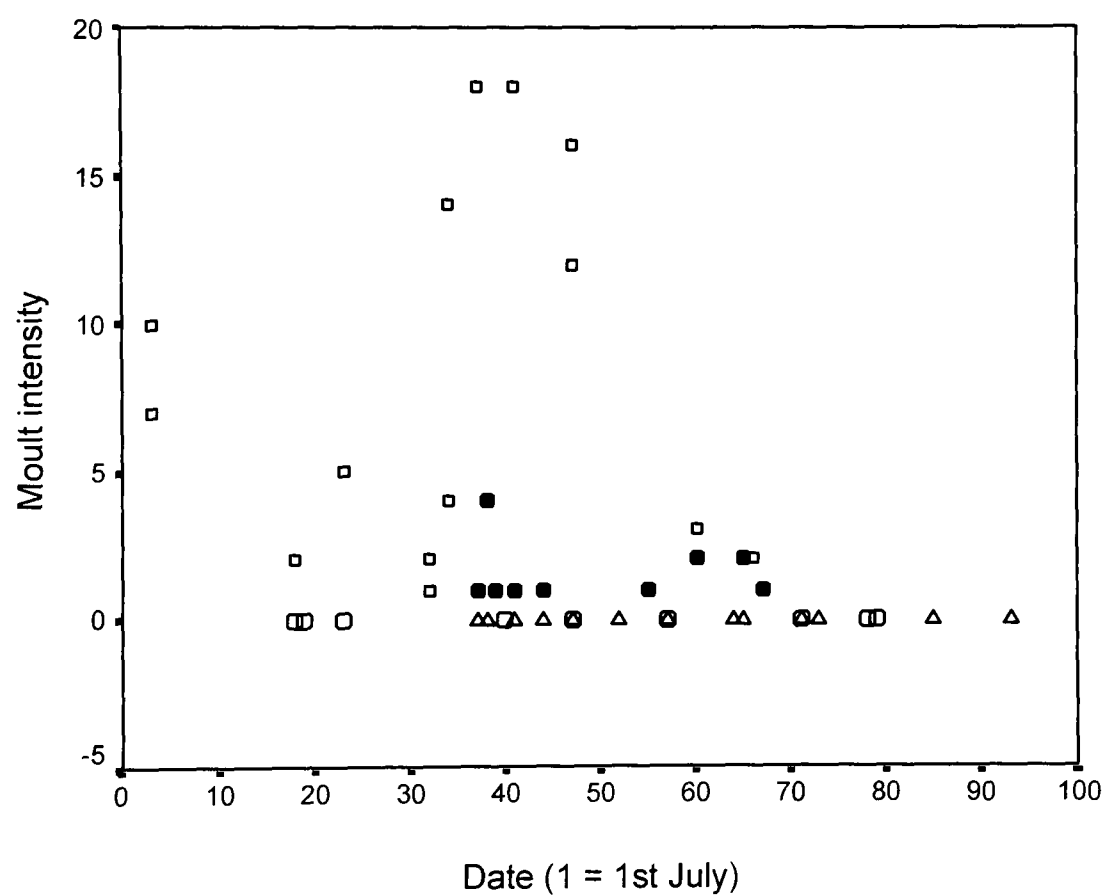


Figure 5.1 – Variation in moult intensity (sum of body moult scores) with date for juveniles captured in 2001 and 2002. Squares – birds undertaking post-juvenile moult; Black dots – birds overlapping filling and post-juvenile moults; Circles – birds undertaking a filling moult; Triangles – birds not moulting.

5.3.2 Post-breeding moult

Seven recaptures of actively moulting adults indicated that the rate of primary moult was 0.707 ± 0.1068 scores per day (range: 0.4 to 1.2143) and, when the secondaries

were included, 1.261 ± 0.1804 moult scores per day (range: 0.4 to 1.7142). Hence, Savi's Warblers took on average 64 days to complete the post-breeding moult or 59 days if the secondaries were included. On average, the post-breeding moult started on 28 July ± 3.38 days (range 6 June-25 August, $n = 28$) and ended on 28 August ± 3.38 days (range 7 July-26 September, $n = 28$). The average moult period of the population (31 days) is shorter than that of individuals (64 days) because of moult interruption (see below). Males started moulting significantly earlier than females (Males: 14 July ± 4.81 , range 6 June-29 July, $n = 11$; Females: 05 August ± 7.18 , range 7 July-25 August, $n = 6$; Mann Whitney U = 8.5, $P = 0.014$). Six males and nine females were caught in July and August while still breeding. Only one female out of nine overlapped breeding and wing moult (mean overlap = 1.3 primary moult scores, range 0-12) and another female was only moulting the primary coverts, whereas all six males were moulting the remiges (mean overlap = 17.2 primary moult scores, range 15-32). The difference between the sexes in moult-breeding overlap is very significant (Mann Whitney U = 1.0, $P = 0.001$).

Table 5.1 – Moult scores of the left and right wings of individuals showing examples of: complementarities between pre-breeding and post-breeding moult (NMYY, WMNG and possibly PMOB and PMGR), typical post-breeding moult sequence (NMNY), suspended moult (NMRO, PMGR) and suspended moult within the breeding quarters (NMYY, PMGN and PMOB). A question mark means that the pattern of wear was not clear. T – tertiary, S – secondary, P – primary.

Bird	Left wing PPPPPPPPPP SSSSSS TTT	Right wing TTT SSSSSS PPPPPPPPPP	Date	Notes
NMYY	5555555000 555555 555	555 555555 0005555555	Spring 2000	Complementarity between pre- and post-breeding moult with suspension
	0000000455 000000 000	000 000000 5550000000	20-07-2000	
NMNY	0001333100 000000 222	122 000000 0013331000	03-08-2001	Typical sequence
NMRO	5344555000 443233 555	555 330344 0005554435	11-09-2001	Suspended moult in a late breeding female
WMNG	5555555000 ?????? 555	555 ?????? 050555555	Spring 2002	Complementarity between pre- and post-breeding moult
	0001444255 105555 542	222 000001 5354442000	12-08-2002	
PMGN	5555555550 555555 555	555 555555 0555555555	26-08-2002	Feather in bold were obviously fresher
PMGR	5455555055 550044 555	555 440455 5505555545	30-08-2002	Uncommon suspension
PMOB	5555555555 555555 555	555 555555 5555555555	23-09-2002	Feathers in bold were more faded - suspension

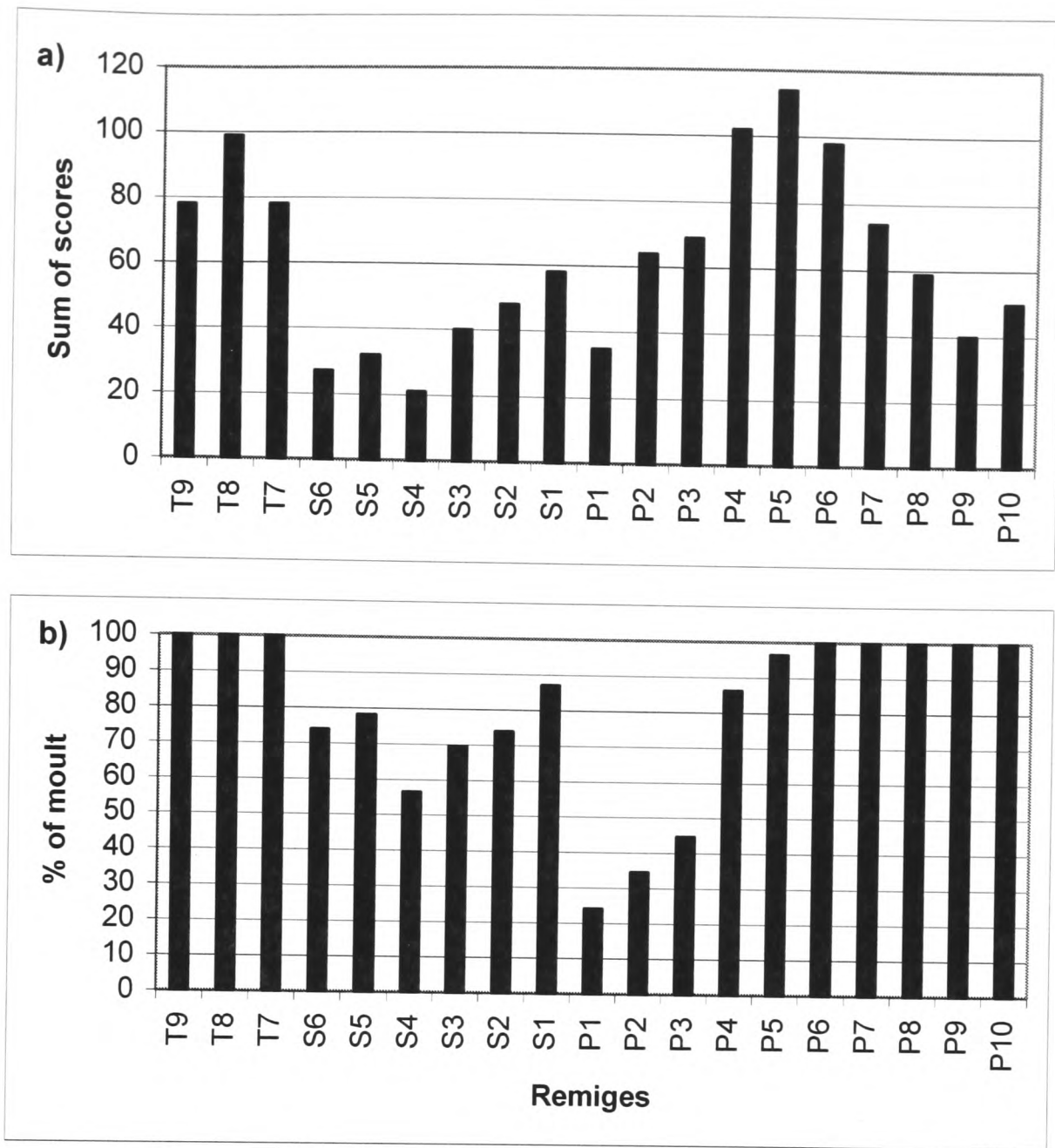


Figure 5.2 – Post-breeding moult sequence (a) and extent (b) in Savi’s Warblers, based on 31 moulting and 29 nearly moulted or moulted individuals, respectively. T-tertiary, S-secondary and P-primary. % of moult refers to the percentage of individuals that replaced a particular remige.

The individual scores of each remex of actively moulting adults were summed to determine the general sequence of moult in this population (Figure 5.2a). The post-breeding moult tended to be initiated in P5 then followed by P4 and P6. The onset of tertiary moult occurred when two to three primaries had been shed, and most often followed the sequence T8-T9-T7 (Figure 5.2a), although occasionally T7 was shed before T9. The secondaries were shed when five or six primaries were already growing and progressed ascendantly in 5 and convergently in 9 out of 19 birds. The tail was moulted

centrifugally, but almost simultaneously, starting at the same time as the tertials. The body moult started in the middle of the dorsal tract soon after the first primary was shed. It then progressed very rapidly to the other feather tracts so that all body tracts had scores of 3 at the same time. The body moult finished after the wing and tail moult.

In at least 27 out of 35 birds (75%) the moult started in the middle of the primary tract. The direction of primary moult was divergent in at least 16 out of 35 actively moulting birds. The first primary to be shed varied greatly among individuals, and in three cases the start of moult was asymmetric. Considering only the right wing, one bird started moulting at P1 and P3 almost simultaneously, one at P2, two at P3, two at P4, four at P5 and two at P6 (see also Figure 5.3b). Those individuals that started moulting the inner primaries, particularly P1 and P2, were probably completing the previous pre-breeding moult, as was the case with two individuals that were retrapped in the same year (NMY Y and WMNG in Table 5.1). The normal post-breeding moult may occur almost at the same time as the ‘complementary pre-breeding moult’ (see WMNG in Table 5.1) or after a short suspension (NMY Y and possibly PMOB in Table 5.1).

Table 5.2 – The extent of post-breeding moult in wing and tail feather tracts. Data are from the right wing of moulted or nearly moulted birds.

	Range	Mean	% all	% some	N
Primaries	5-10	7.9	21	100	29
Secondaries	0-6	4.5	47	95	18
Tertials	3-3	3	100	100	29
Greater coverts	8-10	9.8	89	100	28
Primary coverts	0-6	4.7	0	95	21
Alula	2-3	2.6	83	100	12
Rectrices	12-12	12	100	100	26

The extent of post-breeding moult is described in Figure 5.2b and Table 5.2. Only 21% of the birds moulted all the remiges whereas the vast majority (77%) suspended or arrested moult within the primaries and 66% retained some secondaries (Table 5.2,

NMRO in Table 5.1). The five outer primaries, the tertials and the tail feathers were invariably moulted (Figure 5.2b, Table 5.2). No birds were detected with new inner primaries and old outer primaries, that is, the retained primaries were located in the inner part of the wing and, on one bird, in the middle of the primary tract (this individual had a normal post-breeding moult, but also moulted the two inner primaries. This was apparently a continuation of the previous pre-breeding moult, retaining P3 only, PMGR in Table 5.1). However, even those birds that moulted all remiges retained some very worn outer primary coverts, which did not moult at the same time or in the same sequence as their corresponding primaries. In addition, 14% of the birds retained 1-2 greater coverts and 20% retained 1-2 alula feathers (Table 5.2).

The number of unmoulted primaries increased significantly with the date of moult initiation (Figure 5.3a), i.e., later-moulting birds moulted less extensively. In fact, individuals that undertook a complete moult started moulting sooner than those that suspended or arrested moult (Mann Whitney $U = 0.00$, $P = 0.01$, $n = 13$ with suspended and 6 with complete moult). The effect of date on moult was associated with differences between the sexes because females had a less extensive moult than males (number of unmoulted primaries: Females = 3.29 ± 0.18 , range 3-4, $n = 7$, Males = 1.14 ± 0.46 , range = 0-3, $n = 7$, Mann Whitney $U = 2.5$, $P = 0.03$). Furthermore, early-moulting birds started to moult within the inner primaries whereas later-moulting birds tended to start moulting in P5 or P6 (Figure 5.3b). One individual (PMGN in Table 5.1), that suspended the post-breeding moult, retaining the two inner primaries, and then moulted one more primary (P2), shows that the moult extent is quite plastic. Moult asymmetry was neither related to sex (Mann Whitney $U = 52.5$, $P = 0.193$, $n = 17$ males 9 females) nor to the start of moult (Linear regression: $F_{[1,20]} = 2.17$, $P = 0.157$).

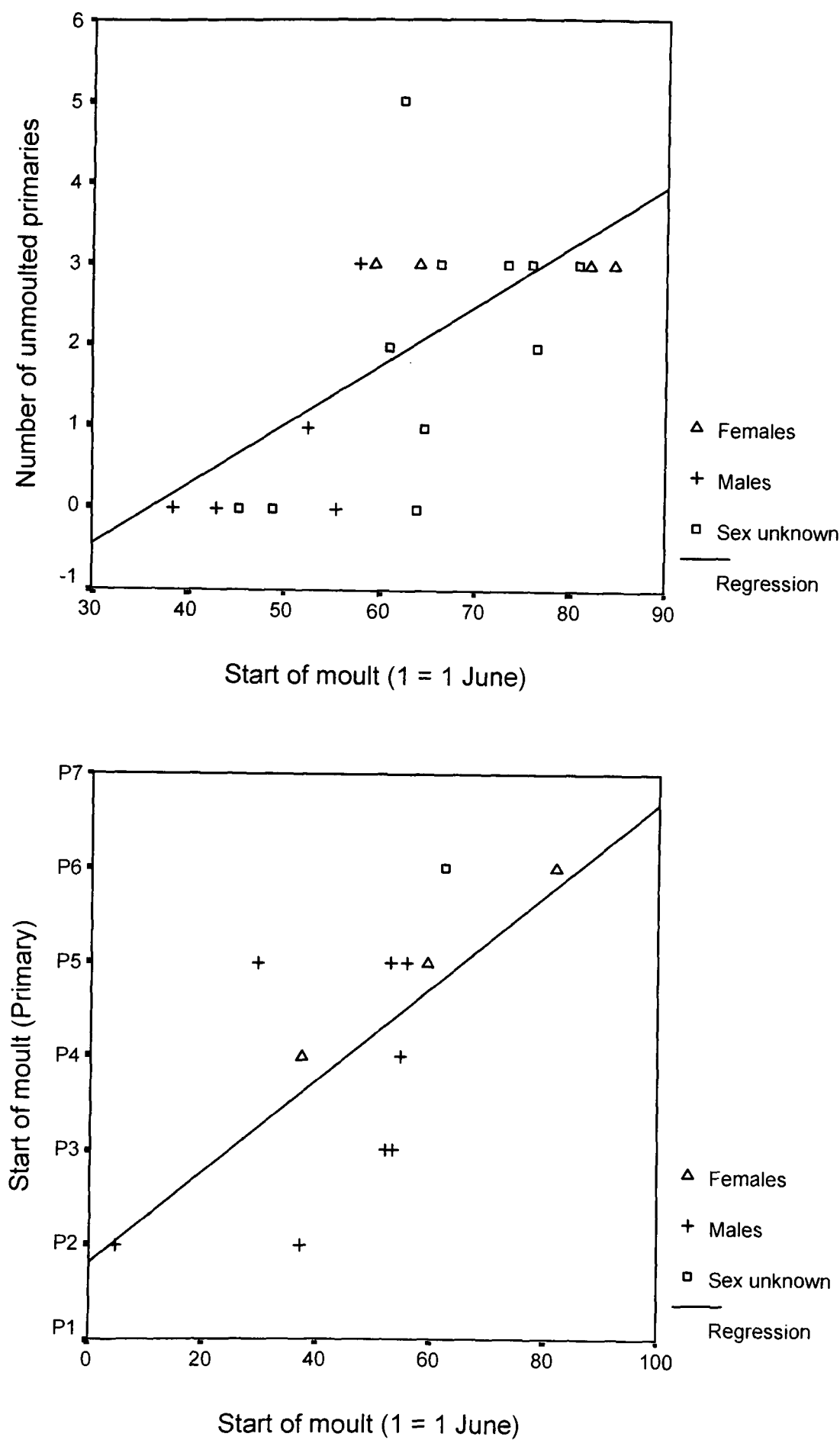


Figure 5.3 – Relationship between the date of start of moult (1 = 1st June) and (a) the number of unmoulted primaries and (b) the primary in which the post-breeding moult started. Regression analysis: (a) Number of unmoulted primaries = 0.0734 (Date of start of moult) – 2.645, $R^2 = 0.402$, $F_{[1,19]} = 12.792$, $P = 0.002$; (b) Primary number = 0.04858 (Date of start of moult) + 1.818, $R^2 = 0.448$, $F_{[1,10]} = 8.113$, $P = 0.017$.

Although body condition (weight / tarsus) increased significantly with the progress of moult (Pearson's correlation: $r = 0.445$, $P = 0.038$, $n = 22$), this relationship was not significant after being standardized for date ($P > 0.05$). Fat score decreased and then increased during the post-breeding moult (Quadratic regression: $R^2 = 0.301$, $F = 4.10$, $P = 0.033$, $d.f. = 19$), still being close to significance when date was taken into account ($R^2 = 0.244$, $F = 3.07$, $P = 0.07$, $d.f. = 19$). Muscle score did not show any pattern of variation throughout moult. A highly significant positive relationship was found between wing raggedness and the body condition index, even after being standardised for date (Figure 5.4), but not for fat or muscle scores.

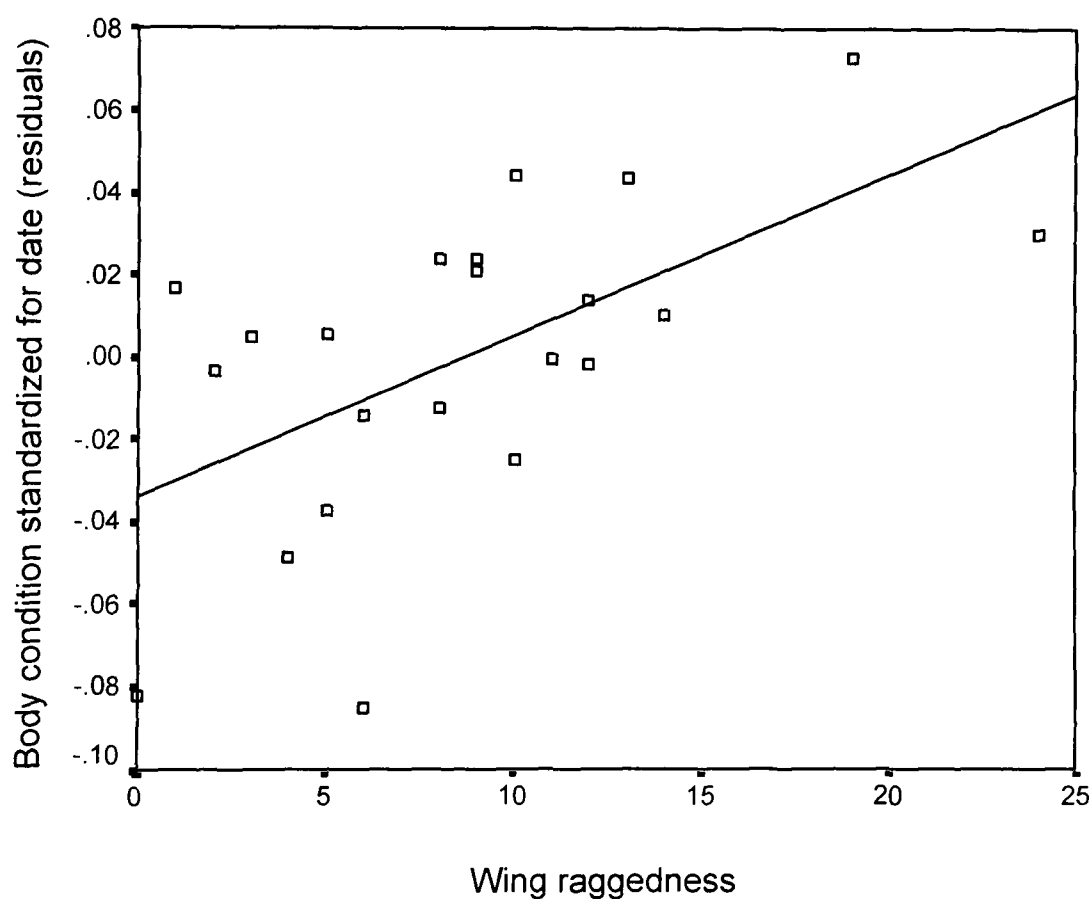


Figure 5.4 – Relationship between the residuals of body condition (weight / Tarsus) vs. date and wing raggedness. Regression analysis: $R^2 = 0.341$, $F_{[1,20]} = 10.344$, $P = 0.004$.

5.4 Discussion

In contrast to Müller (1982) and Heinroth & Heinroth (1924-26 cited by Cramp

1992), who state that Savi's Warblers do not undertake a post-juvenile moult at the breeding quarters in Germany, the data presented here show that, at least in the Portuguese population, this moult may be quite extensive, occasionally involving all body feathers, tertials and greater coverts (see also Trias *et al.* 1982). It is even possible that some juveniles moult a few primaries before the autumn migration, as was apparent in one individual that was replacing one of the primaries, something which has been suggested by experienced ringers working in southwest Portugal (Peter Fearon, personal communication). Hence, there seems to be a marked geographical variation in the post-juvenile moult in this species, which follows the general pattern of a greater moult extent in the south (Jenni & Winkler 1994).

The post-juvenile moult started when the juveniles were about 25-28 days old, whilst the outer primaries and tail were still growing, and the 'filling moult' occurred at least until the fledglings were 38 days old. This timing is similar to the one described for Sedge Warblers (Redfern & Alker 1996). The overlap between the filling and the post-juvenile moults is typical of migratory passerines, which rarely overlap moult and migration and hence are often constrained in the time available for their moult (Jenni & Winkler 1994). Juveniles undertaking a 'filling moult' were captured until 19 September whereas those undertaking a post-juvenile moult were only detected until 5 September. This strongly suggests that later-fledging birds do not carry out a post-juvenile moult. It is probable that the overlap between filling and post-juvenile moult and the post-juvenile moult extent vary with season (which is remarkably long in Savi's Warblers, see §Chapter 3) or fledging date. But, because of the small samples available for analysis, this was not clearly demonstrated.

The post-breeding moult started in early June and in some individuals was carried

out until mid-September. On the basis of retraps, the duration of the complete moult was 64 days, or 59 days if secondaries were taken into account. This is greater than the 55 days estimated by Cramp (1992) from three retraps made in France and presented in Thomas (1977), especially if, as in this study, the outer primary was excluded, then becoming 49 days. The reason for this difference might be that some retraps made in Portugal included breeding birds, which probably moult slowly in comparison to non-breeding birds, but it is also possible that moult rate varies geographically. Males moulted significantly earlier than females, a difference that seems to be associated with differences in breeding effort between the sexes. In fact, from July onwards, all males overlapped breeding and moult, but only 10% of the females did so. The moult-breeding overlap in late breeding individuals, particularly in males, seems to be widespread in passerines (Hemborg 1998, Hemborg *et al.* 2001, Jenni & Winkler 1992, Newton 1966) and causes fitness costs, particularly for the female (Hemborg 1999, Sanz 1999, Svensson & Nilsson 1997).

The post-breeding moult sequence and extent were shown to vary greatly among individuals, even though the sample sizes were rather small. The typical divergent moult sequence of Savi's Warblers is, as far as known, only shared with Brown Shrike, *Lanius cristatus*, (Jenni & Winkler 1994), but probably occurs in other *Locustella*, and must have evolved independently. Interestingly, the individuals that undertook an atypical moult sequence, particularly those that started moulting with the inner primaries or with two different primaries, were in fact completing the previous pre-breeding moult by replacing the contrastingly worn inner primaries, and having a normal post-breeding moult, which occurred almost at the same time or after a short suspension. This shows that in order to interpret moult patterns correctly it is necessary to obtain information on subsequent

moult of the same individuals. This is particularly difficult in migratory species.

Although the sample sizes were small, the post-breeding moult extent seems to vary less among individuals than was previously reported for this species. In fact, the moult may be complete or interrupted within the primaries and/or secondaries, but there is no evidence that some birds do not moult any remiges at all, as was reported by Mead & Watmough (1976) on the basis of one bird with old remiges captured in mid August in Portugal. There are several reasons for supposing that all birds undertake a post-breeding moult in Savi's Warblers: all birds captured later in the season had moulted at least five primaries and all birds that were fattening in preparation for autumn migration, either earlier or later in the season, also had a complete or interrupted moult (per. obs.). In addition, in this population the breeding season may last until 23 August (see §Chapter 3) and the vast majority of the females start moulting only after their nestlings fledge. In fact, a female was still breeding on 12 August and did not overlap breeding and moult was later recaptured while undergoing a normal moult (pers. obs.). Also, the outer primaries of the females were very worn later in the season, apparently due to the nesting activity, and it is extremely unlikely that they would migrate in that condition. Therefore, contrary to what is stated by Jenni & Winkler (1994), the moult strategy referred to as group 10 (complete moult in the winter quarters) and considered by Hall (2003) and Svensson & Hedenström (1999) as Savi's Warbler's main moult strategy, seems not to occur in this species; at least, not in this population.

In migratory birds that have a complete post-breeding moult, time availability often constrains individuals which may either moult fewer feathers, as in the Common Whitethroat (*Sylvia communis*) (Hall & Fransson 2000) and Savi's Warbler (Márton *et al.* 2000, this study), or moult more rapidly, producing shorter feathers (Hall & Fransson

2000, Hall 2002). One of the most interesting results of this study is that, in Savi's Warblers, not only the extent of the post-breeding moult but also the sequence are related to season, i.e., Savi's Warblers are also plastic in moult sequence, being able to start moulting in a variety of locations within the primaries according to season. In fact, early-moulting birds tended to start the post-breeding moult by shedding P2 or P3 whereas later-moulting birds started moult by shedding P5 or P6. This has not been described before, but might occur in other species which regularly have an eccentric moult, i.e., those that start moulting in the middle of the primary tract (see Jenni & Winkler 1994). It is obviously an adaptation to ensure that the most important feathers for flight are replaced before migration.

Although theoretically moult should have an effect on flight performance due to increased wing load (Hedenström & Sunada 1999, Pennycuick 1975), and experimentally induced moult causes impaired escape ability (Lind 2001, Swaddle & Witter 1997), naturally moulting individuals show a surprisingly small effect of moult on flight (Lind 2001, Williams & Swaddle 2003). The reasons for this seem to be that birds are able to compensate physiologically either by reducing weight (Swaddle & Witter 1997) or increasing the relative volume of breast muscle (Lind & Jakobsson 2001). In addition, the apparent absence of additional flight costs associated with moult may reflect a decreased mechanical performance of year-old feathers in comparison with new (fresh) feathers (Williams & Swaddle 2003).

In Savi's Warblers, although the body condition tended to increase with the progress of moult, this relationship was not significant when date was taken into account, a result similar to the one obtained for Great Tits, *Parus major*, by Gosler (1991). The relationship between fat and moult, however, remained close to significance even after

being standardised for date, and tended to decrease at the beginning of moult and then increase at the end, whereas no relationship was found between muscle and progress of moult. It is possible that the increase in fat during the later stage of moult results from the overlap between moult and pre-migratory fattening, as the fat score of at least one individual was typical of a fattening bird. This was shown to occur in Bluethroats, *Luscinia svecica*, under experimental conditions (reduced photoperiod), but not in natural populations (Lindström *et al.* 1994).

There was a strong positive relationship between wing raggedness and body condition, which is the opposite of the relationship found in some birds (e.g. Dunlins, *Calidris alpina*, Holmgren *et al.* 1993), but is similar to others (Chilgren 1977, Newton 1968). This increase may be associated with an increase in water due to feather growth (Chilgren 1977, Newton 1968). Wing raggedness is a good measurement of the speed of moult and is higher in the middle of moult (Bensch & Grahn 1993, pers. obs.), when flight ability is more affected (Hedenström & Sunada 1999, Swaddle & Witter 1997). Therefore, Savi's Warblers do not seem to compensate for an increase in wing load, which given its skulking habits, walking ability (Leisler 1977) and reluctance to fly even at other stages of their annual cycle, is not surprising. In fact, birds with more ragged wings can afford to be heavier because they feed by walking rather than flying, and the dense habitat probably prevents them from being exposed to a high predation risk.

CHAPTER 6

Using stable isotope analysis to determine the winter moult extent in migratory birds: the complex moult of Savi's Warbler

Patterns of feather wear in birds captured in spring have been analysed to describe the winter moult extent of long-distance migrants. However, the interpretation of feather wear may be rendered extremely difficult by the great variation that can exist in individual feather wear due to long moult periods or asynchrony, by the progress of the season, and by the existence of complex moult patterns. Here, stable isotope analysis is used to determine the origin of the wing feather generations present in Savi's Warblers captured in Portugal. Carbon, Nitrogen and Hydrogen isotope ratios of feathers of known European origin differed significantly from those feathers known to have grown in Africa. A discriminant analysis, in which 91.1% of the cross validated samples were correctly classified, was used to determine the origin of tail and wing feathers collected from birds caught when they returned to the breeding quarters. The interpretation of feather wear generally agreed with the stable isotope analysis, but some cases of disagreement were identified. The winter moult extent of Savi's Warblers of known age is described taking into account the origin of feathers as determined by the stable isotope analysis. First-year birds had a complete or interrupted winter moult, in which some inner primaries and secondaries were retained. Five moult types were identified in adults: 12.5% had a complete winter moult, 21.7% had an abridged moult, 25% replaced only a few inner and/or middle primaries and secondaries that

were probably retained on the previous post-breeding moult, 34.4% had a ‘double’ moult, i.e., they seemed to have resumed the post-breeding moult and also replaced some outer primaries, secondaries and tertials, and 6.25% had no winter wing moult at all. The moult strategy of Savi’s Warblers is discussed.

6.1 Introduction

The vast majority of birds, and in particular the passerines, replace (moult) their entire plumage at least once a year. Moult is costly in terms of time, protein and energy, due to the production of new feathers, thermoregulation and impaired flight (Ginn & Melville 1983, Klaassen 1995, Lindström *et al.* 1993, Swaddle & Witter 1997). As a result, breeding, moult (especially the moult of flight feathers) and migration, the most nutrient-demanding periods of a bird’s annual cycle, generally do not overlap in time (Hemborg 1999, Herremans 1990, Jenni & Winkler 1994). In adult resident passerines, the complete annual moult takes place shortly after the breeding season, but this is not always the case for migratory species (Jenni & Winkler 1994). Migratory passerines have a large variety of wing moult strategies allowing them to cope in different ways with breeding and migration. These strategies include: (1) complete summer (post-breeding) moult, which may or may not be followed by a partial winter (pre-breeding) moult, (2) complete pre-breeding moult, which may be preceded by a partial post-breeding moult, (3) seasonally divided moult of remiges, which includes various forms of moult suspension (i.e. when moult is interrupted for some time and then resumed at the point of interruption) within the primaries or secondaries, (4) arrested (i.e. when the moult is interrupted, but is not resumed subsequently) post-breeding moult within remiges

followed by a complete winter moult, and (5) complete biannual moult (reviewed by Jenni & Winkler 1994). Moult seems to be the most plastic of the three nutrient-demanding periods, both at the individual and the species level. Due to this variability and the lack of information about the complementarities of the different moults that take place during the annual cycle (particularly in migratory birds which are rarely retrapped between breeding and wintering quarters), a natural classification of moult types is still lacking (but see Howell *et al.* 2003), and hence, little is known about the evolution of moult strategies (Figueroa & Jovani 2001, Hall 2003, Svensson & Hedenström 1999).

Although the phenology and extent of summer moult are relatively well known for most species, the winter moult of many migratory birds remains little studied (Jenni & Winkler 1994). For example, before the present study moult in Savi's Warblers, *Locustella luscinioides*, for instance, had only been examined in 35 individuals captured in the winter quarters, many of which were not actively moulting or had not started moult (Aidley & Wilkinson 1987, Bensch *et al.* 1991, Nikolaus & Pearson 1991, Stresemann & Stresemann 1970). In addition, winter moult studies are constrained by the fact that it is often impossible to age the birds accurately (the probability of retrapping birds of known age ringed in Europe is extremely low), and the breeding origin of individuals is unknown. It is possible, however, to describe the winter moult extent of birds when they return to the breeding quarters and compare the summer and winter moult extents of individuals belonging to the same population. This is done by observing the wear and bleaching of different sets of feathers, or feather generations, and deducing their origin by the degree of wear. It is also possible to infer the age of individuals by positively identifying retained juvenile feathers, which are weaker and so become worn more quickly than adult feathers (Jenni & Winkler 1994, Svensson 1992). In some individuals and species with simple moult strategies, it seems to be relatively straightforward to

interpret the patterns of feather wear observed in spring, simply by assuming that old feathers grew in the breeding quarters and the new or fresh feathers grew in the winter quarters, although no confirmation of this interpretation has ever been made. However, the moult patterns become very difficult or even impossible to interpret as the season progresses, in species with asynchronous moult or long moult periods (in which the degree of feather wear varies greatly among individuals and almost overlaps between those that had a late post-breeding moult and those that moulted early in the winter quarters) and in species that show more than two feather generations. Savi's Warbler is one of the species for which the spring moult patterns have been interpreted incorrectly, as they were thought to be present in first-year birds only (Roselaar in Cramp 1992). The complexity of its moult patterns seems to result from the long post-breeding moult period (§Chapter 5) and long breeding season (§Chapter 3), which cause great variation in the degree of feather wear of adults and juveniles, and from the occurrence of several moults and suspensions both in the breeding (§Chapter 5) and in the wintering quarters (this study).

In addition to the identification and interpretation of the patterns of feather wear, there are two other methods that can potentially allow the determination of the winter moult extent of birds of known origin. The first method involves the marking of fresh feathers immediately after the post-breeding moult in adults, or the marking of juvenile feathers of first-year birds, and their recapture in the following spring. This permits the correct identification of the retained feathers, i.e., those that were not moulted during winter, and hence describe the winter moult extent and relate both moults in the same individuals. In order to do this, however, it is necessary that a high percentage of individuals are faithful to their moulting/breeding areas so that the sample size is adequate to be analysed statistically. But, retrapping just a few individuals may still be useful to

link both moults in terms of their complementarities and eventually deduce their homology, for instance, by distinguishing between arrested and suspended moults. The return rates of Savi's Warblers are relatively low (§Chapter 3) and only a few of the breeding birds seem to moult in or near to their territories and so the probability of retrapping a moulting or recently moulted bird in the following spring is very low. During this study there were only four such instances.

The second method is based on the fact that stable isotope signatures of intrinsic tissues reflect those of local food webs (Bearhop *et al.* 1999, Mizutani *et al.* 1990, Pearson *et al.* 2003), which, in turn, can vary spatially due to several possible biogeochemical processes. Animals that move between isotopically distinct food webs can retain information on the previous feeding location for periods that depend on the elemental turnover rates of the tissue of interest (Hobson 1999, Pearson *et al.* 2002). Keratinous tissues like hair, feather or nail are metabolically inert once formed, and so their isotopic signatures reflect those of the location where they were synthesized (Mizutani *et al.* 1990). Stable isotope analysis has been used successfully to determine the diet and provenance of feeding (Bearhop *et al.* 1999, Kosh *et al.* 1995, Mizutani *et al.* 1990), to trace the origin or migration of wildlife (Hobson *et al.* 2001, Hobson & Wassenaar 2001, Lott *et al.* 2003, Rubenstein *et al.* 2002, Smith *et al.* 2003, van der Merwe *et al.* 1990) and to link winter and summer events (Marra *et al.* 1998). Therefore, this technique might also be useful to distinguish the feathers that grew in the breeding quarters from those that grew in the wintering quarters of migratory birds, thus allowing the description of the winter moult extent. There are several geographic isotopic patterns that can be used to make this distinction. It has been shown that in North America the stable hydrogen isotopic ratios (δD) of feathers closely reflect the δD of growing season precipitation, which decrease with increasing latitude in a continent-wide gradient

(Chamberlain *et al.* 1997, Hobson & Wassenaar 1997, Hobson *et al.* 2001, Kelly *et al.* 2002, Rubenstein *et al.* 2002, Wassenaar & Hobson 2000). In addition, carbon isotopic ratios ($\delta^{13}\text{C}$) depend on isotopic fractionation associated with plant photosynthetic pathways, which results in distinct signatures between C3 and C4 plants as well as crassulacean acid metabolism (CAM) plants. C4 and CAM plants, which are typically more enriched in ^{13}C in comparison with C3 plants, are associated with hotter and drier habitats and so are more abundant in lower latitudes than C3 plants (Marra *et al.* 2002). Nitrogen isotopic ratios ($\delta^{15}\text{N}$) depend upon a variety of environmental factors (Hobson 1999), tending to be enriched in drier soils and marine ecosystems, but do not vary geographically in a predictable way. They may, nevertheless, be useful to distinguish samples from different sites (Chamberlain *et al.* 2000). Although other isotopes, namely $\delta^{87}\text{Sr}$ and $\delta^{34}\text{S}$ (Chamberlain *et al.* 1997, Hobson *et al.* 1997), may provide additional discrimination between sites, they were not used in this study. In comparison to the American continent, very few data on isotopic signatures of feathers have so far been obtained from the European-African bird migration system. Chamberlain *et al.* (2000) and Evans *et al.* (2003) used both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of feathers grown in Africa to distinguish between wintering sites of migratory birds belonging to different populations. Payne *et al.* (2004) compared the stable isotope signatures of African feathers ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD) between populations breeding at different sites, suggesting that breeding and wintering latitude are correlated in Aquatic Warblers, *Acrocephalus paludicola*.

Savi's Warbler is a long-distance migratory passerine that occurs in marshlands. The breeding distribution ranges from southern Europe to Estonia and from Iberia to Mongolia. The winter distribution is poorly known; the species seems to occur in a narrow belt in sub-Saharan Africa north of the forest area, having been detected in wetlands in Senegal, Nigeria, Ghana, Sudan and Eritrea, but rarely crossing the Equator

(Snow & Perrins 1998). Because of its variable and complex moult, Savi's Warblers were considered by Jenni & Winkler (1992) to be a promising species for further studies on moult strategies. Juvenile Savi's Warblers generally have a limited post-juvenile moult in Europe shortly after fledging, which can be more extensive and variable in the southern part of its distribution, or no moult at all (Müller 1981, Trias *et al.* 1982, §Chapter 5). They seem to have a complete or interrupted (suspended/arrested) moult in the wintering quarters, as when they return to Europe the following spring they have fresh wing feathers, occasionally with a few retained (old) inner primaries and/or secondaries (pers. obs.). Adults have a very variable post-breeding moult, which can be complete, suspended or arrested and some individuals are said not to moult at all (Mead & Watmough 1976, Thomas 1977, Márton *et al.* 2000, but see §Chapter 5). They also moult in Africa (Aidley & Wilkinson 1987, Bensch *et al.* 1991, Nikolaus & Pearson 1991, Stresemann & Stresemann 1970), but it is not known whether the winter moult is complementary to the previous post-breeding moult or if it is a new moult altogether (Jenni & Winkler 1992). Individuals captured in the winter quarters seem to have a pre-breeding moult as variable as the post-breeding moult (Aidley & Wilkinson 1987, Bensch *et al.* 1991, Nikolaus & Pearson 1991, Stresemann & Stresemann 1970), and, in fact, when they arrive in the breeding quarters, adults can have two or three wing feather generations in a variety of patterns including those described for first-year birds (pers. obs.). It is not known how the first pre-breeding moult is related to the adult moult (but see §Chapter 5).

Here, I show for the first time that stable isotope analysis can be used to determine the winter moult extent in long-distance migratory birds. This methodology is compared with the analysis of feather wear, providing a partial confirmation of the general interpretation of moult patterns. In addition, the complex moult of Savi's Warblers is

described in detail and novel isotopic data from the European-African bird migration system is provided.

6.2 Material and methods

6.2.1 Collection of samples

From 2000 to 2003, Savi's Warblers were captured by mist-net in the Salreu marshlands, located in Ria de Aveiro, a coastal lagoon in western Portugal. Most birds were first caught shortly after arrival, from March to April, or when their first nest was found (see §Chapter 2). They were ringed with a metal ring issued by the Portuguese Ringing Centre (CEMPA/ICN) and three colour-rings in a unique combination. The individuals were sexed on the basis of their behaviour and the presence of an incubation patch, and biometric data were obtained (see §Chapter 2). The relative wear of each wing feather generation was recorded as fresh, intermediate or old. Their contrast was recorded as obvious or slight, for instance, individual 'X' had primaries 1, 2 and 3 slightly fresher. The ten primaries were numbered descendantly and the six secondaries and three tertials ascendantly. The degree of wear or bleaching was also recorded as fresh (dark grey-brown), bleached (brownish), slightly worn, moderately worn and very worn, particularly for individuals showing only one generation of feathers and those with particularly fresh or old plumage. The contrasts between the feather generations varied greatly among individuals and could be quite subtle; they had to be observed carefully with the wing almost closed and in good, but diffuse, light. Growing feathers were scored from 1 (feather in pin) to 5 (completely grown feather) following Ginn & Melville (1983). All birds were captured, ringed and the wear pattern observed and recorded by J. M. Neto. It was observed that as in first-year birds, adults could have very obvious moult contrasts

between different wing feather generations, and so the age (first-year or adult) was only determined with certainty for recaptured individuals.

The origin of the different generations of wing feathers was inferred from their pattern and degree of wear, knowing that birds often suspend moult for migration, but also within the breeding or wintering quarters. For birds that had two contrasting generations of wing feathers, it was assumed that the older feathers grew in Europe and the fresh feathers in Africa. However, if both types of feathers were quite fresh (the contrast between them was small), they were presumed to have grown in Africa, and if both were faded and slightly or moderately worn, they were presumed to have grown in Europe. The process of identifying the origin of feathers by their wear was further complicated by the date of capture, as they become increasingly worn with the progress of the season, and the position of the moult limit (i.e., the contrast between different feather generations) within the wing. This is because some feathers (the outer primaries and the tertials) are more prone to abrasion than others (the inner primaries and secondaries), which are more 'protected' (Svensson 1992). Therefore, the expected contrast in wear between the feather generations varied with their position. Some individuals had three generations of flight feathers, in which the intermediate was considered to be of unknown origin, although it was usually quite fresh; the fresher feathers were assumed to be African and the older to be European.

From 2001 onwards, one tail feather of each bird was collected, and in 2003, one remex of each generation of feathers was collected and stored in a self-sealing plastic bag. This did not seem to cause any harm to the birds as the replacement feathers started to grow shortly after sampling, and the birds bred successfully (per. obs.). The isotopic signatures of the remiges of different generations were compared with those of feathers of

known origin, which were obtained from several sources. European-grown feathers were collected in Portugal from post-breeding adult and juvenile Savi's Warblers ($n = 13$), juvenile Reed Warblers, *Acrocephalus scirpaceus*, ($n = 10$) and juvenile Great Reed Warblers, *Acrocephalus arundinaceus*, ($n = 3$). African-grown feathers were taken from adult Reed Warblers ($n = 8$), Great Reed Warblers ($n = 1$), Sedge Warblers, *Acrocephalus schoenobaenus*, ($n = 1$) and from first-year Savi's Warblers captured in spring ($n = 7$). With rare exceptions, these *Acrocephalus* species are known to undertake a complete moult in the winter quarters (Jenni & Winkler 1994), which also lie in wetlands in sub-Saharan Africa. In addition, they are also insectivorous passerines, hence, the isotopic signatures are not expected to differ between these species. The wintering ranges of these *Acrocephalus* species are larger than that of the Savi's Warbler, but they overlap completely with it. Therefore, the range of the isotopic signatures of the African feather samples probably overestimates the one expected for Savi's Warblers.

6.2.2 Stable isotope analysis

Each feather was cut into small pieces, ground and homogenised. For the Carbon and Nitrogen analysis, approximately 0.55 mg of the ground feather sample was folded into a clean tin capsule and combusted in a Carlo Erba C/N/S analyser interfaced with a Finnigan Tracer Matt continuous flow isotope ratio mass spectrometer (CF-IRMS). Hydrogen analysis was performed with an Elemental Analyser Pyrolysis Isotope Ratio Mass Spectrometry (EA-Pyr-IRMS) using 0.75 mg of ground feather sample folded in a clean silver capsule. All stable isotope ratios are reported in permil (‰) using the δ notation: $\delta_{\text{sample}} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where the δ_{sample} is the isotope ratio of the sample relative to the standard, R_{sample} and R_{standard} are the fractions of heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^2\text{H}/^1\text{H}$) in the sample and standard, respectively.

6.2.3 Statistical analysis

Univariate comparisons were made between the isotopic signatures of known African and European feathers. As the variables were approximately normally distributed, judging from their rather low skewness and kurtosis, the parametric *t*-test was used. The results of non-parametric tests did not differ from the parametric ones. A discriminant analysis was performed, in which $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and δD were predictors and the origin of feathers (Europe or Africa) was the classification or group variable. As this analysis is very sensitive to the presence of outliers (Tabachnick & Fidell 2001), two models were created: one including all isotopic measurements (the complete model) and another excluding the outliers of the stable isotope ratio statistical distributions for each group.

The origin of all feathers was determined by the discriminant classification model. In order to assess whether the analysis of feather wear was consistent with the stable isotope analysis, the isotopic signatures of presumed African (fresher) and European (older) feathers were compared by *t*-test with those of known origin. In addition, for each sample, the presumed origin of feathers inferred from their degree of wear was compared with its origin as established by the classification of the discriminant analysis and assumed to be inconsistent if the probability of membership to the group was very high (>0.95) and there were no unusual values of stable isotope ratios.

6.3 Results

6.3.1 Discrimination between African and European feathers

The descriptive statistics of the isotopic signatures of feathers that grew in Europe and Africa as well as those with different degrees of wear are presented in Table 6.1. δD

was slightly higher in European than in African feathers (*t*-test: $t_{44} = 2.058$, $P = 0.046$). This was unexpected because of the decrease in precipitation δD with latitude. African feathers had significantly higher $\delta^{13}C$ (unequal variance *t*-test: $t_{22.7} = 5.759$, $P < 0.001$), which indicates the influence of C4 plants, and significantly lower $\delta^{15}N$ (*t*-test: $t_{44} = 6.953$, $P < 0.001$).

Table 6.1 – Isotopic signatures (mean $\pm$ SE) of wing and tail feathers of known origin (European and African) and different wear patterns. The percentage feathers classified as African or European by the discriminant analysis is also presented.

Feather type	δD (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N	Classification
European	-58.22 ± 1.619	-23.11 ± 0.348	12.76 ± 0.293	26	94.7% European
Older (worn)	-57.63 ± 1.609	-20.62 ± 0.763	12.35 ± 0.521	38	73.7% European
Intermediate	-65.27 ± 3.304	-17.81 ± 1.113	8.96 ± 0.531	7	100% African
Fresher	-59.22 ± 1.106	-18.79 ± 0.668	10.06 ± 0.373	46	80.4% African
African	-63.61 ± 2.121	-16.36 ± 1.119	9.71 ± 0.324	20	92.3% African

The discriminant model in which the origin was the group variable and δD , $\delta^{13}C$ and $\delta^{15}N$ were predictors was highly significant (Canonical function 1: eigenvalue = 2.617, canonical correlation = 0.851, Wilk’s Lambda = 0.276, Chi-square = 53.352, d.f. = 3, $P < 0.001$), correctly classifying 91.1% of the cross-validated group cases. If the outliers of the distributions of the stable isotope ratios of each group were excluded, the classification improved to 97.4% of the cross-validated cases. However, only four of the 168 feathers analysed changed their classification (from African to European). All of these were intermediate in their isotopic ratios and so the probability of membership to any of the groups was quite low. Likewise, when only Savi’s Warbler’s feathers were included in the model, the classification improved to 95%, but only four feathers changed their classification, all of which had a relatively low probability of membership to any of the groups. In addition, as some of the feathers of unknown origin also had unusual values, the complete model was chosen to classify them also.

6.3.2 Comparison of the isotopic signatures with feather wear

The interpretation of the patterns of feather wear and the isotope signatures seem generally to agree, because 73.7% of the older feathers and 80.4% of the fresher feathers were classified as European and African, respectively, by the discriminant model (Table 6.1). However, these percentages of correct classification seem to be lower than the ones found for the feathers of known origin, suggesting that the interpretation of the feather wear involved some errors. This is further supported by the fact that the $\delta^{13}\text{C}$ of older feathers, i.e., those presumed to have been grown in Europe, is significantly higher than the $\delta^{13}\text{C}$ of known European feathers (unequal variance *t*-test: $t_{50.8} = 2.98$, $P = 0.004$). Therefore, some of the feathers that were faded and slightly to moderately worn early in spring were not grown in Europe, as has been predicted from their abrasion, but in Africa. This occurred in four individuals out of the 31 that had their wing feathers analysed, all of which seemed to have had a complete moult in Africa and not in Europe as predicted from their degree of wear. In fact, they had high values of carbon isotope ratios (see Figure 6.1) and a high probability (> 0.999) of belonging to the African group.

The δD of fresher feathers also tended to be higher than that of the African feathers (*t*-test: $t_{64} = 2.01$, $P = 0.048$) and the $\delta^{13}\text{C}$ lower (*t*-test: $t_{64} = 1.94$, $P = 0.057$). Hence, some fresher feathers might have grown in Europe or at a stopover site during the pre-breeding migration, rather than in Africa. This seemed to have occurred in one bird, which had the three inner primaries contrastingly worn, and so it was assumed that it had had a suspended moult in Africa. However, the isotopic ratios strongly indicated that both feather generations were European (probability of membership to the European group > 0.99), which means that the older inner primaries must have grown some time before the post-breeding moult in which the outer primaries were moulted. In addition, in one bird

with three generations of feathers, the isotopic ratios indicated that not just the older but also the fresher feathers grew in Europe whereas the intermediate ones were African. As most birds were captured early in spring, these fresher feathers probably started growing at a stopover site during the spring migration. In fact, some birds apparently overlapped spring migration and pre-breeding moult because three individuals still had growing wing feathers when they arrived in the breeding quarters.

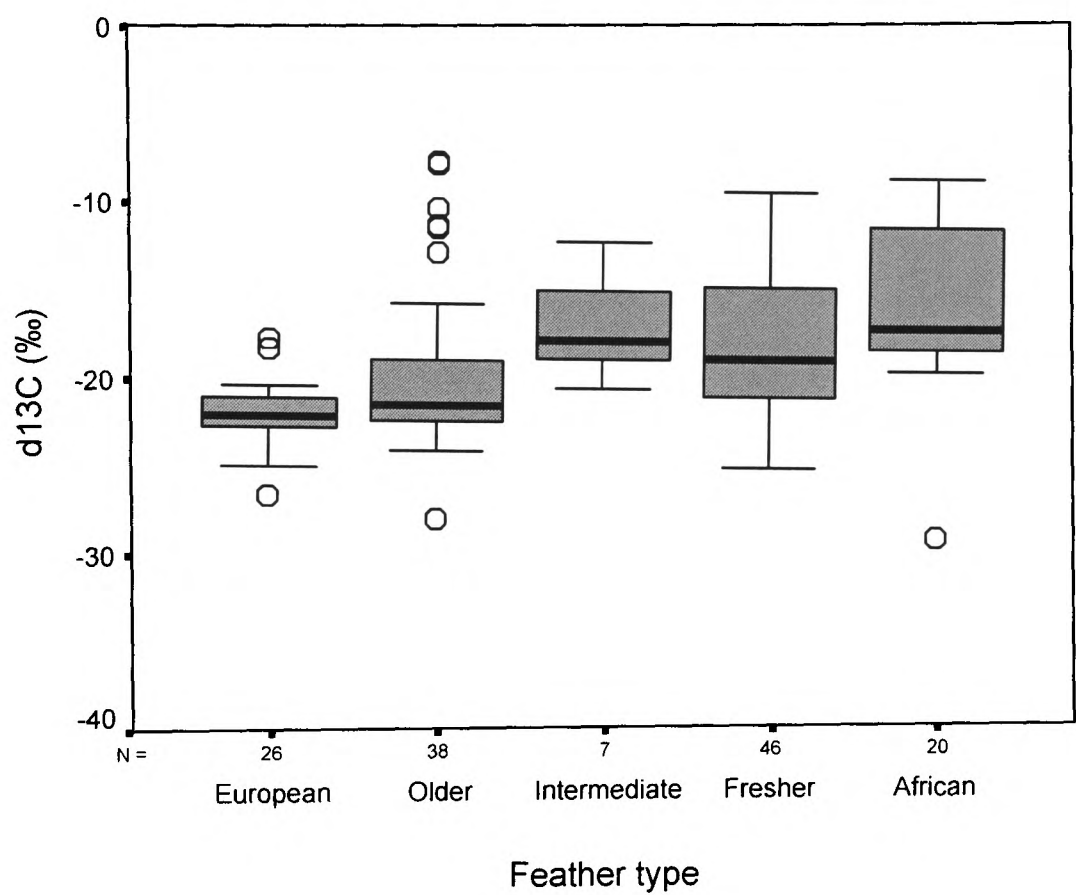


Figure 6.1 – Boxplot of $\delta^{13}\text{C}$ (d13C) for each feather type, showing the median, quartiles, range and outliers. European feathers have lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ than African feathers. The upper $\delta^{13}\text{C}$ outliers of older feathers represent African feathers that were quite worn earlier in spring and so their pattern of wear was presumably wrongly interpreted. The lower median and minimum $\delta^{13}\text{C}$ of fresher feathers also indicate that some fresher feathers probably grew in Europe or at a stopover site during the pre-breeding migration.

Other instances of disagreement between the interpretation of feather wear and the discriminant classification were not strongly supported by the latter method, according to the relatively low probability of membership to the group calculated from the

discriminant classification, and may be explained by errors associated with the discriminant analysis.

6.3.3 Winter moult extent in Savi's Warblers

The extent of the pre-breeding wing moult varied greatly among individuals. Five types of winter moult were identified (see Figure 6.2): (1) 'complete' moult, in which all wing feathers had isotopic signatures typical of the wintering quarters, even though contrasts between the wear of wing feathers could be seen in some birds indicating temporal moult suspension during winter; (2) interrupted moult, in which only the outer primaries, and some or all secondaries and tertials were replaced during winter; (3) 'post-breeding winter' moult, in which only a few inner and/or middle primaries and secondaries were replaced during winter, suggesting that it was a continuation of a suspended post-breeding moult; (4) 'double' moult, which was characterized by the replacement of some inner primaries and secondaries as in the 'post-breeding winter' moult, but a variable number of outer primaries and tertials were also shed, apparently in an independent, but almost simultaneous, interrupted pre-breeding moult; and (5) no wing moult, although as in the previous types of moult, some or all body feathers and even the tail may have been replaced during winter. The frequencies of these types of moult were expected to differ between the age classes because most, if not all, adults have a complete or interrupted post-breeding moult, whereas the juveniles only moult the body feathers and occasionally the tertials before the autumn migration (§Chapter 5). Hence, the average winter moult extent of the population would depend on the frequency of birds of each age class and moult type, and so the winter moult extent was only described for individuals of known age (retraps).

Only four birds ringed as juveniles were recaptured in the next spring: two had a

complete moult in Africa (WMPG and PMYR in Table 6.2), and two showed signs of moult suspension having three generations of feathers (YMRB and WMBN in Table 6.2). The latter resumed the suspended moult later in winter and one of them (WMBN) still had two tertials growing when it arrived in the breeding quarters.

Table 6.2 – Moult pattern of known first-year birds, that is, individuals ringed as nestlings or juveniles and retrapped on the following year. P, S and T represent primary, secondary and tertiary wing feathers. Growing feather scores vary from 0 (old feather) to 5 (fresh feather). Numbers in bold indicate feathers contrastingly fresher than those with score 5.

Individual	Left wing			Right wing			Moult type
	PPPPPPPPPP	SSSSSS	TTT	TTT	SSSSSS	PPPPPPPPPP	
WMPG	5555555555	555555	555	555	555555	5555555555	Complete
YMRB	5555555 555	000000	555	544	000000	5555555555	Suspended
WMBN	555555555 5	550055	555	555	550555	5555555555	Suspended
PMYR	5555555555	555555	555	555	555555	5555555555	Complete

Only 15 out of the 32 known adults that returned to the study area had some feathers analysed for stable isotopes. Four out of 15 adult birds (27%) moulted all wing feathers in Africa, three of which had some fresher feathers that indicated suspension of the pre-breeding moult within Africa. Four (27%) had an abridged moult, in which some inner primaries and secondaries were retained (see Figure 6.2a); two of these birds also suspended moult within Africa, having three generations of feathers. Two individuals (13%) moulted only 2-3 inner or middle primaries and 0-2 secondaries, apparently resuming a suspended post-breeding moult (see Figure 6.2b). The remaining five individuals (33%) retained zero to six secondaries and two to four inner and/or middle primaries, and seemed to have had a ‘double’ moult. If all 32 known adults were taken into account (Figure 6.2), four birds (12.5%) had a complete winter moult, seven (21.7%) had an interrupted moult, 11 (34.4%) had a double moult, eight (25%) seemed to have resumed the post-breeding moult and two (6.25%) had no winter wing moult at all. It is possible that some individuals that replaced all their feathers during the winter actually

resumed the post-breeding moult and then replaced the remaining feathers during the pre-breeding moult, thus having a 'complete double' moult. According to the isotopic signatures, the four first-year birds moulted the tail feathers in the winter quarters, but in adults, 11 out of 32 birds did not moult the tail in the winter quarters, although three of them had some fresher (African) tail feathers admixed.

Some birds were captured during both the post-breeding moult and the next spring and so their winter moult extent could be related to the previous moult. Two males, which had a complete post-breeding moult, had a subsequent interrupted pre-breeding moult. One female that had a suspended post-breeding moult, in which the three inner primaries on both wings and one secondary on the right wing were retained, moulted all the wing feathers of the left wing and the retained feathers plus P5 on the right wing, as well as the tail. Finally, another male, which had a suspended post-breeding moult, retaining the three inner primaries, S3 and S5 of the left wing and S2 and S3 of the right wing, had a 'double' winter moult, replacing the two old secondaries and three inner primaries plus five outer primaries and two inner tertials on the left wing, and all the primaries, the four outer secondaries and two inner tertials on the right wing.

This clearly shows that the interrupted post-breeding moult is suspended during the autumn migration and resumed during winter, and also that an additional pre-breeding moult may be undertaken, involving a variable number of primaries, secondaries and tail feathers, which are moulted twice a year.

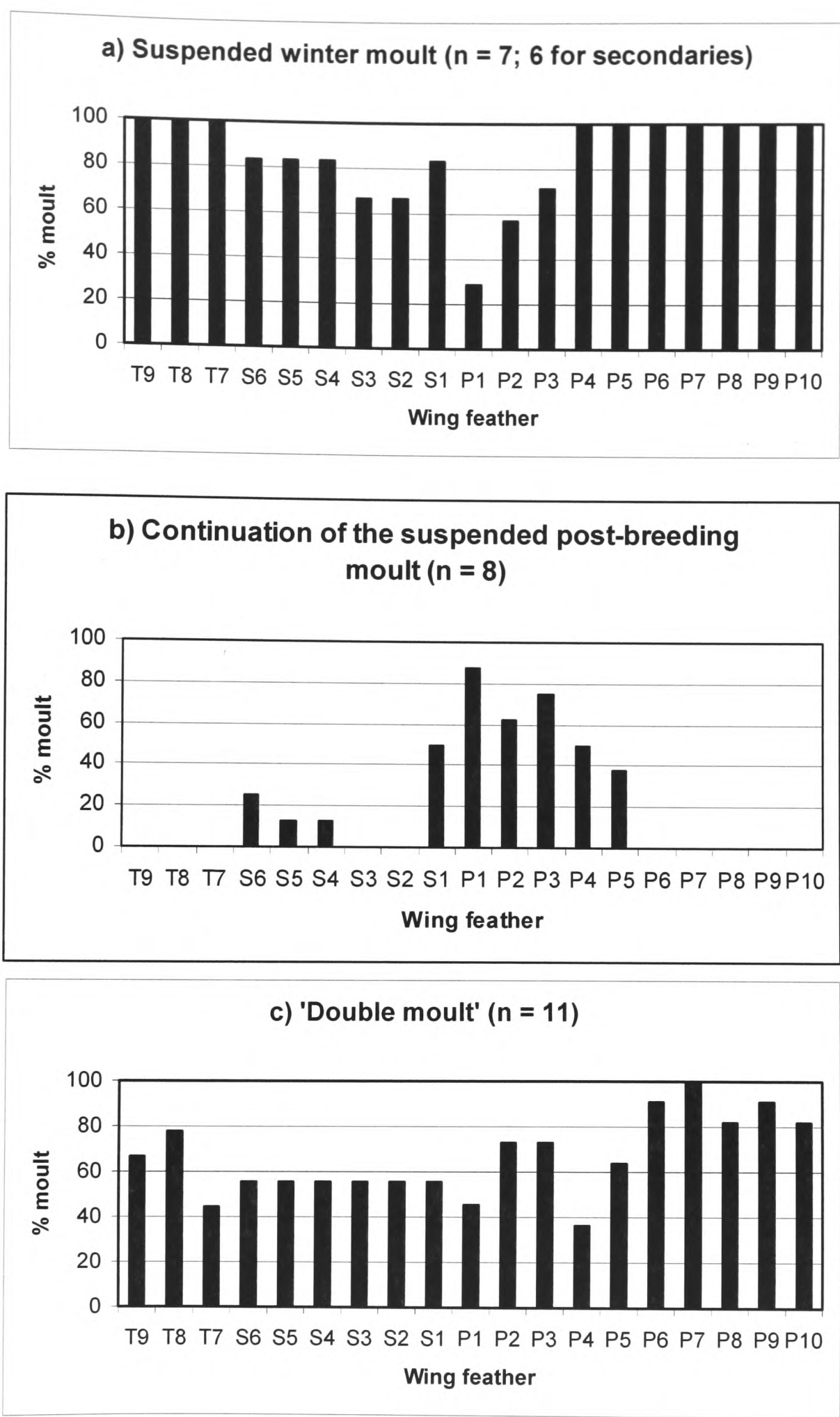


Figure 6.2 – Winter moult extent of the right wing of 32 adult Savi’s Warblers recaptured in spring. % of moult refers to the percentage of individuals that replaced each remige. In addition to the patterns shown, four birds had a complete winter moult (% moult = 100 for all feathers) and two birds did not moult in the winter quarters (% moult = 0). P, S and T refer to primary, secondary and tertial wing feathers, respectively.

6.4 Discussion

The isotopic signatures of African and European feathers of reedbed warblers were differentiated by all stable isotope systems measured in this investigation. As expected, African feathers showed a distinct influence of C4 plants, having a greater relative abundance of ^{13}C . Interestingly, although $\delta^{15}\text{N}$ does not vary geographically in a consistent way, this stable isotope ratio was significantly lower in Africa than in Europe. This difference is the opposite of the one expected from the influence of aridity, and could have resulted from the fact that ^{15}N is more abundant in marine ecosystems (Hobson 1999), which could have had some influence, as the Portuguese study site is located near the coast. Another possible explanation is the effect of fertilisers used in the Portuguese location, although this is difficult to quantify. The difference in $\delta^{15}\text{N}$ may also indicate that these birds are higher in the food chain in Europe, thus showing a stronger enrichment in the heavier isotope (Hobson 1999). Feather samples from other sites within Europe are needed to determine whether the differences found between African and European feathers are consistent.

The difference between African and European feathers was less marked in δD than in the other isotopes analysed. In North American passerines, the δD of feathers is strongly correlated with the growing season average δD values in precipitation (Chamberlain *et al.* 1997, Hobson *et al.* 2001, Hobson & Wassenaar 1997, Wassenaar & Hobson 2000), which decreases with increasing latitude. Therefore, it was expected that the δD of African feathers would be greater than that of European feathers. However, the opposite was true. This probably resulted from the fact that precipitation is scarce in Western sub-Saharan Africa and so the δD of standing water might be quite different from the δD of precipitation, for instance due to differential evaporation.

The discrimination between African and European feathers was quite high, allowing the origin of most unknown feathers to be identified with confidence. However, the accuracy of the discriminant classification could reach even higher levels if the African feathers were collected from the same species and at known wintering sites. This is because the wintering range varies between the species analysed and although they generally occupy the same habitat, there might be small differences in diet and microhabitat selection that could influence the isotopic signatures of feathers. Therefore, the value of stable isotope analysis to determine the origin of feathers and the winter moult extent is expected to increase when more feather samples of the study species from known winter sites become available. In other species and populations, the discrimination between juvenile or post-breeding feathers and pre-breeding feathers is expected to depend on the wintering range and on changes in diet, habitat and distance between breeding and wintering areas.

It was shown that the origin of most feathers were correctly identified by the analysis of feather wear. Therefore, the stable isotope analysis provided an independent confirmation of the interpretation of feather wear. However, the inaccuracy of the discriminant model do not seem to fully explain the differences in the percentage of correct classification between the feathers of known and unknown origin (Table 6.1). That is, the presumed origin of a few feather generations, as inferred from their pattern of wear, was not consistent with the stable isotope analysis. This was further supported by the presence of significant differences in $\delta^{13}\text{C}$ between the presumed and known European feathers, and, to a lesser extent, by differences in $\delta^{13}\text{C}$ and δD between presumed and known African feathers.

A detailed inspection of the results for each sample showed that four out of 31

birds had a complete moult in Africa and not in Europe, as had been inferred from their worn plumage. In addition, in one bird with a contrast in wear within the remiges, both feather generations had isotope signatures typical of European feathers. Therefore, the patterns of feather wear may in some circumstances be wrongly interpreted in a way that precludes an accurate description of the winter moult extent. The other instance of an inconsistency between the two methods (an individual with three feather generations in which the fresher feathers probably grew in Europe), would not influence the description of the winter moult extent. The inconsistencies between the results of the two methods were probably caused by the fact that the time window between the end of the post-breeding and beginning of the pre-breeding moults is quite small at the population level, and so the feathers produced during these moults may be very similar in terms of wear. In addition, passerines that live in closed habitats like reedbeds probably wear faster than others, hence, even feathers that grew in the winter quarters may be unexpectedly worn next spring. The possibility that Savi's Warblers moult a few or all retained feathers during the spring migration either at stopover sites, during active migration or as soon as they reach the breeding quarters, also seemed to have caused inconsistencies between the two methods.

In order to describe the winter moult extent of Savi's Warblers, the stable isotope analysis was taken into account to correct wrongly interpreted wear patterns. A great variety of moult types was observed, and as these might differ between the age classes, the winter moult extent was described only for birds of known age. As expected, first-year birds had a complete or interrupted wing moult in Africa, in which the tail was also replaced. The existence of contrasts between the wing feathers strongly suggests that the first pre-breeding moult is often suspended within the winter quarters. This could be

related to moving between sites or temporal shortages of food.

Only two out of 32 adults (6.25%) did not moult any remiges in the winter quarters. The percentage might, in fact, be even lower because these two adults were not analysed for stable isotopes. But, the existence of this moult type was supported by one individual of unknown age (probably an adult because otherwise its feathers would have been extremely worn, which they were not), in which the isotopic signatures showed that it did not have a wing moult in the winter quarters. Many adults (25%) moulted only 1-3 primaries located in the inner or middle primary tract and/or 0-2 secondaries. This pattern suggests strongly that these individuals resumed a suspended post-breeding moult because the location of the fresher feathers corresponds to the primaries more often retained after the post-breeding moult (§Chapter 5).

This was further supported by the recapture of two adults in which the pre-breeding moult was largely complementary with the previous suspended post-breeding moult. The majority of adults (34.4%) seemed to have resumed a suspended post-breeding moult, but also replaced additional primary, secondary, tertial and tail feathers in an interrupted pre-breeding moult. A complete winter moult was observed in 12.5% of the adults, but at least some of these individuals may have resumed a suspended post-breeding moult and then replaced the remaining feathers in an interrupted pre-breeding moult, in a similar way to that observed in some birds in autumn (§Chapter 5). With the exception of the adults that resumed the post-breeding moult, both adults and first-year birds replaced the outer primaries, suggesting that the onset of moult took place in the middle of the primary tract. In fact, three adults moulted only 1-3 middle primaries in addition to the replacement of a few retained inner primaries and secondaries, which caused a peak in the percentage of moult in P6 and P7 in Figure 6.2c. As moult

suspension is very common in this species, the start of moult in the middle of the primary tract seems to be an adaptation to replace the outer primaries, which are the most important for flight, before migration. This sequence of moult was observed both in the breeding and winter quarters (Aidley & Wilkinson 1987, Bensch *et al.* 1991, Márton *et al.* 2000, Mead & Watmough 1976, Nikolaus & Pearson 1991, Thomas 1977, §Chapter 5).

Although the post-breeding and pre-breeding moult extents of the Savi's Warbler is now relatively well described, particularly for adults, its moult strategy is not yet fully understood. This is because few data exist on the complementarities between the different moults carried out by Savi's Warblers. More retraps between subsequent moult events are needed before these can be understood. It is necessary to determine whether the winter moult is suspended or arrested although some data suggest that at least the first pre-breeding moult is suspended (§Chapter 5). In addition, it is not clear whether some adults undertake a biannual moult of remiges, as the post-breeding moult of those that had a complete pre-breeding moult is unknown. However, the moult strategy of Savi's Warbler is probably best described as a partial biannual moult of remiges, which, in contrast to strategy five of Jenni & Winkler (1994), does not involve an arrested post-breeding moult and a complete pre-breeding moult, which occurs in *Locustella fluviatilis* (Pearson & Backhurst 1983), but a suspended post-breeding moult and an arrested or suspended pre-breeding moult.

The individual and year-to-year variation probably ranges from a complete post-breeding moult followed by a partial pre-breeding moult (strategy 2 in Jenni & Winkler 1994) to a biannual wing moult (strategy 6). Even within individuals, the moult strategy recorded may depend which wing is analysed because the wing moult can be very asymmetric. As all individuals seem to moult at least some wing feathers before the

autumn migration (§Chapter 5), a complete winter moult (strategy 3 in Jenni & Winkler 1994) probably does not occur in this species. If the pre-breeding moult proves to be suspended, as was suggested by some retraps (§Chapter 5), the most common moult strategy of Savi's Warbler should be described as a seasonally divided biannual complete moult, i.e. two annual complete moults often suspended for migration, a strategy which has not been previously described for any species. However, this moult strategy might occur in other species with complex moults (for instance in *Sylvia communis*, some species of *Lanius* and other *Locustella* species).

Independently of the exact moult strategy of the Savi's Warbler, it is clear that most birds replace many of the wing feathers, particularly the outer primaries, twice a year. This probably reflects the fact that the birds inhabit a very dense habitat and the feathers become worn rather easily. The difference in their moult in relation to other species that occur in the same habitat, such as the *Acrocephalus* warblers that undertake a complete winter moult, may result from the way they explore their habitat, being particularly skulking, and the fact that their wings are more pointed, which makes the outer primaries more prone to wear.

In conclusion, the stable isotope analysis provided a partial support of the interpretation of feather wear, but also identified cases of misinterpretation allowing an accurate description of the winter moult extent in a migratory bird with a complex moult. It was shown that the moult strategies of Savi's Warblers vary greatly between individuals, ranging from a complete post-breeding moult, probably followed by a partial pre-breeding moult, to complete biannual moult, but the complementarities of its moults are not fully understood.

CHAPTER 7

Pre-migratory fuelling, flight range and post-breeding migration strategy of Savi's Warblers in Portugal

In order to determine the migration strategy of Savi's Warblers, the data from 913 first captures and 232 retraps held by the Portuguese Ringing Centre were analysed. Juveniles started migrating in early August, before the later nestlings fledged, whereas adults departed at the end of August. As both age classes occurred in Portugal until early October, the migration of adults seems to be more synchronous than that of juveniles. In contrast to juveniles, adults showed a marked increase in weight and fat from the end of August and had a significantly higher fuel deposition rate. Accordingly, in adults, the average potential still-air flight range increased dramatically in September (1500 Km), in comparison with August (600 Km), whereas in juveniles it remained the same (150 Km). The fattest adults, i.e., those captured at the end of the fuelling period, had an average flight range of 2750 km, and so, were able to reach the winter quarters without refuelling. These data indicate that Savi's Warblers are time-selected migrants. The early departure of juveniles with lower fuel stores is in agreement with their lower fuel deposition rates under time-minimising migration models, as is overloading and by-passing of potential staging sites in adults. However, energy-selected migration cannot be excluded for juveniles as they left the breeding quarters with only enough fuel to reach the next staging site.

7.1 Introduction

According to optimal migration theory (Alerstam & Lindström 1990, Lindström & Alerstam 1992), the three selective forces acting on migratory birds are time, energy and mortality. Many long-distance migrants are believed to be 'time-minimizers', that is, they are selected to undertake the migration, including stopovers and flight, in the least possible time. This is particularly true for the pre-breeding migration, in which the early arrival on the breeding territory may have important fitness consequences (Hasselquist 1998, §Chapter 4). However, in the European-African bird migration system, the populations or species that winter in the west sub-Saharan Africa, particularly those that moult in the winter quarters and/or defend winter territories, might also be under time pressure. This is because the dry season arrives comparatively early in West Africa and so the period of abundant food is relatively short, which was shown to be associated with rapid moult among Palaearctic migrants (Bensch *et al.* 1991). As migration involves periods of stopover and flight, the speed of refuelling is very important for the overall migration speed. In fact, the main prediction of the time-minimization hypothesis is that the amount of fuel with which a time-minimizing migrant leaves a given stopover site is determined by the rate of fuelling at that site and by the conditions expected further along the migration route (Alerstam & Lindström 1990). Hence, overloading, i.e., deposition of larger fuel stores than needed for covering the flight distance to the next destination, and by-passing of possible, but low-quality, staging sites is expected from time-selected migrants (Gudmundur *et al.* 1991).

The Savi's Warbler, *Locustella luscinioides*, is a long-distance migratory passerine that breeds in wetlands in the Palaearctic region, from Portugal, NW France and SE England to Mongolia and from Estonia in the north to Morocco (Cramp 1992,

Hagemeijer & Blair 1997, Snow & Perrins 1998). The winter quarters are poorly known. Although there are a few records of wintering birds north of the Sahara, namely in Algeria, its main winter distribution lies in sub-Saharan Africa north of the forest zone. It seems to occur in a narrow belt extending discontinuously from Senegal to Eritrea. The nominate subspecies is regular in Senegal, southern Mali, northern Ghana, northern Nigeria and irregular in southern Mauritania (Cramp 1992, Hedenström *et al.* 1991). Further east, both nominate and *fusca* occur (and presumably *samartica*), being common in Sudan, western Ethiopia and probably regular in eastern lowlands of Eritrea (Cramp 1992). Two records in Ngulia, south-east Kenya, suggest that small numbers may cross the Equator (Pearson 1988).

Very little is known about the migration of Savi's Warblers. West European birds are believed to head south or southwest in autumn (reverse in spring), and there seems to be a migratory divide in central Europe (Cramp 1992). However, there are very few ringing data supporting this hypothesis: two birds ringed in the Netherlands were recovered in eastern France, one ringed in France was retrapped in Barcelona, and a bird ringed in East Germany was recovered in Athens (Aymí & Tomás 1998, Cramp 1992, Dürr *et al.* 1995). There is only one recapture from sub-Saharan Africa: one individual ringed in Barcelona was recovered in Senegal (Aymí & Tomás 1998). Western populations leave the breeding grounds from August to mid-September, and, in contrast to the larger eastern populations, the passage is inconspicuous (Cramp 1992). Although all the retrapped individuals decreased in weight during August, the capture of a few heavy birds in late August indicate that pre-migratory fattening might occur in western France (Bibby & Green 1983). There are no published data on fuel deposition rates for this species. Low abundance at migratory stopovers, even at well studied sites (Cramp 1992) strongly suggests that migration takes place in long, non-stop flights. It has been

recorded in small numbers in western Mauritania in September-November, but the arrival at wintering sites is rather late, namely southern Mali (November) and Senegal (December), and the whereabouts of late-autumn stopover sites are not known (Cramp 1992).

Here, the ringing data held by the Portuguese Ringing Centre (CEMPA/ICN) are analysed in order to determine the timing of migration and fat deposition of adult and juvenile Savi's Warblers. In addition, the fuel deposition rate of both age classes and the potential still-air flight range are estimated, and the migration strategy is discussed.

7.2 Material and methods

I analysed the data from all Savi's Warblers captured and recaptured in Portugal from 1997 to 2002, which were entered in the Portuguese Ringing Centre database. The birds had been ringed and measured following the standard procedure, in particular, the weight was measured with a Pesola spring balance accurate to 0.1 g and the visible fat was scored from zero to five. The age (juvenile or first-year birds are used with the same meaning, and adults) was determined following the criteria described by Svensson (1992). Two retrapped individuals for which the age at retrapping disagreed with the age at capture were excluded, as were records for which measurements were outside the species expected range as these were probably erroneous.

Although Savi's Warblers were captured at six sites in Portugal, the vast majority of birds were ringed at two sites only: 47% of the individuals were first captured at Lagoa de Santo André (see Catry *et al.* 2004 for a description of this area and the ringing procedure) and 50% at Ria de Aveiro (described above), both of which are coastal lagoons located in western Portugal about 350 Km apart. From July to September, the

ringing effort at these two sites was approximately constant for any given year, and so the total number of captures and recaptures is considered comparable. However, as song playback was used to capture birds during spring at Ria de Aveiro (see §Chapter 3), the number of individuals trapped during spring is not comparable to those trapped during summer. The vast majority of individuals (over 99%) were caught during the morning, and so time of day did not influence the weight, and no correction was made. Although the wing length, weight and fat score of adults and the wing and fat of first-year birds captured from 1 August onwards were significantly greater at Ria de Aveiro (t -test, $P < 0.05$), the data were pooled because this does not influence the differences between age classes.

The within-year recaptures made during the pre-migratory period (particularly those involving individuals first captured during August or September, which were not moulting or breeding and which had increased in weight), were used to determine the fuel deposition rate (FDR). The FDR was estimated from the regression line between the weight change and the number of days between capture and recapture. Due to small sample sizes, the FDR was assumed to be similar at the two sites and the data were pooled.

The potential flight ranges were estimated by using the program Flight 1.11 (see Pennycuick 1998 for details of the model) with still-air and altitude zero. The flight model was set to estimate maximum range, and the muscle burn criteria were set to hold specific work constant, i.e., during the simulated migratory flight not only all the fat would be used as fuel, but also part of the protein so that the pectoral muscle maintained its efficiency for flight. In addition to the mass of the birds ('all-up mass', as mentioned in the model), four other variables were entered: muscle and fat fractions, wing span and

wing area (Pennycuick 1998); all the other variables were as default. As in Pennycuick (1998), it was assumed that the muscle fraction was constant (0.16) for all individuals. The fat fraction was estimated with the following formula: Fat fraction = $[(\text{All-up mass} - \text{LBM}) - 0.16(\text{All-up mass} - \text{LBM})] / \text{All-up mass}$, in which LBM is the lean body mass of individuals with zero fat score (adults: 13.24 g; juveniles: 13.08 g). This is because it has long been known that the LBM increases during the fuelling period (Lindström & Piersma 1993, Piersma 1990), and it was shown that the avian pectoral muscle rapidly tracks body mass changes during fasting (Klaassen & Biebach 1994, Klaassen *et al.* 1997), experimental induced flights (Lindström *et al.* 2000) and in wild populations (Schwilch *et al.* 2002). Therefore, this model is probably more accurate than previous versions, although there are still some problems and assumptions that have not yet been adequately addressed (Pennycuick 1998).

The potential still-air flight range was estimated for adult and first-year birds captured in August and September using the average wing span and wing area measured in five adults captured in September at Ria de Aveiro (mean $\pm$ S.E.: Wing span: 0.195 ± 0.00254 m, range 0.189 to 0.203; Wing area: 0.003517 ± 0.0000971 m², range 0.003165 to 0.003700). This is likely to overestimate the flight range of first-year birds since they have slightly shorter wings (§Chapter 3) and presumably smaller wing span and area. The five adults mentioned above were at the later stages of fattening, and were used to estimate more accurately the potential still-air flight range of individuals just before departure to the winter quarters. The flight range of individuals at departure was also estimated from the upper quartile of the weights of birds captured during September at both main sites separately.

7.3 Results

7.3.1 Phenology of fuelling and migration

In total, 913 captures and 232 retraps were analysed. No birds were retrapped between ringing sites. The number of adults, juveniles and nestlings captured per five-day period (pentade) are presented in Figure 7.1. The adults were captured from mid-March to early October. The peak in abundance, and presumably the departure date of the Portuguese population, was at pentade 49 (29 August-2 September, counting pentade 1 as from 1 January), after which the number of adults declined dramatically. Juveniles were captured from mid-May to the beginning of October. They were more abundant in pentade 44 (4-8 August), that is, about three weeks earlier than adults, and their numbers did not drop so steeply. Therefore, on average adults departed to the winter quarters later than juveniles and migrated more synchronously.

As is shown in Figure 7.1c, some birds were still in the nest in mid-August, just after the peak in juvenile abundance, which shows that there is some overlap between breeding and the onset of juvenile autumn migration. The high percentage of retraps particularly of adults during summer, suggests that most individuals were local breeders.

The variation in the weight and fat score of adults and juveniles captured in Portugal is presented in Figure 7.2. Adults were lower weight and had less fat in July and early August, presumably associated with the end of the breeding season and moult, and increased dramatically in both weight and fat from late August, which correspond with the time when their abundance declined. The regression analysis of both weight and fat on date (day 1 = 1 August) are highly significant (Weight = $0.07(\text{Date}) + 13.292$, $R^2 = 0.220$, $F_{[1,104]} = 29.387$, $P < 0.001$; Fat = $0.06078(\text{Date}) + 0.0098$, $R^2 = 0.307$, $F_{[1,94]} = 41.62$, $P < 0.001$).

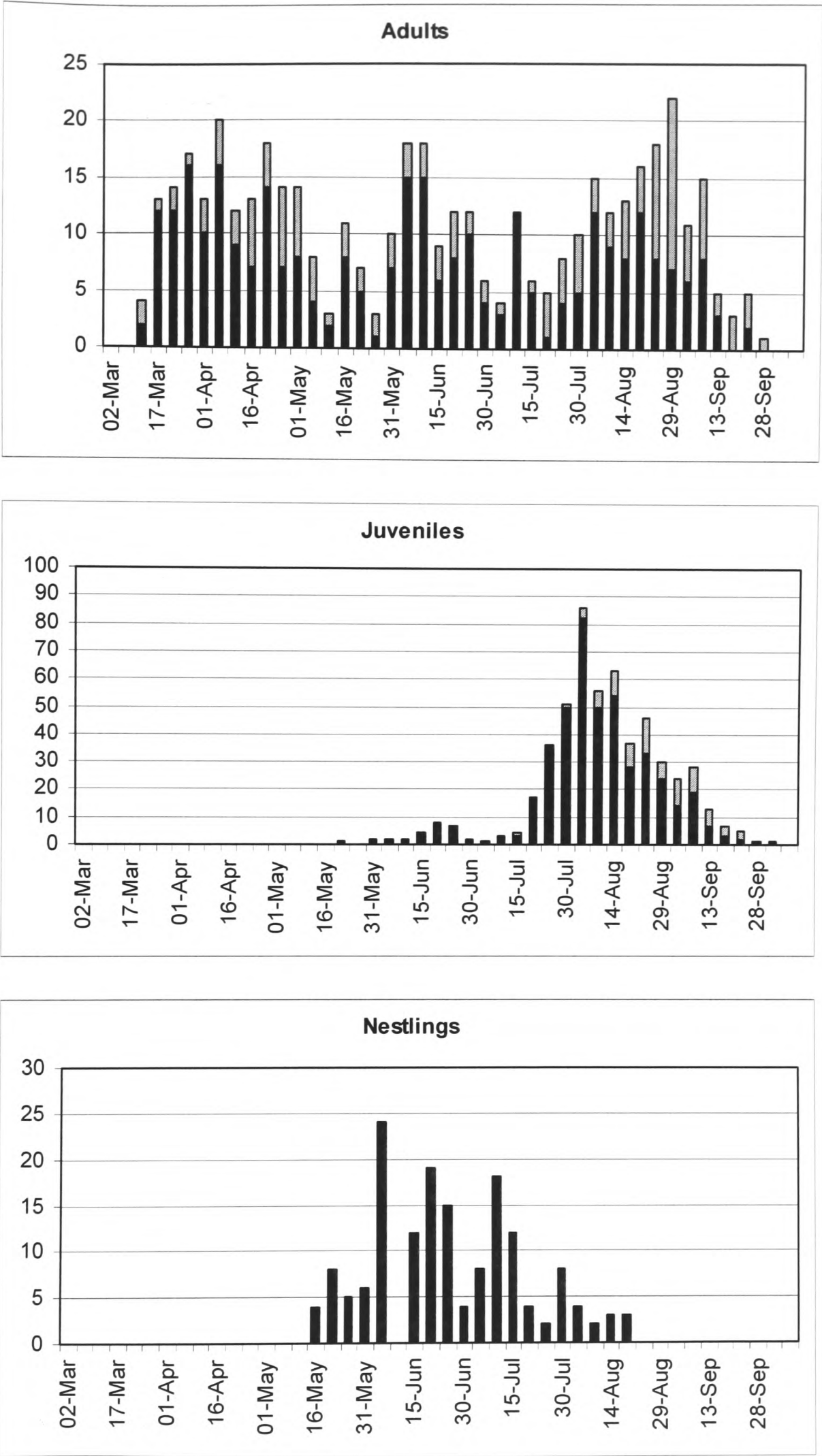


Figure 7.1 – Seasonal variation in the number of adults, juveniles and nestlings captured (black) and retrapped (grey) in Portugal from 1997 to 2002.

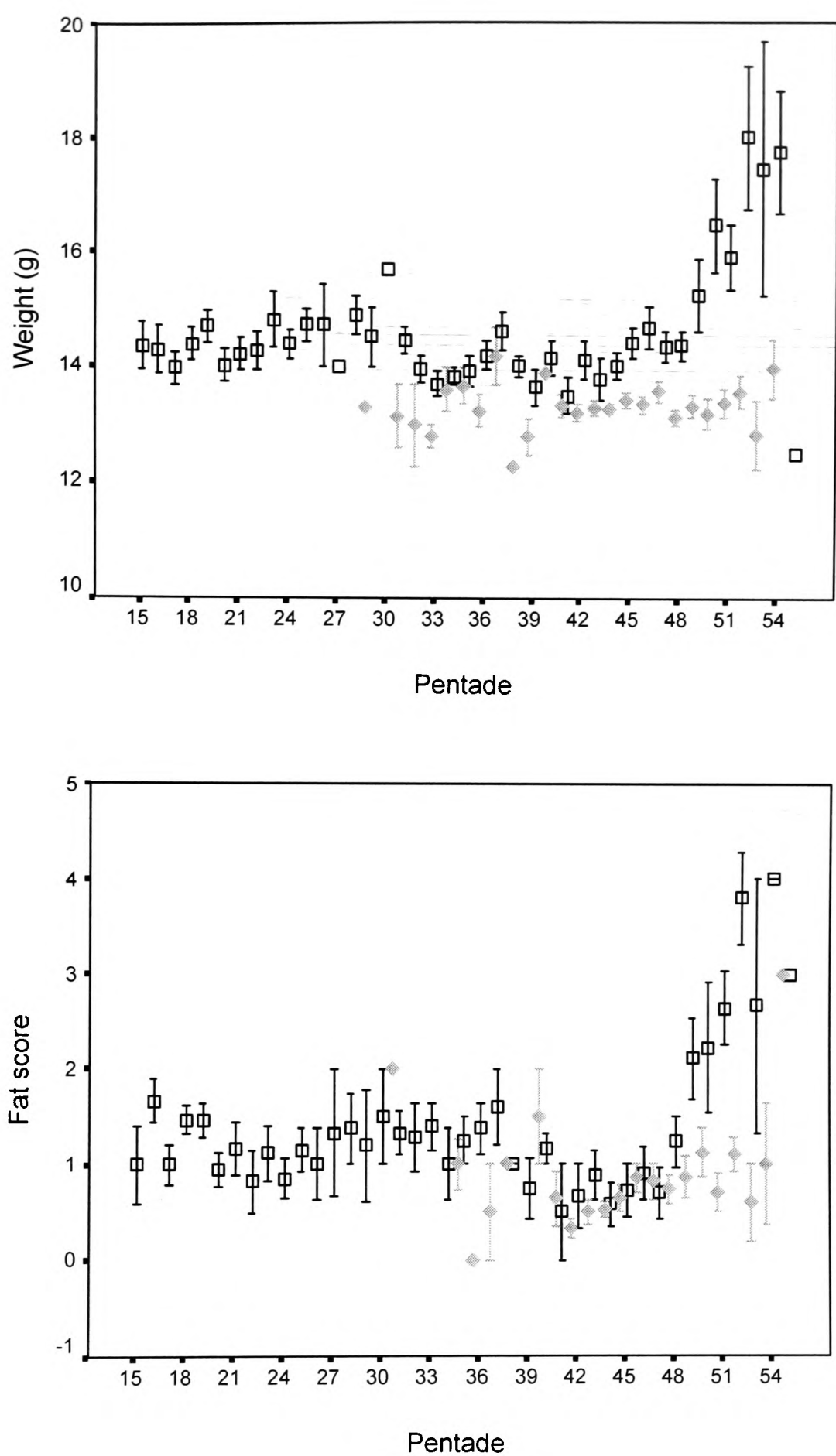


Figure 7.2 – Seasonal variation in weight and fat score (average and S.E. per five-day period) of adult (black) and juvenile (grey) Savi's Warblers captured in Portugal from 1997 to 2002.

Juveniles seem to have increased slightly in weight and especially in fat in mid-

June and from August onwards (Figure 7.2), but it is not clear whether this represents two migratory or dispersal waves, or if the first peak was caused by the generally greater weight and fat of fledglings, which tend to decrease in fat and weight during the first few weeks after leaving the nest (pers. obs.). In contrast to adults, juveniles did not increase in weight, but increased significantly in fat, from August onwards (Regression analysis: $\text{Weight} = 0.0024(\text{Date}) + 13.339$, $R^2 = 0.001$, $F_{[1,349]} = 0.31$, $P = 0.578$); $\text{Fat} = 0.0147(\text{Date}) + 0.494$, $R^2 = 0.035$, $F_{[1,325]} = 11.923$, $P = 0.01$). In a GLM analysis using the weight as the dependent variable and wing length, year and site as covariates (Model: $F = 5.8$, $P < 0.001$), age ($F = 103.0$, $P < 0.001$), date ($F = 4.0$, $P < 0.001$) and their interaction ($F = 3.6$, $P < 0.001$) were highly significant, as the adults were heavier than juveniles and increased in weight, whereas the weight of juveniles remained constant. When fat is used as the dependent variable, the result is similar (Model: $F = 3.091$, $P < 0.001$; Age: $F = 29.61$, $P < 0.001$; Date: $F = 2.377$, $P < 0.001$; Age vs Date: $F = 1.697$, $P = 0.009$).

As the heavier and fatter individuals caught in late summer could differ from the ones captured earlier, for instance if they were passage migrants, the within-year recaptures were analysed to determine the changes in body condition within individuals during the pre-migratory period (Figure 7.3). Adults tended to decrease in weight in June, July and early August, whereas most recaptures made in late August and September indicated the occurrence of pre-migratory fuelling. This contrasts with juveniles, which showed a great variation in body weight change during summer: some individuals obviously fattened up in July, August and September, whereas others seemed to decrease in weight even in September. Hence, fattening in juvenile birds seems to be asynchronous, whereas adults are remarkably synchronous. The existence of long-term within-year retraps indicates that many of the individuals were local birds, which fattened

up in their breeding quarters shortly after moult.

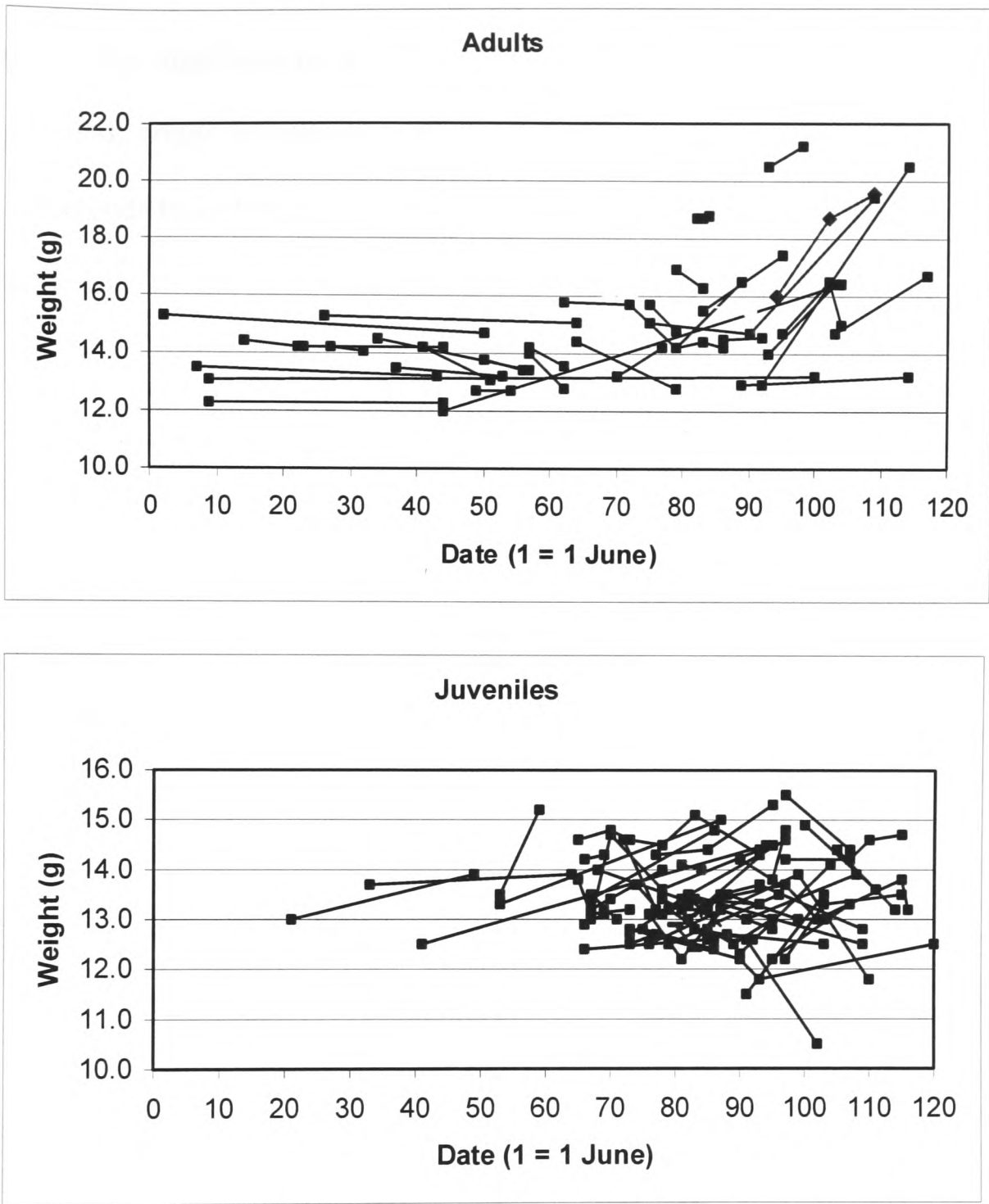


Figure 7.3 – Changes in weight of adult and juvenile Savi’s Warblers recaptured during summer in Portugal.

7.3.2 Fuel deposition rates

The body mass changes of adults and juveniles recaptured in the same year during the fuelling period are presented in Figure 7.4. The FDR of both age classes were

estimated from the regression lines presented in Figure 7.4 (Adults: Body mass change = $0.347(\text{Number of days}) - 1.328$, $R^2 = 0.826$, $F_{[1,10]} = 47.5$, $P < 0.001$; Juveniles: Body mass change = $0.0434(\text{Number of days}) - 0.568$, $R^2 = 0.197$, $F_{[1,15]} = 3.67$, $P < 0.074$.) The slopes differ significantly ($t_s = 5.5$, $P < 0.01$). Hence, during the pre-migratory fattening period, adults increased 0.347 g day^{-1} and juveniles 0.043 g day^{-1} in weight, which corresponds to 2.59% and 0.33% of the LBM, respectively. The FDRs were related neither to the date of first capture nor to the initial body weight ($P > 0.05$).

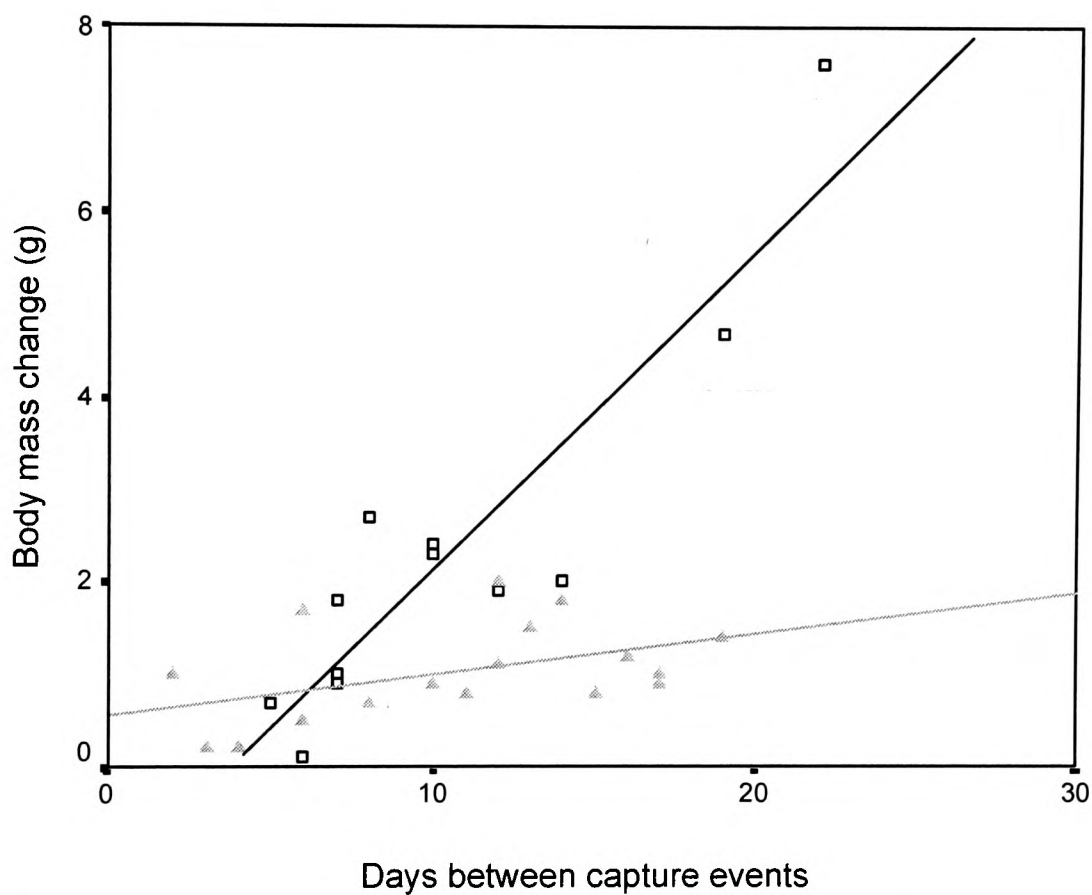


Figure 7.4 – Body mass change between capture and recapture in adult (black) and juvenile (grey) Savi’s Warblers captured during the pre-migratory (fuelling) period. Regression lines, adults: Body mass change = $0.347(\text{Number of days}) - 1.328$, $R^2 = 0.826$, $F_{[1,10]} = 47.5$, $P < 0.001$; Juveniles: Body mass change = $0.0434(\text{Number of days}) - 0.568$, $R^2 = 0.197$, $F_{[1,15]} = 3.67$, $P < 0.074$.

7.3.3 Potential still-air flight range

The pre-migratory season was divided into four periods: two before the adult onset of migration (August 1 and 2) and two afterwards (September 1 and 2, see Table

7.1). The weight and average potential still-air flight range of adults and juveniles captured and recaptured during these periods are presented in Table 7.1. Juveniles had an average flight range of about 125 Km, increasing only slightly at the end of September, whereas adults had much greater average flight ranges (from 530 to 1630 Km), which increased throughout the pre-migratory period, particularly from August to September.

Table 7.1 – Weight and average potential still-air flight range of adult (age 4) and first-year (age 3) Savi’s Warblers captured in Portugal. August 1 corresponds to the pentades 44-46, August 2 to 47-49, September 1 to 50-52 and September 2 to 53-55. The weight was significantly greater in adults during all periods (Mann-Whitney U test, $P < 0.01$ except in September 2 in which $P = 0.095$).

Period	Age	Weight $\pm$ s.e. (g)	Range (g)	n	Flight range (Km)
August 1	3	13.354 $\pm$ 0.066	11.5 – 16.0	184	125
August 2	3	13.339 $\pm$ 0.0974	11.5 – 16.6	106	121
September 1	3	13.350 $\pm$ 0.160	10.1 – 15.5	54	125
September 2	3	13.427 $\pm$ 0.413	10.5 – 15.5	11	160
August 1	4	14.379 $\pm$ 0.168	12.7 – 16.9	33	529
August 2	4	14.698 $\pm$ 0.253	12.5 – 20.5	45	680
September 1	4	16.446 $\pm$ 0.449	12.5 – 21.2	28	1497
September 2	4	16.733 $\pm$ 1.326	12.5 – 20.5	6	1628

The potential still-air flight range estimated for the five individuals captured in September at Ria de Aveiro, which had their wing area and span measured and were closer to the later stages of fuelling, varied from 1567 to 3469 km (mean = 2749 $\pm$ 342 Km). This corresponded to flight times of 36.5 to 72.6 hours (mean = 60 $\pm$ 6.5 Hours). The flight range of the heaviest birds captured at Ria de Aveiro during September (upper quartile: adults – 19.9 g, juveniles – 14.2 g) was 2972 Km for adults and 538 Km for juveniles. The corresponding flight ranges for birds trapped at Lagoa de Santo André (upper quartile: adults – 18.5 g, juveniles – 14.55 g) were 2397 and 694 Km. Therefore, a large percentage of adults captured during September at both sites would be able to cross the Mediterranean and the Sahara without refuelling whereas the juveniles would only be able to reach the north African coast. The difference between the two main sites seems to

be negligible.

7.4 Discussion

The phenology of migration differed between age classes. Juveniles departed for the winter quarters about three weeks earlier than adults, and both age classes were captured in Portugal until early October. Therefore, adults were more synchronous in their post-breeding migration schedule. Dürr *et al.* (1995) suggested that juveniles ringed in eastern Germany departed in mid-August whereas adults migrated earlier (from mid-July). However, this is unlikely because Savi's Warblers in Germany also breed until August and many individuals seem to undertake a post-breeding moult (Dittberner & Dittberner 1991, Steiner 1970, Stresemann & Stresemann 1970). It is probable that, as in Portugal, adults migrated from the end of August, when a peak in abundance was reached and from which the number of birds ringed dropped steeply (Figure 3 in Dürr *et al.* 1995).

Although breeding is quite asynchronous in this species (§Chapter 3), the variable post-breeding moult undertaken by most, if not all, adults seems to be an important factor in synchronising the adult migration, because the sequence and extent of this moult is adjusted to the time available between breeding and migration (§Chapter 5). Due to the long breeding season, juveniles also fledge very asynchronously, but the variation in the extent of the post-juvenile moult does not seem to be enough to synchronise juveniles in relation to migration. In fact, some juveniles departed to the winter quarters while others were still at the nest, thus having a more extended departure period than adults. The same factor might be important in explaining the differences in migration schedule between the age classes of different species. It is possible that the occurrence of a complete post-breeding moult delays the migration of adults, which may leave the breeding quarters at

the same time as juveniles or even later. This occurs in Savi's Warblers and many of the *Sylvia* species, whereas in species that do not have a complete moult in the breeding quarters (*Acrocephalus* warblers, *Sylvia borin*), adults seem to migrate earlier than juveniles (Basciautti *et al.* 1997, Fransson 1995, Neto 2003).

As expected, the date of departure of adults corresponded closely to the appearance of heavy individuals, which were presumably ready to make a long-distance flight. Although the within-year retraps of adults indicated the occurrence of fuelling only after the departure date (possibly due to small sample size), fuelling probably started in mid-August. Juveniles showed a very different pattern of pre-migratory fuelling, being very asynchronous and increasing only slightly in weight. In fact, juveniles never reached weights over 17 g (maximum recorded 16.6 g), whereas adults often weighed more than 19 g (maximum 21.2 g). In addition, within-year retraps indicated that the FDR is much higher in adults than in juveniles (2.59% and 0.33% of the LBM, respectively). Although these values were estimated from relatively small samples taken from more than one site, the difference in fuelling between the age classes seems to be realistic. Even if the retraps of juveniles included periods during which no fuelling was taking place, because the fuelling period is more difficult to identify in juveniles since they may not moult and gain less weight and fat than adults, it is clear from the data that the age classes build up different amounts of body reserves before migration, and at different rates. The lower FDR of juveniles may be associated with a lower feeding efficiency, or may result from competition (Lindström *et al.* 1990, Moore & Yong 1991). There are few published data on FDR of different age and sex classes. Ellegren (1991) found no difference in FDR between adult and juvenile Bluethroats, *Luscinia svecica*, at a stopover site in Sweden. Rguibi-Idrissi *et al.* (2003) found identical FDR in Reed Warblers, *Acrocephalus scirpaceus*, in one site in Morocco at both seasons, but in another site the adults tended to

have lower FDR. Presumably, the difference in the FDR of adults and juveniles changes along the migration route. For instance, juvenile Savi's Warblers stopping over in North Africa are expected to have higher FDR than adults, as they leave the breeding quarters with low fuel stores and need to fatten up before crossing the Sahara.

As the maximum FDR of passerines the size of Savi's Warblers is approximately 6% of LBM, the FDR found in this study indicates that in Portugal Savi's Warblers are 'foraging-time limited' migrants, i.e., they are limited by food availability (Lindström 1991). However, Alerstam & Lindström (1990) pooled data on FDR from a large number of studies of natural populations and found a median value of 2.4%, which is quite similar to the one found for adult Savi's Warblers.

The fact that juveniles leave with lower fuel stores than adults is in agreement with their lower FDR according to the models of time-minimization during migration (Alerstam & Lindström 1990, Lindström & Alerstam 1992, Weber & Houston 1997), which predict a positive relationship between FDR and fuel load at departure. They also leave earlier, which could be a strategy to avoid competition at the stopover sites along the migration route, as juveniles are subdominant, and dominance influences FDR at stopovers (Lindström *et al.* 1990). There are few potential stopover sites along the migration route from Portugal to sub-Saharan West Africa. Furthermore, at the end of summer, the food availability is probably smaller at these sites than it is in the wetlands of the Iberian Peninsula, because of drought conditions. However, in contrast to adults, juveniles might have a greater FDR at this sites than at the breeding sites due to lower competition. Therefore, accordingly to time-selected migration models, they should leave the breeding sites in Portugal as soon as they have enough fuel to reach the next staging areas for which the FDR would be higher. The short flight range of juveniles estimated in

this study agrees with this prediction. But, the FDR of juveniles at stopovers in Africa is unknown and so the energy-selected migration model cannot be excluded (Alerstam & Lindström 1990).

The flight range estimates strongly suggest that the majority of the adults would be able to reach sub-Saharan Africa or the wintering sites without refuelling, particularly if the wind speed is taken into consideration. Therefore, adults showed signs of overloading and probably by-pass potential staging sites, which is expected in time-selected migrants (Gudmundur *et al.* 1991). Studies of FDR at several stopover sites along the migration route, as the ones made by Schaub & Jenni (2000, 2001), but using individuals of both age classes, are needed to understand the difference in migration strategies between adults and juveniles, and the effect of competition. Judging from the migration strategies described here, it is predicted that the proportion of juveniles at stopover sites in south Europe and North Africa is much larger than at breeding sites, and they should have greater FDR and fuel loads. It remains to be shown whether eastern and northern populations have similar migration strategies to the Portuguese population.

CHAPTER 8

General discussion

Several aspects of the breeding biology, moult and migration of a Portuguese population of Savi's Warblers were addressed in this study, with the purpose of increasing our knowledge of this little-studied species and of comparing it with other populations and species. A particular effort was made to describe its unusual moult strategy. In fact, the most interesting results of this study arose from the application of stable isotope techniques to determine the winter moult extent in migratory birds, as this is a novel application of this method.

It was shown that Savi's Warblers arrived in the breeding quarters earlier than other reedbed migratory passerines. They also bred earlier, which suggests that the early arrival of Savi's Warblers is not caused by competition for territories being stronger than that in other species. It was also shown that although this species only occurred in large reedbeds and fed in pure reed stands, its nesting habitat was much denser, usually of *Juncus* or *Scirpus* with or without reeds. Its nesting habitat might be the reason why Savi's Warblers breed earlier than, for instance the *Acrocephalus* warblers, as the latter species are dependent on the growth of reeds to build their nests. This was also suggested by Schulze-Hagen *et al.* (1996) to explain the later breeding season of *Acrocephalus palustris*, which builds its nests in a habitat of reeds and nettles, available only later in the season. Other potential ecological reasons for the differences in breeding phenology between reedbed species involve the variations in diet and feeding technique, which may result in different peaks of food abundance for each species.

As in other species, the date of arrival and breeding was negatively correlated with

the breeding success. Early-arriving males had shorter tarsus and bill and more fat, which suggests that smaller males invested more in getting a good territory due to their low resource-holding power. The greater fat, however, may be related to starvation risk early in the season rather than to male quality or condition. Although the growth bars of the tail feathers are very conspicuous, the ptilochronology method does not seem to be appropriate to measure body condition in this species, because many of the adults, but not all, and the first-year birds moult the tail feathers during the winter and thus are not directly comparable. The mating system is 'facultative polygynous', as some males mated with two females even though the sex ratio was very biased towards males. Although Aebischer *et al.* (1996) suggested that females may settle randomly on arrival within occupied territories, polygynous females seem to choose the early-arriving males or their territories, because these females are largely responsible for the higher breeding success of early-arriving males. Therefore, there must be some cues that females use to assess male or habitat quality.

The return rate was rather low for adult birds, but this could have resulted from the fact that the study area was located within a large reedbed, which could not be fully surveyed. The return rate of first-year birds was extremely low, as would be expected in a species that has a metapopulation structure.

Both clutch and egg size were smaller in the Portuguese population than those reported elsewhere. Egg size was dependent on female size, as measured by tarsus length. A comparison with the Swiss birds strongly suggested that the difference in egg size between populations is almost fully explained by differences in female size, but other populations and factors should be considered in future comparisons. Clutch size might follow a latitudinal gradient, but may also be dependent on predation risk and food

availability. Interestingly, clutch size seemed to increase and then decrease during the season, as in resident birds. This may be related to the early arrival and long breeding season of this migratory species.

The major cause of nest loss was predation, being responsible for a nesting success of only 40%. However, the relatively high number of clutches per female in each season resulted in a relatively high productivity (5.38 fledglings per season), which is sufficient to compensate for the typical adult and juvenile mortality in passerines. Therefore, the population studied is probably a source population. This also seems to be the case for other populations studied, which are generally less heavily predated and have larger clutches. This species seems to be dependent on or constrained by the available habitat, which has been decreasing markedly in Europe. The facts that a few breeding females were predated with their nestlings, and that females decreased more in body condition (particularly in muscle score) than males during the breeding season, may be responsible for the biased sex ratio of the adult population.

Many juveniles had a partial moult shortly after fledging, which could include the tertials and greater coverts, but others (probably later fledging birds) did not moult at all prior to migration. Retraps showed that first-year birds had a complete or interrupted moult in Africa, retaining a few inner primaries and/or secondaries. Those that retained some feathers started the first post-breeding moult by replacing them, which probably caused the appearance of birds with an unusual moult sequence for this species. The moult strategy of the first-year birds seems to involve one or two moults, the first being partial and the second almost complete. However, it is possible that the partial post-juvenile moult is actually suspended during the autumn migration, and so they may have only one complete or almost complete moult during their first year. It was suggested that

some first-year birds replace some flight feathers before autumn migration (Peter Fearon, personal communication) and, in fact, one juvenile replaced at least one primary, which supports the second hypothesis (suspension of post-juvenile moult).

From July onwards, all males and very few females overlapped breeding and migration. This suggests that for later breeding birds, there is a conflict between breeding duties and moult, and this is partly compensated in males by overlapping these activities. Females started moulting later and replaced fewer remiges. Another adjustment to the short time available, which has not been recorded for other species, was the sequence of moult: later moulting birds tended to start moulting in a primary located in the outer wing rather than in the inner wing, and so the outer primaries were always replaced before migration. In Savi's Warblers, the impaired flight due to the post-breeding moult was not compensated for by reducing the weight and, in fact, birds with large gaps in their wings were significantly heavier, probably because of an increase in water associated with feather growth.

Stable isotope analysis of feathers taken from known adult birds showed that the pre-breeding moult is very variable in extent. A few birds did not moult the remiges even though they seemed to have moulted some body feathers and even rectrices. Others replaced the retained remiges from the previous post-breeding moult and also moulted the body feathers, some or all rectrices and a variable number of additional remiges, rarely all. From these data it seems that the moult strategy of most adults is a partial biannual moult of remiges, in which the post-breeding moult is suspended and the pre-breeding moult suspended or arrested, which was not described by Jenni & Winkler (1994) in their review of moult strategies. However, the individual variation in the extent of the moults indicate that the strategy might vary from a complete biannual moult to complete post-

breeding moult followed by partial pre-breeding moult. It is not clear whether some individuals migrate to the winter quarters without moulting the remiges, as was suggested by Mead & Watmough (1976), but this seems unlikely. According to Howell *et al.* (2003), the Savi's Warbler moult strategy should be described as a 'Simple Alternate Strategy' or a 'Complex Alternate Strategy' depending on whether the post-juvenile moult and the first pre-breeding moult were considered the same (suspended) or different, respectively.

The post-breeding, and apparently first pre-breeding and pre-breeding moults of Savi's Warblers are adapted to replace the most important feathers for migration (the outer primaries), which are often shed twice a year. This probably results from their skulking behaviour, very dense habitat and very pointed wings, which may become more worn than those of other species. The moults of the other *Locustella* warblers are also variable and rather unusual, but their strategies are not well known, except, maybe, the River Warbler, *Locustella fluviatilis*, which is an eastern migrant that occurs in a different habitat. They also have a partial biannual moult of remiges, but the post-breeding moult occurs in Africa and is arrested within the primaries, following the normal sequence. Its pre-breeding moult occurs further south in Africa and is complete (Pearson & Blackhurst 1983). Therefore, there is a huge variation, even among closely related species, both in sequence and extent of moult. In order to determine which ecological factors influenced the evolution of this adaptation, more data are needed about the moult strategies of many of the reedbed species. The use of stable isotope analysis might prove to be very useful for this purpose, as they are migratory passerines that moult at isotopically distinct sites, but data from retraps and detailed observation of moult contrasts are also essential.

As was suggested by Cramp (1992) the migration strategy of the Savi's Warbler

involves a large accumulation of fuel and long non-stop flights. Juveniles, however, did not build up large fuel reserves despite the major ecological barriers (the Mediterranean sea or the Atlantic, depending on the direction of flight, and the Sahara desert) being so close to Portugal. Juveniles departed for the winter quarters earlier than adults, which is the opposite of what occurs in *Acrocephalus* species (Neto 2003). For instance, adult Reed Warblers occur in the study area until the beginning of September whereas juveniles occur until October. It seems that the migration of adult Savi's Warblers was delayed by the post-breeding moult. This may be a general phenomenon, which is supported by the phenology of migration of other Sylviid species (Basciautti *et al.* 1997, Fransson 1995, Neto 2003).

The migration strategy of other populations of the Savi's Warbler, particularly those that winter in central and eastern Africa might be quite different. Studies of fuelling rate and migration strategy across the Savi's Warbler's range, might prove to be very interesting, particularly if moult studies are also performed in the same populations. This would allow a better understanding of the ecological factors that influence the variations in moult, migration and the interaction between these activities. Estimates of fuel deposition rates at stopover sites would also permit a better understanding of the differences in migration strategy between age classes.

CHAPTER 9

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