Managing time in a changing world
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Chapter 1

Managing time in a changing world: timing of avian annual cycle stages under climate change

Barbara M. Tomotani
Timing of stages in complex annual cycles

Organisms are exposed to changes in their environment that can be predictable, especially if they occur periodically. Predictable changes are particularly common in temperate environments which have large annual variations in day length, temperature and precipitation. Organisms can then use cues to time their activities over the year in such a way that they maximise their fitness. We observe these as within-individual phenotypic changes in morphology, physiology and behaviour, i.e. as rhythms (biological events that repeat themselves in a periodic way), which are tuned to the environmental conditions (Enright 1970; Moore-Ede et al. 1982; Gwinner & Pévet 1987; Jacobs & Wingfield 2000; Dawson 2008; Wingfield 2008).

It is possible to identify particular sets (or “syndromes” sensu Wingfield 2008) of annual phenotypic changes expressed during a specific time of the year. These sets involve a suite of behavioural/morphological/physiological features related to a specific process that usually progresses in a regular way (Jacobs & Wingfield 2000; McNamara & Houston 2008; Wingfield 2008). They are termed “annual routines”, “life-history stages”, “life-cycle stages” or “annual cycle stages” (Jacobs & Wingfield 2000; Helm & Visser 2006; Dawson 2008; McNamara & Houston 2008; Wingfield 2008). In this thesis, I will use “annual cycle stages” to denote stages during adult life unless explicitly stated otherwise (e.g., Chapter 8). Thus, these stages should not be confused with the “life-stages” denoting development (e.g., larvae, pupae, nestling, adult etc.). Some typical examples of annual cycle stages in animals are breeding, migration and moult.

There is a relatively large diversity of strategies in allocation of annual cycle stages (Jenni & Winkler 1994; Helm et al. 2005). These different strategies generate diversity in the complexity of annual cycles: while some annual cycles are fairly simple, with only a transition from breeding to a non-breeding stage, others present several events in a single year. Because the period for allocating all stages remains one year, but the time available for each stage is reduced, a more complex annual cycle will also be a more time constrained one (Pengelley 1974; Jacobs & Wingfield 2000; Helm et al. 2005; Dawson 2008; Wingfield 2008).

Regardless of the complexity of the cycle, each stage has to be timed to the appropriate environmental conditions. Because the optimal windows may be very narrow, the timing of annual cycle stages can be under considerable selection pressure: if stages are allocated too early or too late in time, they will incur fitness costs (Verhulst & Nilsson 2008). For example, in some birds the offspring value declines with the progression of the season and late born offspring are less likely to recruit (Drent & Daan 1980; Daan et al. 1988; Verhulst & Nilsson 2008), as timing of breeding may depend on a narrow peak of food availability for the offspring (Perrins 1970; Drent & Daan 1980; van Noordwijk et al. 1995; Visser et al. 2006). However, laying eggs too early may be costly
due to the energetic constraints of producing an egg early in the season, related to food availability and inappropriate weather conditions (Perrins 1970; Drent & Daan 1980; Visser et al. 2012).

Apart from being synchronised to the environmental cycles and constrained by short optimal windows, different stages must also be appropriately timed in relation to one another. The energetic requirements of different stages often make their concomitant occurrence incompatible (Rutledge 1974; Piersma 2002; McNamara & Houston 2008; Wingfield 2008). Migratory birds are an example of this conflict as the reproductive and migratory states often require incompatible behavioural and morphological changes, such as nesting behaviour, courtship, plumage and egg production during reproduction versus organ reduction (including the reproductive system), muscle and fat storage and the migration itself (Dietz et al. 1999; Piersma 2002; Ramenofsky & Wingfield 2006). If the organism possesses a complex annual cycle and needs to allocate several of these energetically demanding activities in a single year, the time constraints may force the adoption of potentially costly time-saving strategies, such as overlapping stages, shortening their duration, or shifting the stage in time and potentially moving it outside its optimal window (Dawson 2004, 2008; Crozier et al. 2008; Wingfield 2008; McNamara et al. 2011).

Complex annual cycles thus require a balance between two aspects: on the one hand, organisms need to avoid being too early or too late, matching the occurrence of stages to the optimal environmental conditions. On the other hand, they should avoid overlapping or shortening stages (Fig. 1.1). So how could they ensure this balance?

**How do animals organise their annual cycle stages?**

To be able to appropriately allocate each of their annual cycle stages, individuals must track the within- and between-year variation of the optimal windows to adjust their stages accordingly. Allocating each stage, however, is not a straightforward decision: many stages require preparation, such as physiological changes or accumulation of resources, so decisions have to be made well in advance (Enright 1970; Gwinner & Pévet 1987; van Noordwijk & Müller 1994; Visser et al. 2004, 2010).

**Animals use an internal “calendar”**

Biological rhythms are present in virtually all organisms, from bacteria to multicellular eukaryotes. The mechanisms of synchronisation between biological rhythms and environmental cycles are well known for daily rhythms. For instance, it is widely recognised that a great number of daily rhythms (e.g., activity/rest) are endogenously generated by a circadian clock, as these rhythms persist even in the absence of temporal
cues (Pittendrigh & Daan 1976; Aschoff 1981). The existence of circadian clocks allows the organism to track the time of day even when environmental cycles are not immediately available (Enright 1970).

The mechanisms involved in the timing of annual rhythms are much less understood and may be strongly species-specific or even case-specific (Menaker 1974; Paul et al. 2008; Visser et al. 2010). For instance, seasonal endogenous rhythms that persist in constant conditions have already been reported for bird migratory activity, fuelling, feather replacement and gonadal development/regression (Holberton & Able 1992; Dietz et al. 1999; Gwinner 2003; Maggini & Bairlein 2010). This suggests the existence of a circannual clock analogous to a circadian clock (Nisimura & Numata 2001). Other studies, however, show that this mechanism is not the single explanation for all annual rhythms or for all taxa (Zucker 2001; Paul et al. 2008).

The analogy between annual rhythms and the circadian system may become particularly oversimplified when organisms with several annual cycle stages are considered (Pengelley 1967, 1974; Menaker 1974; Mrosovsky 1970). The system could still have a single main oscillator or each stage could involve a different oscillator. Studies with birds in captivity show that when individuals are kept at constant day length, distinct annual rhythms free-run with different endogenous periods (Gwinner 1986; Karagicheva et al. 2016). This points out that, at least in these birds, different rhythms could be regulated by distinct endogenous oscillators with different periods. It was also debated, however, that the temporal allocation of consecutive stages could be dictated solely by the timing of the previous stage and, thus, the next stage would initiate only upon completion of the previous one (Pengelley 1967; Enright 1970; Mrosovsky 1970). The “finite-state machine” (Jacobs & Wingfield 2000), is yet another proposed mechanism in which organisms express a finite number of functionally different stages defined by a combination of appropriate sub-stages and the transition to the next stage occurs only when certain conditions are met (Jacobs & Wingfield 2000).

Regardless of the mechanism involved in generating annual rhythms, there is consensus that the variation in day length (photoperiod) is one of the major cues used by organisms to time their annual cycle stages (Gwinner 1989b). Organisms are able to precisely track and interpret the changes in the proportion of light and darkness (Gwinner 1989b; Bradshaw & Holzapfel 2007; Dawson 2015), even the small variation in the tropics (Hau 2001). And, in contrast with the previous debate, how animals measure photoperiod is well understood at the molecular level at least for the timing of reproduction of birds and mammals (e.g., Yoshimura et al. 2003; Hanon et al. 2008; Nakane & Yoshimura 2014), but less so for other vertebrate annual cycle stages (but see Hut et al. 2014). The importance of photoperiod as a cue synchronising annual rhythms becomes evident in studies with captive birds, in which a shortening of the period of the photoperiodic cycle from 12 months to one and a half month resulted in a more or less equivalent
shortening in the period of the rhythms of moult and testes sizes of European starlings (*Sturnus vulgaris*) (Gwinner 1981). These birds displayed up to four complete cycles of moult and gonadal regression/growth in six months (thus in one year they would have eight complete cycles instead of a single one).

The photoperiodic cycle is virtually invariable between years and, therefore, constitutes the most precise environmental cue. The timing of several seasonal rhythms in birds (*e.g.*, moult, migration and gonadal development) is strongly dependent on photoperiodic information (Gwinner 1989b; Dawson 2008). Photoperiod allows the organisms to know the time of year. It thus allows long-term prediction for the within-year transitions, for instance, by triggering gonadal development in anticipation of the breeding season (Gwinner 1989b; Silverin *et al.* 1993; Wingfield 2008; Hut & Beersma 2011; Dawson 2015). Even during the migration of birds, when the photoperiodic exposure is almost literally chosen by the individual, day length is known to serve as a source of information for the time of year and cause advances and delays on the timing of events at the breeding grounds (Helm & Gwinner 2006).

**Supplementary cues allow tracking optimal conditions**

While photoperiod gives important information to track the time of year, it cannot be used to track year-to-year changes in optimal conditions due to its lack of year-to-year variation. But the variation in the timing of annual cycle stages is still observed from one year to the next. For example, birds lay their eggs earlier when it is warmer (Meijer *et al.* 1999; Visser *et al.* 2009) and warmer temperatures also correlate with an earlier peak in insect availability, an important event for birds that need to feed their chicks (van Noordwijk *et al.* 1995; Visser *et al.* 1998, 2006; Visser & Both 2005). Thus, “supplementary cues” are important and organisms need to be able to time their stages also in relation to them.

Supplementary cues allow tracking optimal conditions

The same genotype can produce different phenotypes, depending on the environment, in a non-random way; this is termed phenotypic plasticity (Stearns 1989; van Noordwijk & Müller 1994). It is the case for traits that are produced once in a lifetime (*e.g.*, the age of maturity, growth; Stearns 1989) or several times (*e.g.*, laying dates; Stearns 1989). In this definition, the timing of annual cycle stages can be seen as plastic traits that are produced multiple times in an organism’s lifetime. Thus, the year-to-year variation in timing of stages (*e.g.*, laying dates in birds) is due to plasticity, which allows the organism to fine-tune its activities to the variation in the conditions of the environment (Fig. 1.1: “drivers of selection”; Visser *et al.* 2004). In different years, the relation between fitness (*e.g.*, number of recruits) and a given trait (*e.g.*, egg-laying date) is different (*i.e.*, the fitness landscape varies), because the drivers of selection also vary from year to year (*e.g.*, food peak or weather conditions; Visser *et al.* 2004).
An important point to consider is that the environment in which the animals are making the decisions is rarely the same in which selection will take place (van Noordwijk & Müller 1994; Visser et al. 2004; Visser 2008) as decisions are made weeks in advance. In the egg-laying date example above, to use the peak in food availability for the chicks, females need to make the decision on when to lay their eggs weeks before the food peak is actually there, as the interval between egg-laying and chick hatching (which should coincide with this food peak) may be longer than one month. Thus, they must use cues from the present environment (environment of decision making) to predict conditions of the future environment (environment of selection). Therefore, to be useful, these cues need to correlate in some way to the variation in the drivers of selection (van Noordwijk & Müller 1994; Visser et al. 2004; Visser 2008). As explained above, photoperiod is invariable from one year to the next, so it cannot be a supplementary cue. Temperatures, however, are useful cues, as they either correlate with or actually determine the phase of the year when environmental conditions are best suited for the allocation of stages (van Noordwijk & Müller 1994; Visser et al. 1998, 2004, 2009; Visser 2008). Moreover, temperatures are particularly important cues in temperate regions, being used by many organisms for fine-tuning their stages (Visser 2008).

As an analogy, we can think that the photoperiodic variation serves as a calendar, allowing organisms to track the time of the year. However, it is the supplementary cue (e.g., temperature) that will tell when it is the correct moment for a certain activity to take place. In this case, the photoperiod is responsible for triggering the development of a stage in preparation for the moment of decision-making when the supplementary cue will be present, which will then trigger the event itself (Wingfield 2008). Another way of looking at this is that the photoperiod modifies the way the supplementary cue affects the event. For example, high temperatures early in the season have a stronger effect in triggering laying dates than later in the season (Gienapp et al. 2005). Finally, the endogenous timekeeping mechanism will allow the organism to keep track on time even when cues are not readily available (Enright 1970; Hedenström et al. 2007; Paul et al. 2008).

Because the drivers of selection and the fitness landscape are species-specific, the supplementary cue used for fine-tuning will equally depend on the species and/or population in question (Dawson 2008). For example, rainfall is an important supplementary cue in some cases; for instance, it signals grass growth and thus optimal breeding time for zebra finches (*Taeniopygia guttata*; Zann et al. 1995) similarly, wind may be an important cue for the optimal timing of migration in birds, signalling en-route conditions (Erni et al. 2005; Sinelschikova et al. 2007; Bauer et al. 2008; Eikenaar & Schmaljohann 2014).
Causal relation between stages aids their temporal segregation

Yet another way that animals could achieve temporal segregation between annual cycle stages is via physiological mechanisms that causally connect one stage to the other. In this case, the onset of one stage depends on the termination of the previous one; for instance, the pleiotropic effect of hormones may help organisms to segregate annual cycle stages (Jacobs & Wingfield 2000; Dawson 2006; Williams 2012). When this happens, if one stage is advanced/delayed by an abnormal weather event, for example, the consecutive stages are expected to shift at the exact same rate; but the time interval between them remains constant. This would be a way of preventing incompatible or energy-demanding annual cycle stages (e.g., moult and breeding) from overlapping too much (Hemborg & Lundberg 1998; Dawson 2006). This topic was studied to some extent in birds, especially in laboratory conditions. For instance, experimentally elevated testosterone levels suppress migratory activity in house sparrows (Passer domesticus; Schwabl & Farner 1989) and regulate post-breeding moult in European starlings (Dawson et al. 2000). Post-breeding moult is also related to a prolactin decline in starlings (Dawson 2006). Both testosterone and prolactin are correlated with gonadal regression and, thus, the termination of reproduction. Finally, in captive juvenile Eurasian blackcaps (Sylvia atricapilla), migration only initiates after the completion of moult (Pulido & Coppack 2004).

While the preservation of intervals between annual cycle stages could be interesting to prevent large overlaps, if stages are causally linked, a change in one stage may desynchronise its linked stages with the environmental cycles and/or reduce the time for their occurrence. This would likewise be undesirable, as a loss in fitness could occur even without the overlapping of stages. For example, a change in the timing of breeding in birds may reduce the time available for moult; a faster moult, in turn, is related to the loss of feather quality and thus has additional thermoregulatory costs (Dawson et al. 2000).

Organising the annual cycle stages

The organisation of annual cycle stages depends on (at least) three aspects: (a) a way to detect time of year (e.g. a synchronised internal calendar), (b) a way to know when the optimal conditions will be present (e.g. via supplementary cues predictive of the peak in optimal conditions) and (c) a way to segregate energetically demanding activities (e.g. via hormonal control). Timing mechanisms involved in the organisation of annual cycle stages were selected in specific environments to respond to a certain range of environmental conditions, for example, temperatures. What happens, then, under contemporary climate change, when the global temperatures rapidly increase?
Timing of annual cycle stages under climate change

Climate change causes increases in global temperature and has profound effects on biological systems (McCarty 2001; Walther et al. 2002; Visser 2008; Chapter 2). One of the best-reported effects is the change in timing of stages. For example, advancements in timing of avian breeding (Crick et al. 1997; Brown et al. 1999; Visser et al. 2004; Both & te Marvelde 2007; Charmantier & Gienapp 2014), migration (Cotton 2003; Ahola et al. 2004; Marra et al. 2005; Gienapp et al. 2007; Charmantier & Gienapp 2014; Usui et al. 2016) and moult (Møller et al. 2011; Morrison et al. 2015) have already been reported. However, such advancements are not always sufficient to track the concomitant changes in optimal environmental conditions (Visser et al. 2004, 2006; Visser & Both 2005; Visser 2008). In great tits (Parus major), for example, there has been increased selection on earlier egg-laying dates, suggesting that these birds are not adapting fast enough to changes in climate (Visser et al. 1998). In European pied flycatchers (Ficedula hypoleuca), on the other hand, the selection for early egg-laying had increased in the past but dampened in recent years (Visser et al. 2015).

As discussed before, the annual cycle stages of some organisms show a large degree of plastic responses to temperature. For example, egg-laying in birds is earlier when temperatures are higher (van Noordwijk et al. 1995; Meijer et al. 1999; Visser et al. 2009). However, plasticity alone may not be enough for organisms to cope with climate change (Gienapp et al. 2008; Visser 2008). Apart from the fact that the range of possible plastic responses may not be sufficient (Gienapp et al. 2008), a large part of the problem is caused by the unequal increases in temperature in both time and space (Easterling et al. 1997; Visser et al. 1998; Walther et al. 2002; Vose et al. 2005; Serreze & Francis 2006; Stocker et al. 2013). This means that temperatures will change at different rates at the moment of decision making and at the moment of selection, thus uncoupling the supplementary cues from the drivers of selection. This will make the cues less reliable predictors of the fitness landscapes (Visser et al. 2004; Gienapp et al. 2008; Visser 2008; Fig. 1.1).

A further complication appears when organisms with complex annual cycles are considered. Multiple annual cycle stages mean multiple cues at different points of the year for each stage and some may depend more temperature than others. Thus, a desynchronisation among annual cycle stages could be expected. In fact, even if all stages were affected by temperatures in the same way, the unequal warming around the globe would still cause a desynchronisation.

The problem is: organisms with complex cycles are supposedly unable to accommodate large changes in the timing of stages. Because the total period available for the allocation of stages obviously cannot surpass one year, if the rate of change is different for each stage, intervals between them may get more or less constrained (Hedenström et al.
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2007; Crozier et al. 2008; Dawson 2008; Wingfield 2008). Therefore, climate change may affect the balance between being on time and preserving the intervals between consecutive stages (Fig. 1.1).

The number of studies that looked at how intervals between annual cycle stages changed is relatively limited. One of the reasons for this is the need of good datasets comprising multiple stages. Among the few studies dealing with this topic, it was reported that marmots (Marmota flaviventris) advanced both the termination of hibernation and weaning, giving more time for the offspring to grow (Ozgul et al. 2010). In red deer (Cervus elaphus), on the other hand, unequal advancement of reproductive stages of males and females suggest that males are unable to track advancements in oestrous of females. Moreover, the termination dates of males’ rut (display/breeding season) advanced more than the initiation dates, which resulted in a shorter breeding window and likely reduced breeding performance (Moyes et al. 2011). In birds, long-term data on date of arrival and breeding of migratory pied flycatchers suggest that timing of breeding and migration may not respond the same way to climate change: there are reports of both shorter (Both & Visser 2001) and longer (Ahola et al. 2004; Valtonen et

Figure 1.1. Simplified schematic representation of the concepts discussed in this introduction. Because the decisions of when certain stages should be allocated are not made in the same environment of selection, animals use cues at the time of decision making to predict conditions of the environment at the time of selection. Different stages depend on different cues and climate change affects both cues and drivers of selection, but not necessarily in the same way (as indicated by the different types of arrows). Finally, the amount of overlap between annual cycle stages is affected by the decisions made for each stage, and overlap also affects fitness. Adapted from (Crozier et al. 2008; van Noordwijk & Müller 1994; Visser 2008; Visser et al. 2004, 2010; Wingfield 2008).
al. 2016) intervals between arrival and breeding. Finally, barnacle geese (*Branta leucopsis*) have rapidly expanded their breeding range to temperate areas and highly increased the interval between breeding and moult; this resulted in changed body mass dynamics (van der Jeugd *et al.* 2009; Eichhorn *et al.* 2010). For these animals with complex annual cycles, the assessment of the impacts of climate change is considerably more challenging, because apart from the need to track optimal conditions in the environment, unequal changes in the timing of stages may also affect their fitness (Fig. 1.1).

**Thesis’ goals**

Predictions on whether or not species will adapt to changes in climate rely on efforts to integrate mechanistic and evolutionary knowledge (Visser *et al.* 2010). Understanding the effects of climate change on organisms with complex annual cycles, however, also requires looking at these aspects throughout the annual cycle. It is necessary to investigate how each stage responds to temperature, to investigate the mechanisms that regulate the transitions between stages and, finally, to assess the fitness consequences of being too early or too late and of overlapping stages. This allows a more accurate forecast of the impact of climate change and is important to assess how much temperatures can increase without damaging wild populations (Gienapp *et al.* 2013; Visser 2008). Therefore, my main goal with this thesis was to investigate avian seasonal timing in an annual cycle perspective. More specifically, I wanted to understand: (1) if different stages of the annual cycle indeed shift at different rates due to climate change; (2) the causes of unequal shifts and (3) the fitness consequences of these shifts in terms of mismatches with the environment and of mistimed or overlapping stages.

To address these questions, I used an experimental approach, both in the wild and in captivity. When this was not possible, I looked at correlations using standardised long-term datasets. The combination of these various approaches allowed me to assess the causes and consequences of the observed patterns of unequal shifts caused by climate change.

**The annual cycle of the European pied flycatcher**

The European pied flycatcher, *Ficedula hypoleuca* ([Pallas], 1764), was the model system chosen for this study. I focused heavily on the stages that occur on the breeding grounds in the Netherlands, without looking at events from the wintering sites in Africa. However, all the events on the breeding grounds (arrival, breeding, moult and then departure) must happen in circa four months (Fig. 1.2). Thus, this is a good case of the allocation of multiple stages in a supposedly very constrained time span.
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study, this issue is not as problematic, since our focus was on a single population in the Netherlands. This population is currently recognised as *F. h. hypoleuca*, but it likely belongs to the sometimes-recognised *F. h. muscipeta* of central Europe (Chapter 9). To aid future research into these aspects, and complying with scientific standards (Schilthuizen *et al.* 2015), three male flycatchers collected in the study area were deposited as voucher material in the ornithology collection of the Naturalis Biodiversity Centre (Leiden, The Netherlands; inventory numbers RMNH 592347, 592348 and 592349).

I studied the flycatcher breeding population of the Hoge Veluwe National Park (The Netherlands; 05°51’E 52°02’N), which has been monitored for more than 40 years. In this forest, we provide around 400 nest boxes year-round (in an area of 171 ha) that are regularly monitored in spring. Apart from the timing of breeding, I, alongside our group, also collected data on individual arrival dates (see Chapters 3 to 5 for more information), moult onset (Chapter 3) and departure dates using geolocators (Chapters 3 and 6). Female arrival dates were derived from nest initiation dates and longer term data of male mean arrival dates was obtained via a collaboration with the Vogelwerkgroep Arnhem, who collect data in Arnhem (The Netherlands), a location close to our study area (Chapter 3).

The use of geolocators to track migration in pied flycatchers is relatively recent (Ouwelhand *et al.* 2016); consequently, deployment requires extra caution to prevent detrimental effects (Ouwelhand & Both 2017). For instance, when I deployed geolocators for the first time, the birds got entangled in the harness due to the attachment method that was chosen. Luckily, this was done in captivity and thus quickly noticed and corrected.

Pied flycatchers are small passerines (about 12 g) that reproduce in Europe and Western Asia and winter in West Africa. Due to their acceptance of artificial nest-boxes and low nest desertion rates, they are suitable for studies involving field experiments. Moreover, they allow to precisely monitor the timing of their annual cycle stages and breeding success.

The taxonomy of *Ficedula hypoleuca* is confusing and presently insufficiently investigated (reviewed in Chapter 9), requiring awareness on how to treat different populations and subspecies in comparative studies. For the present study, this issue is not as problematic, since our focus was on a single population in the Netherlands. This population is currently recognised as *F. h. hypoleuca*, but it likely belongs to the sometimes-recognised *F. h. muscipeta* of central Europe (Chapter 9). To aid future research into these aspects, and complying with scientific standards (Schilthuizen *et al.* 2015), three male flycatchers collected in the study area were deposited as voucher material in the ornithology collection of the Naturalis Biodiversity Centre (Leiden, The Netherlands; inventory numbers RMNH 592347, 592348 and 592349).

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without further harm to the birds. This led to a pre-deployment test to assess which attachment method would be the most appropriate for the flycatchers (Chapter 10). The leg-loop harness, made with elastane (elastic material), was selected and successfully used.

The pied flycatcher annual cycle stages shifted at different rates in response to climate change

The timing of the annual cycle stages of pied flycatchers changed at different rates over time (Chapter 3). While male arrival dates did not advance, the timing of breeding and male moult did (with a greater advancement of the latter). This caused the intervals between arrival and breeding and between breeding and moult to shorten over time. Curiously, a similar pattern was not observed for the interval between female arrival and breeding, with a fairly fixed interval through time (Fig. 1.3). This was probably caused by the fact that different temperature intervals were important for the timing of each of these stages, with moult not being affected by any of the analysed climatic variables (Chapter 3).

While other studies with pied flycatchers reported that arrival time advanced due to climate change (Ahola et al. 2004; Both et al. 2016; Valtonen et al. 2016), we did not observe this for our population. Instead, our updated data using the Arnhem arrival dates still corroborates what was reported 15 years ago (Both & Visser 2001): there has been no change in male arrival dates, but females advanced their egg-laying dates and, thus, until 2008 the interval between arrival and breeding got shorter (Fig. 1.3: orange line). However, this pattern seems to have changed again in the last eight years (Fig. 1.3: orange line): after 2008, the interval increased again (albeit not significantly). The recent data of male arrival dates and female laying dates collected in the Hoge Veluwe (2005–
2015) also point to a non-significant increase in this interval (Fig. 1.3: dashed grey line). It is then possible that in recent years the arrival dates of the males are advancing and the females’ breeding date delaying but with too large inter-annual variation to be picked up by the analyses.

The cause for the different trends in arrival and breeding, however, is not immediately evident. We would expect that the temperatures important for the laying dates of the females changed in time. However, we only observed a significant change in the temperatures explaining the variation in the female arrival dates, but not the laying dates. There was also no change through time in the temperatures important for timing of male arrival. So even if the timing of male arrival is affected by temperatures, arrival dates did not advance, as temperatures did not change (Chapter 3).

It is curious to notice that this pattern is almost the exact opposite of what was reported for pied flycatchers in Finland, where arrival dates advanced but breeding dates did not (Ahola et al. 2004; Valtonen et al. 2016). These differences in populations are not surprising if we consider that pied flycatchers in Finland breed and winter in different localities (Ouwehand et al. 2016); moreover, they have a different proportion of colour morphs compared to the Dutch flycatcher population and might belong to a different subspecies (Chapter 9). Furthermore, Valtonen et al. (2016) reported that the arrival of Finnish flycatchers was more strongly related to temperatures in the Baltic region. Thus the factors that best explain the arrival of these birds are also not the same as those for the Dutch population (Chapter 3 and 4).

While the intervals between the timing of arrival and breeding and between breeding and moult got shorter, there was no effect on adult survival (neither males nor females). We also did not observe any significant decline in the number of breeding pairs between 1980 and 2016 (since 1980 the number of boxes remained constant, with no limitation in nesting sites and the majority of the flycatchers in the Netherlands breed in nest boxes; Both et al. 2006; Fig. 1.4).

Reproductive success also does not seem to be affected by the shortening of the interval. Actually, the shorter interval was correlated with a higher proportion of fledged chicks that recruited, probably due to the earlier laying dates in these years (Fig. 1.5).
The costs of shortening the arrival-breeding and breeding-moult intervals are, therefore, presently not evident neither in terms of adult survival nor in reproductive success. This is an intriguing result, as a decrease in the interval between arrival and breeding is expected to reduce the time to develop the “arrival biology”, important for coping with local conditions (Ramenofsky & Wingfield 2006, 2016), and that the overlap between breeding and moult would be energetically costly (Hemborg & Lundberg 1998; Echeverry-Galvis & Hau 2012, 2013; but see Williams 2012). It becomes then important to investigate these stages in more detail to determine what could be the cause of the lack of advancement in arrival, why the overlap between moult and breeding increases and if there are more subtle costs of those unequal shifts that the previous analysis could not pick up.

### Male arrival dates

**Causes: African temperatures are important cues for male arrival time**

The non-advancement of male arrival dates in contrast to female egg-laying dates is probably related to the absence of a shift in the relevant temperatures for arrival (Chapter 3), while the temperatures relevant for the egg-laying dates shifted. This difference can be caused by the unequal effects of climate change on global temperatures (Easterling *et al.* 1997; Visser *et al.* 1998; Vose *et al.* 2005; Serreze & Francis 2006). Another possibility, however, would be a lack in plasticity to respond to temperature cues (Both & Visser 2001; Coppack & Pulido 2004).

Long-distance migratory birds spend the winter in a location very far from their breeding grounds, it is therefore often assumed that it is impossible for them to track the
variation in environmental conditions at their breeding grounds (Gwinner 1996; Visser et al. 2004). They would rely only on the endogenous clocks and photoperiodic cues to time their migration (Gwinner 1989b, 1996; Dawson 2002; Coppack & Pulido 2004; Pulido 2007; Ramenofsky & Wingfield 2016), lacking plastic responses in the timing of migration. Still, some migrants arrive earlier in warmer springs (Walther et al. 2002) and departure, passage dates and arrival times are correlated with conditions on the wintering grounds, breeding grounds and en route (Ahola et al. 2004; Saino et al. 2004, 2007; Hüppop & Winkel 2006; Gienapp et al. 2007; Bauer et al. 2008). This suggests that even for long distance migrants the use of supplementary cues for fine tuning the timing of migration could be important (Gienapp et al. 2007).

We tested whether precipitation and temperatures from the breeding and wintering grounds could be important for male spring arrival dates in our population (Chapter 4). We showed that not only the year-to-year variation in male arrival time is explained by the variation in temperatures in Africa prior to arrival (Fig. 1.6a, warmer temperatures are related to a late arrival), but also that those temperatures predict the fitness landscape at the breeding grounds (Fig. 1.6b, warmer temperatures are related to a later peak of the fitness landscape). Thus, there is a potential for the pied flycatchers to track changes in temperatures (in Africa) and adequately time their arrival at the breeding grounds. African temperatures, in this case, would act as cues for the birds to time their arrival dates. Interestingly, the relation between fitness and temperature (Fig. 1.6b) is steeper than one between arrival dates and temperature (Fig. 1.6a). This suggests that the optimal arrival time responds more strongly to the temperature than the trait arrival date when temperatures increase. This follows the expectations of Gienapp et al. (2014) who suggested that the trait (“consumer”) reaction norm is always flatter (less plastic) than the optimal time (“resource”, which is unknown in this case) reaction norm because the cue does not perfectly predict the fitness landscape. As a consequence, even if the temperatures affecting “consumer” and “resource” (or breeding and wintering grounds) change equally, there will be selection on the trait (Gienapp et al. 2014).

Our results, together with those previously reported in the literature (Ahola et al. 2004; Hüppop & Winkel 2006; Saino et al. 2004) challenge the view that migratory birds only rely on photoperiodic cues for timing of migration, being unable to use other cues from the wintering or breeding grounds (Gwinner 1989b, 1996; Dawson 2002; Coppack & Pulido 2004; Gienapp et al. 2007; Pulido 2007; Ramenofsky & Wingfield 2016).

Because we do not have data on departure dates from Africa for most years we analysed, we were unable to distinguish whether this variation in arrival dates correspond to differences in departure dates or other adjustments after departure. Departure dates could still be mainly regulated by photoperiodic cues and occur every year at the same moment. The variation in arrival time, in this case, would correspond to decisions made en route (Both 2010a; Tøttrup et al. 2012). Ouwehand & Both (2017) compared
individual migration schedules of pied flycatchers in a single year and suggested that variation in arrival is mainly due to variation in departure. If this could be extrapolated to multiple years, it would support the idea that pied flycatchers can use cues from their wintering grounds to time their departure.

At the moment, we cannot explain why the African temperatures are related to the fitness landscape at the breeding grounds (i.e., whether it is a correlation or a causal effect). This missing piece of information is important because a mere correlation between conditions from the breeding and wintering grounds will make the use of temperature cues unreliable since climate change will affect them in a different way. And, unfortunately, a proper manipulation of the timing of migration, even to simply understand whether individuals suffer costs from arriving too early or too late, is a very challenging task (see Box 1).

**Consequences: early arrival is important for males to obtain fertilisations**

Male arrival date is a trait with major fitness consequences in pied flycatchers (Velmala *et al.* 2015). It correlates, for example, with how many chicks the males will fledge (Chapter 4) and with the laying date of the females, as early arriving males obtain early laying females (Canal *et al.* 2012a; Velmala *et al.* 2015).

It would be important to use experimental manipulations on the timing of arrival to understand the fitness consequences of arrival dates (Verhulst & Nilsson 2008). But, as mentioned before, manipulating timing of arrival has proven to be a very challenging task.

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**Figure 1.6.**

*a)* Date when 50% of birds arrived in relation to the mean African temperature.  
*b)* Peak date of fitness (of arrival date) in relation to the mean African temperature. The points in these two figures do not correspond to raw data. In Fig. 1.6a they are estimates from a proportional hazards model of the day when 50% of the individuals arrived in each year (from model of Fig. S4.1, Chapter 4). In Fig. 1.6b they are estimates from a linear mixed effect model of the day when the curves peak in each year (from model of Fig. 4.2b, Chapter 4).
Timing of stages under climate change

Chapter 1

We assessed the probability of males to obtain fertilisations (in their own nest or via extra-pair paternity) depending on when they arrived (Chapter 5). While arrival dates did not correlate with gain or loss in extra-pair paternity, early-arriving males had a higher chance of obtaining fertilisations and the majority of bachelors (males without a female) were late arriving males (Fig. 1.7). However, a few bachelors were still able to fertilise eggs via extra-pair paternity (Fig. 1.7). This is interesting as it demonstrates that even if the bird is unable to secure his own female, it does not necessarily have zero fitness by the end of the season. It is common for pied flycatcher males to be caught as a breeders only two years after they are born (Visser et al. 2015). Sometimes, males are recovered as breeders one year after they are born, but this does not occur very often in our study area. It is still possible that the young birds do return to the breeding grounds one year after they are born, stay in the breeding area, but are unable to obtain a social female. In extra-pair paternity studies, the identity of a few extra-pair fathers remained unsolved, even with a high proportion of the breeding birds identified (e.g., Canal et al. 2011); they could be those unpaired males. Thus, if there is a chance for birds to still obtain paternity one year after they are born, even if they do not secure a female, it may still pay-off to return to the breeding grounds.

It is important to notice that there is no year-to-year variation in probability to obtain a female and that this probability is neither related to variation in temperature or precipitation. The probability just declines with late arrival dates and early males are always more likely to obtain a female (Chapter 4). Thus, obtaining a female may only depend on the focal individual arrival in relation to the decision of the other individuals of the population, which are roughly exposed to the same climatic variation. This may be driven by the density of breeding birds rather than by climatic conditions. If this is the case, the effects of climate change on the probability to obtain a female will only be relevant if climate change also affects the density of breeders (Parmesan 2006; Wiens 2016).

The consequences of reducing the interval between male arrival and breeding are, thus, still not clear. It is possible that the transition from arrival to breeding is not as energetically demanding in pied flycatchers from the Netherlands in comparison to birds that breed further North and face harsher and more unpredictable weather (Ramenofsky & Wingfield 2006, 2016). The costs could be more subtle as well: for example, females may need to be much faster in choosing a male (Dale et al. 1992; Dale & Slagsvold 1995), building the nest and laying eggs. This could, for example, result in less time for females to assess male quality (Alatalo et al. 1984) and increase the probability of pairing with a male that already has another female, which is costly for the females (Lubjuhn et al. 2000). At the population level, we observed a trend in the
clutch sizes which were previously increasing but changed in recent years (Chapter 3). The shorter time interval between male arrival and breeding may, therefore, in the long run, affect the females’ body condition upon arrival and affect the costly egg-laying stage (Visser & Lessells 2001).

Breeding, moult and departure

Causes: timing of male moult does not depend on the termination of breeding

The shortening of the interval between breeding and male moult suggests that timing of moult is independent of the timing of breeding (Fig. 1.3), otherwise, the two stages would shift together. However, it is important to directly test for a causal relation between the termination of breeding and the onset of moult, controlling for potential confounding effects (such as individual quality). One way of doing this is to manipulate the timing of the first stage and look for cascading effects on consecutive stages.

We tested whether the moult onset of males would be causally related to the termination of breeding with manipulations of the hatching date of the chicks, which is a stage that serves as a proxy for termination of breeding due to its high correlation with fledging date and end of parental care. The advancements and delays were achieved with an egg-swapping/cross-fostering experiment that modified the incubation length of the females and made the chicks hatch four days earlier or later than when unmanipulated (thus, the adults finished breeding four days earlier or later as well, with eight days of difference between Advanced and Delayed broods; see Chapter 6 for more details).

The manipulations did not affect the timing of moult. In fact, moult occurred at a certain calendar day for most birds. Thus, Delayed birds had more moult-breeding overlap than Control (that finished breeding at the expected time) and Advanced ones. This suggests that moult is either independent of any stage or that the decision to start moulting is made at an earlier, un-manipulated, stage. We tested these two possibilities using the data of males for which we had moult and original hatching date in 2013.
and 2014 to test whether this difference in moult onset would be related to a previous stage, such as laying date (Fig. 1.8, hypothetical line a) or would happen at a certain day, independent of a previous stage (Fig. 1.8, hypothetical line b). We found that moult onset is more likely to be independent of any previous stage (slope was not significantly different from 0, $F_{1,8} = 2.92$, $p$-value $= 0.13$; but was from 1, $F_{1,8} = 21.2$, $p$-value $< 0.01$). But it is interesting to notice that it also does not happen exactly at the same day in all years (intercept significantly different from 0, estimate $= -7.38$, $p$-value $< 0.01$). Both the data from these individuals with multiple captures and the average moult from all individuals demonstrates that the onset of moult differed in these two years by a few days (earlier in 2014 than in 2013). Thus, it is likely that timing of moult is not solely regulated by photoperiod and is also related to something that presents year-to-year variation. This is also supported by the advancement in moult onsets observed in the long-term dataset (Fig. 1.3).

Both the male moult being independent of the timing of breeding and the unequal advancement of moult and breeding, argues against a system in which onsets of stages are governed solely by the termination of previous stages (Enright 1970; Mrosovsky 1970), at least for the transition between breeding and moult. This, however, contradicts previous studies that suggest that onset of male moult is tied to male gonadal regression and thus, to the termination of breeding (Dawson 2006). It is important to remember, however, that in our study the interval is not measured from the gonadal regression to the onset of moult, but from the chick fledging date to the onset of moult. The manipulations in the present study affected the hatching date of the chicks and thus, their fledging dates; however, if termination of breeding in males is actually defined as gonadal recrudescence, then moult onset could still be related to the termination of breeding (Dawson 2006). At the mechanistic level, the use of gonadal regression may be more appropriate. But the fledging date marks the point when males stop coming back to the nest to feed the chicks, although post-fledgeling care continues for some
days (Visser et al. 2015). Thus, if a male starts to moult before the chick fledging date, it will surely overlap moult and breeding, potentially suffering fitness costs due to the high energetic demands of the two stages. Moreover, gonadal size does not correlate with egg-laying dates even when measured in females (Schaper et al. 2012; Caro et al. 2013). Therefore, for the purpose of understanding the effects of climate change on the time interval available for different stages the use of hatching dates seem to be more appropriate.

Since our experiment suggests that timing of moult does not depend on a previous stage, this explains why moult and breeding shift at different rates, increasing the amount of moult breeding overlap. Our experiments, however, do not explain why moult advances more than breeding (Fig. 1.3), which is also not explained by any trend in temperatures.

The timing of the whole cycle, including moult onset, could be set by the rearing conditions, like the photoperiod upon hatching (Coppack et al. 2001; Coppack & Pulido 2009; Both 2010a). If the timing of stages is set when individuals are born, and hatching dates advance in time, this could explain why moult onset also advanced. A similar explanation is given for the advancement in arrival time of both flycatchers and other bird populations (Both 2010a; Gill et al. 2014), but not observed in our study population. Because timing of migration is much more affected by the environment than timing of moult (e.g., Ahola et al. 2004; Erni et al. 2005; Sinelschikova et al. 2007; Eikenaar & Schmaljohann 2014; Both et al. 2016; Teitelbaum et al. 2016), moult would be a relatively cleaner expression (i.e. less masking) of the individuals’ endogenous timing (Gwinner 1996). Therefore, if the timing of moult is determined upon birth, the effects will supposedly be more detectable in the timing of moult rather than in the timing of arrival. This possibility, however, is not the case in barnacle geese (Larsson 1996) but could work differently in small passerines (Coppack et al. 2001).

Our analysis of long-term trends (Chapter 3) and the experiment (Chapter 6) had their main focus on the male moult. Females were scored for moult progression as well, but in the majority of cases, they were not mouling. In the experiment described in Chapter 6, a few females were also taken to captivity in 2014 and their moult onset was pinpointed similarly to the males. The inclusion of the females on the moult-breeding overlap analysis (originally done only with males) curiously resulted in a very similar result as the original analysis without an effect of sex. This hints that the onset of female moult also occurs at a certain calendar date. It is, unfortunately, not possible to go into further details due to the low amount of data available for the females.

Another study that used hatching date manipulations (Hemborg & Lundberg 1998) suggested a different control for the moult onset of males and females (see also Chapter 7). Hemborg & Lundberg (1998) showed that females were unable to adjust the moult onset depending on their chick age while males did moult when their chicks had a certain
age. Curiously, the results they reported for females are similar to what we observed for the males, as males in our experiment moulted at a certain day, not adjusting moult to hatching date (Chapter 6). Because the two experimental designs are not exactly the same (e.g., their design depends on broods that lay eggs a number of days apart, while our experiment used broods with same laying dates), it is difficult to determine what caused these differences. It is also possible that instead of methodological differences, the two populations differ in their control of moult. Pied flycatchers from Sweden breed later than birds from the Netherlands and are supposedly more time constrained to moult; thus, they may also require different strategies to be able to migrate in time (Hemborg et al. 1998, 2001).

While the independence of moult from the previous stages does not prevent the moult-breeding overlap, the increase in moult-breeding overlap also allows an earlier moult or a longer time to moult for males. Both cases could result in fitness benefits in terms of earliness or plumage quality. Thus, the consequences of advancement of moult for the males depends on how costly it is for them to increase the overlap between moult and breeding. The lack of a possibility for fine adjustments of the timing of male moult in relation to the termination of breeding already implies a lower cost of moult-breeding overlap than potential costs of moult in time, nevertheless, it is important to directly test for these costs.

**Consequences: females pay the cost of males’ moult-breeding overlap**

Although the previous experiment (Chapter 6) suggests some costs of moult-breeding overlap, these costs were confounded by the effects of timing per se, because Delayed breeders were also those birds with the largest amount of overlap. In order to test for the overlap effects alone, it was necessary to experimentally simulate moult while the birds were feeding their chicks, controlling for other effects such as the brood size and hatching date (Chapter 7).

We experimentally simulated moult gap by plucking the two innermost primary feathers of the males, leaving other males with same brood size and hatch dates intact as Controls (see chapter 7 for more details). When we simulated moult gap, we observed that moult was costly in terms of flight performance. Males with a simulated moult gap had a reduction of 5% on their displacement per wing beat, possibly having to adjust their flight to achieve the same speed as Controls (Chapter 7). Thus, it was expected that sustaining this additional cost with the need to feed their chicks would be costly for these males (Hemborg & Lundberg 1998; Echeverry-Galvis & Hau 2013). However, measurements of the adult or chick condition did not reveal differences in costs between Control and manipulated individuals. Interestingly, when comparing parental care between treatments (using number of visits to the nest), it could be seen that males and
females responded differently to the treatments. While males with simulated moult gaps decreased the number of nest visits, their females (which were not plucked) increased the number of visits (Fig. 1.9). Thus, females with plucked males completely compensated the male’s reduced visitation. On the next year, we found that fewer females from the Plucked-males group survived; however, the difference was not significant.

Interestingly, this is consistent with the results of Hemborg & Lundberg (1998), where both sexes were plucked and females from Plucked groups suffered a reduced survival in the next year. Our experiments add to this previous study by showing that even when females are not moulting themselves, they may suffer costs from the moult-breeding overlap of their partners. Williams (2012) suggested a lack of empirical evidence supporting the costs of moult-breeding overlap, stating that these costs could appear at a later stage. The present study (Chapters 6 and 7) supports this idea: males seem to be relieved from the immediate costs of moult-breeding overlap because their females pay the costs.

Because even when they are not moulting themselves, the females are paying a cost for their partners’ moult-breeding overlap, another potential consequence of the decreased interval between breeding and male moult is an enhanced sexual conflict between males and females during parental care (Hemborg & Merilä 1998). The results of this experiment also raise the question of why males do not always overlap moult and breeding since this would potentially allow them to either depart earlier or have more time to complete moult prior to departure. The cost for males could potentially appear as a larger proportion of divorces in pairs in which one individual is moulting (Hemborg & Merilä 1998). Anecdotally, only birds in the Plucked group deserted their nests after the treatment.

Finally, such effects of an increased moult breeding overlap may not have an immediate observable impact on adult survival or reproductive success. For example, the effect on female survival, although in the expected direction, was not significant. Selection against moult-breeding overlap can be dependent on environmental conditions. Optimal food and weather

Figure 1.9. Average number of visits to the nest (with standard errors) by males (circles) and females (squares) in Plucked (red) or Control (blue) treatments. The interaction between sex and treatment is significant. Reproduced from Chapter 7.
conditions may allow some degree of resilience. Thus, in the long run, the breeding costs of moult-breeding overlap may appear only in sub-optimal environmental conditions, such as unpredictable extended periods of inclement weather.

Consequences: wintering location is affected by the hatching date of the chicks

Apart from understanding the effects of the termination of breeding on moult, it is also interesting to look at how the termination of breeding affects departure dates and wintering location (Chapter 6). For that, light-level geolocators were used to determine when the pied flycatchers leave to the wintering grounds and where they winter. The treatments (Advanced, Delayed and Control) from Chapter 6 did not affect the departure dates of the birds. The location where these birds wintered, however, differed among treatments, with Delayed animals wintering further west than those from the other two treatments (Fig. 1.10). This suggests that while costs of moult-breeding overlap may be initially very low for the males (Chapter 7), differences may appear later in other annual cycle stages, carrying over to the wintering grounds (Marra et al. 1998; Williams 2012).

This result mimics the natural pattern observed for different populations of pied flycatchers (Ouwehand et al. 2016): birds from northern populations (that naturally breed later) tend to winter further west than those from southern populations (that naturally breed earlier). This pattern is not observed in birds from the same population that naturally breed earlier or later (Chapter 6; Ouwehand & Both 2017). In conjunction, this suggests that the delay caused by the treatments either forced the birds to adopt a different wintering location or are a result of a selective mortality of individuals from Delayed groups that wintered in their “usual” location. This effect may be related to the larger moult-breeding overlap for Delayed birds, which could lead to low-quality feathers or reduce the individual’s condition on a whole, making it less competitive in securing a wintering territory (Dawson et al. 2000; Dawson 2004; de la Hera et al. 2010; Echeverry-Galvis & Hau 2013). Unfortunately, too few Plucked birds were deployed and recovered (Chapter 7) for a proper comparison on whether moult gap during breeding independent on timing would have caused a similar pattern.

In American redstarts (Setophaga ruticilla), the arrival at the wintering grounds is important for securing resources during winter, resulting in improved body reserves that carry-over to the next season (Marra et al. 1998, 2015b; Norris et al. 2004; Reudink et al. 2009). So birds that winter in good quality habitats also perform better in the breeding season. In the present study, however, treatments did not differ in the probability of finding the manipulated birds the next year ($\chi^2 = 0.83$, $p$-value = 0.66) or any of following years (up to 2016, $\chi^2 = 1.04$, $p$-value = 0.59). The breeding date of males and females in the following year was also not affected by treatment ($F_{1,26} = 0.39$, $p$-value = 0.68), only by their un-manipulated laying date in the previous year (estimate
Thus, manipulating hatching date of the chicks, with a change in the amount of moult-breeding overlap, causes changes in the males' wintering location with no apparent carry-over effect to the next breeding season. If this change is mainly caused by the amount of moult-breeding overlap, a potential consequence of the decreased interval between breeding and moult through time would be a change in the wintering location of the males, especially for the late-breeding ones. With no carry-over effect in the male probability of survival to the next year or their breeding date.

Figure 1.10. Effects of treatments on winter longitude of males. Each point on the map represents an individual; each colour represents a treatment (pink = Advanced, grey = Control, blue = Delayed); the orange point represents the breeding grounds. Longitude (East-West axis) is accurate, while latitude (North-South axis) is not. The smaller panel shows the average winter longitude for each treatment with the standard error (colours as above). Image produced in the open platform www.carto.com, based on data from Chapter 6.
Juveniles

Causes and consequences: early hatching of the chicks increases the time available for their development

One of the consequences of the advancement in laying dates is the fact that chicks are born earlier in the year (Chapter 8). Hatching earlier may mean that chicks gain more time to develop prior to migration if the timing of juvenile migration does not change. Alternatively, chicks could also be able to depart earlier, in case timing of juvenile autumn migration advances as well. In both cases, an earlier hatching date would give more time for the chicks. To test this possibility, we experimentally manipulated the hatching dates of sibling chicks in the field by combining egg-swapping/cross-fostering methods (see Chapter 8 for more details) and then measured the timing of the annual cycle stages of these chicks during their first year (until migration) in controlled laboratory conditions. Chicks born earlier had more time to moult and gain weight/accumulate fat because the timing of migration did not differ between early- and late-born birds. This created differences in weight by the time the birds were migrating (i.e., developed migratory restlessness in laboratory settings) between genetically similar chicks that were born earlier or later in the season, with delayed chicks being lighter (Fig. 1.11).

Despite not being the original goal of this experiment, it is worthwhile to compare the timing of post-juvenile moult and juvenile migration (Chapter 8) with the timing of adult moult and adult departure (Chapter 6). While the timing of adult moult onset seems to be fairly fixed to a certain calendar date (unless the bird was a very late breeder; Chapter 6), the timing of moult in chicks depended on when they were born, occurring at various times of the season (Chapter 8). The timing of migration, however, was independent of the hatching date in both cases. The results for the juveniles is (initially) contradictory to the observation of self-sustained annual rhythms (suggesting a true circannual clock) of moult, fattening and migration that were demonstrated in several bird species, including pied flycatchers (Gwinner & Schwabl-Benzinger 1982; Holberton & Able 1992; Gwinner 1996, 2003; Karagicheva et al. 2016). Instead, what was observed for the chicks is more consistent with a “developmental timer”, or timing that is dictated solely by the previous stages (Mrosovsky 1970); thus, their timing is related to the interval necessary for development, instead of endogenous oscillator(s) that would give information on the time of year (Pittendrigh & Skopik 1970). Therefore, it is possible that the control of juvenile moult differs from the adult moult. The fact that in the juveniles the timing of migration is independent of hatching dates is consistent with what is observed in adults.

The timing of adult migration was also related to the timing of an unmanipulated previous stage, which was not the hatching date. This suggests that the adult departure from the breeding grounds is determined earlier in the season, maybe upon arrival or
laying date of their females. On the other hand, in juveniles, although we observed genetic variation in timing of migration, there was no genetic covariation between their migration time and the laying dates of their mothers (Chapter 8). Thus, some aspects of the control of the timing of migration of these birds may also differ between juveniles and adults. For example, a larger effect of previous stages on the adults’ timing of migration.

It would be interesting to know whether the time from chick hatching to departure would also reflect on the probability of that chick to recruit as a breeder. Unfortunately, this data is not available, as we do not have long-term data available on chick departure. It is possible, though, to use the laying dates of the females as a proxy for the interval between hatching and departure. This is shown in Figure 1.5b, in which an earlier egg-laying correlated with a higher proportion of recruited chicks. Thus, the gain in time for development may be one of the factors, obviously among many others (Perrins 1970; Drent & Daan 1980; Daan et al. 1988; Verhulst & Nilsson 2008), explaining why a higher proportion of chicks recruits with an earlier average laying date of the females (Fig. 1.5b).

Thus, this experiment shows that, in the case of juveniles, because earlier egg-laying gives more time for chicks to develop, climate change may actually lift time constraints and give more time to late-born between hatching and migrating.

Conclusion

In this thesis, I investigated how climate change affects the organisation of the annual cycle stages. The results show that complex interactions between annual cycle events do exist, especially during the restricted time the birds have at their breeding grounds.

The pied flycatcher’s annual cycle stages undergo unequal shifts caused by climate change. The potential cause is the use of different cues by different stages and the fact that those cues do not shift at the same rate. Moreover, we did not detect causal links between the termination of breeding and moult onset or departure dates that prevented or reduced the magnitude of those unequal shifts. These shifts (and their associated costs)
are also dependent on the sex and life stage of a bird. On the whole, the advancement of breeding time of females will benefit the offspring, which will gain more time for development. The males seem to have the largest amount of overlapping of stages (both during arrival and breeding and during breeding and moult). However, males do not seem to pay costs in terms of either survival or reproductive success even if moult does impair their flight performance. In fact, most of the potential costs evaluated in this study seem to fall upon the females, even if they do not overlap stages themselves.

While females do benefit from the increased probability to recruit their offspring when breeding earlier, this may be at the cost of having less time to choose a male, build a nest and lay eggs. Moreover, they may also suffer increased costs as male moult advances faster than breeding, since females pay the costs when their partners overlap moult and breeding. Costs for males may only get evident as the overlap increases: for example, divorces may become more common if costs become too high for the females. Costs may also appear much later when the birds move to their wintering area. Finally, moult gaps closer to the edges of the wing, characteristics of the late stages of moult, are more detrimental for the flight (Hedenström & Sunada 1999), possibly limiting the amount of moult-breeding overlap a male can tolerate (Hemborg et al. 2001). Thus, the impacts of climate change, at least for the aspects studied here, only become apparent if we look at those more subtle costs of overlapping and mismatches, particularly at the individual level. Impacts that affect larger-scale patterns of adult survival or reproductive success may only appear if the overlap is much more extensive or in sub-optimal environmental conditions. This, to a certain extent, reflects the generally stable number of breeding pairs in this population for the past 37 years (Fig. 1.4).

It is important to point out that while some of the results of my study suggest that climate change may have some beneficial effects for this particular species, they should not be taken as an evidence that climate change is an unimportant issue. Instead, they demonstrate how complex (and often subtle) the impacts may be, even when we focus just on a small portion of the annual cycle of a species. It also serves as a warning that our knowledge is far from complete as, even in this extremely well-studied population, there are still important gaps that hinder our capacity to fully grasp the extent of the problem. What to expect of less studied populations and species? Apart from the changes in timing, there are also other consequences of climate change that may impose increased challenges for the survival of different (migratory) species. For example, increased temperatures may affect the suitability of breeding and wintering grounds and migratory routes. If wintering locations or migration routes become less suitable for the birds due to the increased anthropic effects, we could also expect population declines in the future but due to very different causes (Both et al. 2006, 2009; Saino et al. 2011; Bairlein 2016).
As a final message, I would like to stress two aspects that have been recently emphasised by several authors and were essential in the theoretical and practical development of this thesis. I consider them to be of the greatest importance in moving the field forward. The first one is the importance of the continued long-term standardised monitoring of individuals, especially of the same population (Visser 2008, 2016), pedigreed (Gienapp et al. 2008; Visser 2008), and in particular, if the data collection involves more than a single stage. Good long-term datasets will be essential to grasp the complexity of interactions across annual cycles and how they change throughout the decades as we enter the Anthropocene (Waters et al. 2016). The second aspect is the need to understand the causes and consequences of any observed environmental change, not only via experimental manipulations that allow the measurement of fitness (Verhulst & Nilsson 2008), but also via multidisciplinary studies that improve our mechanistic understanding of the control of annual cycle stages (Dawson 2008; Wingfield 2008). This is essential to understand how species will respond when changes move them outside their natural range of environmental conditions (Wingfield et al. 2008; Visser et al. 2010; Stevenson et al. 2015).

Even if the past, present and future studies of how climate change affects wild organisms will neither serve as a starting point to reverse our current predicament nor steer the public perception in the right direction (Kahan et al. 2012), may they at least guide the decision of how much (more) biodiversity we are willing to sacrifice. At the very least, from a purely scientific perspective, they will serve as “natural” experiments to help us to understand how organisms are able to manage time in a changing environment.
Part I.
General patterns