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Low temperature and a shorter duration of food availability both delay testicular regression and affect the daily cycle in body temperature in a songbird

Running title: Temperature, food and testicular regression

What is already known:

Although photoperiod is the major environmental cue used by birds to time the annual gonadal cycle, food and ambient temperature can modulate photoperiodic through unknown pathways.

What this study adds:

The time of food availability and ambient temperature both modulate photoperiodic responses so that long-photoperiod induced gonadal regression is delayed. Both also affect the daily cycle in body temperature to give it characteristics of a shorter photoperiod, suggesting that the daily cycle in body temperature may play a role in photoperiodic responses.

Keywords: starling; testis; annual cycle; body temperature; energy; food; photoperiod; temperature; thyroid

Abstract

Photoperiodic control of reproduction in birds is based on two processes, a positive effect leading to gonadal maturation and an inhibitory effect subsequently inducing regression. Non-photoperiodic cues can modulate photoperiodic control, particularly the inhibitory process. In previous studies on common starlings *Sturnus vulgaris* (1) restriction of food availability to 8h after dawn had little effect on testicular maturation but dramatically delayed subsequent regression and (2) lower ambient temperature also had little effect during maturation but delayed regression. Could the effects of food restriction and temperature share a common underlying mechanism? Four groups of starlings were kept on a simulated natural cycle in photoperiod in a 2x2 factorial experimental design. Two groups were held under an ambient temperature of 16 ^{0}C and the other two at 6 ^{0}C. One of each of these groups had food provided *ad lib* and in the other two groups access to food was denied 7h after dawn. In both *ad lib* food groups and food-restricted groups, lower temperature had little effect on testicular maturation but delayed subsequent regression and molt. In both the 16 ^{0}C and 6 ^{0}C groups, food restriction had no effect on testicular maturation but delayed regression and molt. The daily cycle in body temperature was recorded in all groups when photoperiod had reached 12L:12D, the photoperiod at which regression is initiated. In both 6 ^{0}C groups, night-time body temperature was lower than in the 16 ^{0}C groups, a characteristic of shorter photoperiods. In the two *ad lib* food groups, high daytime temperature was maintained until dusk whereas in the two food restricted groups, body temperature began to decrease after food withdrawal. Thus both lower temperature and food restriction delayed regression, as if photoperiod was shorter than it actually was, and both resulted in daily cycles in body temperature that reflected cycles under shorter photoperiods. This implies that the daily cycle in body temperature is possibly a common pathway through which non-photoperiodic cues may operate.

1. Introduction

The predominant environmental cue to time the annual cycle in gonadal maturity in birds is photoperiod. However, since gonadal cycles are rarely if ever symmetrical with photoperiod, at least two photoperiodic mechanisms must be implicated. For a considerable time, these have been referred to as photostimulation and photorefractoriness (Burger, 1947), and these were thought to act sequentially to time gonadal maturation and regression respectively. A more recent hypothesis is that there is a stimulatory process and an inhibitory process acting in tandem at all times to control GnRH secretion and the net difference between these two determines the rate of maturation or regression (Dawson, 2015). Differences between species in terms of the timing and duration of gonadal maturity (e.g. absolute and relative photorefractoriness) can be accounted for entirely by differences in sensitivity of the inhibitory process to photoperiod. The neuroendocrine pathway through which long photoperiods induce stimulation of GnRH secretion is fairly well understood e.g. (Ikegami and Yoshimura, 2016), but this is not true of the inhibitory process.

Although photoperiod is the primary environmental cue, other factors can have modifying effects. These are more important in species with less predictable breeding schedules (Wingfield et al., 1992) and may act directly on GnRH or through GnIH (Bedecarrats et al., 2016; Ernst et al., 2016; Tsutsui and Ubuka, 2016). Many studies have investigated the effects of ambient temperature on photoperiodically induced testicular maturation in birds. In a few cases, testicular maturation was accelerated by higher temperature (Perfito et al., 2005; Silverin et al., 2008; Wingfield et al., 2003); in the majority it was not (Caro et al., 2013; Caro and Visser, 2009; Dawson, 2005; Perfito et al., 2005; Silverin et al., 2008; Soumalainen, 1938; Visser et al., 2011; Wingfield et al., 1996; Wingfield et al., 1997). However, in all of the studies where subsequent regression was also monitored, high temperature advanced regression (Dawson, 2005; Dawson and Sharp, 2010;

Visser et al., 2011; Wingfield et al., 2003; Wingfield et al., 1997). The photoperiodic inhibitory process leading to gonadal regression is therefore more amenable to modulation by temperature than is photoperiodic stimulation.

Food restriction also has effects on the reproductive axis (Davies and Deviche 2014). Hahn (1995) photostimulated Red Crossbills *Loxia curvirostra* but restricted their food consumption to short photoperiod levels. This delayed the increase in LH but did not affect testis size after 30 days. Perfito et al. (2008) found that testis size, but not LH, was affected by similar food restriction in Zebra finches *Taeniopygia guttata*. Food-deprived house finches *Haemorhous mexicanus* had under developed testes which was thought to result from decreased GnRH secretion (Valle et al. 2015). The daily timing of food availability does influence the photoperiodic inhibitory process. I did a study a (Dawson, 1986) involving three groups of common starlings (*Sturnus vulgaris*). One was kept under a short photoperiod 8L:16D with food provided *ad lib*. Another was transferred to a long photoperiod (16L:8D) with food *ad lib*. The third group was also transferred to a long photoperiod (16L:8L) but food was removed 8 h after dawn, so a feeding day of the same duration as the short photoperiod group. There was little difference in the rate of testicular maturation between the two long photoperiod groups, demonstrating that food had no influence on photoperiodic gonadal stimulation. However, there was a dramatic delay in regression. For most birds, regression was delayed by about 2 weeks (regression started 6 weeks after transfer to long photoperiods as opposed to 4 weeks in birds with ad lib food). Remarkably, two birds had shown no regression by the end of the study (12 weeks after transfer). Thus, as with temperature, the photoperiodic inhibitory process is more amenable to modulation by food availability than is photoperiodic stimulation.

Lower ambient temperature and decreased food availability had similar effects on the testicular cycle in starlings; neither influenced testicular maturation, but both delayed

98 regression (Dawson, 1986, 2005). It is perhaps counterintuitive that a decrease in food
99 availability and a decrease in ambient temperature do not have negative effects on the
100 reproductive system; they both extend rather than inhibit it.

101 Starlings have a robust and marked daily cycle in body temperature (T_b) which is
102 closely related to photoperiod (Dawson, 2017). T_b starts to increase before dawn, remains
103 high throughout daylight hours, and then decreases rapidly at dusk. The amplitude of the
104 cycle increases as photoperiod decreases. Night-time hypothermia is thought to be an energy
105 conserving mechanism and ambient temperature affects depth of nocturnal hypothermia
106 (Reinertsen and Haftorn, 1986). Several studies have shown that food deprivation, or energy
107 deficiency, can also increase the depth of nocturnal hypothermia (Ben-Hamo et al., 2010;
108 Cooper and Gessaman, 2005; McKechnie and Lovegrove, 2002; Noakes et al., 2013; Nord et
109 al., 2009; Pravosudov and Lucas, 2000; Prinzinger et al., 1991; Reinertsen and Haftorn, 1986;
110 Reinertsen et al., 1988; Waite, 1991; Welton et al., 2002). Importantly, food removal in
111 Japanese quail *Coturnix coturnix japonica* led to diurnal hypothermia (Laurila et al., 2005).

112 Since food restriction and ambient temperature affect both reproduction by delaying
113 gonadal regression, and both can also affect the daily cycle in T_b, could the effects of food
114 and temperature act through a common mechanism, the daily cycle in T_b, and that this
115 influences the timing of gonadal regression? The aim of this study was to confirm that low
116 temperature and food restriction have similar effects on gonadal cycles i.e. little effect on
117 maturation but delaying regression, and to determine whether either or both affect the daily
118 cycle in T_b. The results show that food restriction and ambient temperature both changed the
119 characteristics of the daily cycle in T_b to that of a shorter photoperiod. A shorter photoperiod
120 would also delay gonadal regression. Thus food and ambient temperature may act through a
121 common pathway to influence the plastic photoperiodic inhibitory process, but not the more
122 resilient photoperiodic stimulatory process.

2. Materials and methods

2.1 Birds

Juvenile starlings were caught at Berwick upon Tweed, UK, 55.8 ^{0}N during August 2014. Only males were kept; females were released. Birds were kept in four environmentally controlled chambers (2.5 m x 2.0 m and 2.2 m high) in which they were allowed to fly freely. They were provided with turkey starter crumbs *ad lib*. All birds were treated with a single dose of anti-helminthic (Flubenvet) in food to eliminate gut parasites and 1% Ivermectin on the skin to remove ectoparasites.

Temperature was initially kept at 14 ^{0}C with 5 changes of air per hour. The time that lights came on and went off each day was controlled by a Lutron GRAFIK Eye QS system. Light intensity increased from zero to 1000 lux during a 1 min period at dawn and decreased during 1 min at dusk. Photoperiod for all groups was changed daily and simulated the natural changes at 56 ^{0}N (minimum at the winter solstice 7L:17D; maximum at summer solstice 17L:7D).

Food was provided in commercial poultry feeders. Each feeder was placed in a solid enclosure (1m x 0.8m x 0.8m) such that access to the food was through two openings at floor level. In two of the chambers the opening could be closed with a metal plate that slid down at pre-determined times (Chicken Guard, used in free-range chicken coups to prevent nocturnal access by predators).

2.2 Experimental design

In early December, birds were laparotomized under isofluorane anaesthesia and the dimensions of the left testis measured to the nearest 0.1 mm using a binocular microscope.

Testicular volume was calculated as $4/3\ \pi a^2 b$ where a is half the width and b is half the length. Birds were laparotomized at approximately three week intervals during the study.

At the winter solstice, temperature was decreased to 6 ^{0}C in two chambers and in the other two it was increased to 16 ^{0}C. In one of the chambers at 6 ^{0}C and one at 16 ^{0}C, access to food was prevented by the Chicken Guard door closing 7h after dawn (which was at dusk at the winter solstice). In the other two chambers, food was available *ad lib*. Sample numbers in the four groups were: 6 ^{0}C *ad lib* food, n = 7; 6 ^{0}C restricted food n = 9; 16 ^{0}C *ad lib* food, n = 8; 16 ^{0}C restricted food, n = 8. Dawn was kept at a fixed time (08:00 GMT), so that the time when access to food was prevented, also occurred at the same time each day (15:00 GMT). Photoperiod was increased to simulate the natural changes in photoperiod by extending dusk.

The inhibitory photoperiodic process leading to testicular regression (otherwise known as photorefractoriness) starts when photoperiod has increased to 12L: 12D (Dawson, 2015). Changes in the daily cycle in T_b which may be involved should therefore be apparent then. The daily cycle in T_b was assessed using DST nano-T temperature data loggers (Star-Oddi Ltd, Iceland) in which recorded data is stored in the logger's internal memory with a real time clock reference for each measurement. The loggers were synchronised with the same computer as the GRAFIK Eye lighting controls. The loggers were 17 mm x 6 mm, weighing 1 g. Each bird was anesthetised with Isoflurane, a 10 mm incision was made in the skin of the nape of the neck, and a logger inserted sub-dermally. The incision was closed with tissue adhesive (Vetbond). The loggers were implanted 5 days before photoperiod had increased to 12L:12D, and programmed to start recording five days after implantation. Temperature was recorded every 5 min. Birds were again anesthetised, the loggers removed and data downloaded using the SeaStar program (Star-Oddi Ltd, Iceland).

The start of molt is closely associated with the time of testicular regression (Dawson, 2006). Since the start of molt can be measured exactly, unlike the gradual decrease in testis size, it provides an accurate quantifiable data point which is related to the timing of regression. Birds began to molt during May. The progress of molt was assessed using a molt scoring system which corrects for primary feather mass (Dawson and Newton, 2004). For each bird, a series of linear regressions was used to calculate molt start date and the date that subsequent increments in molt score occurred.

The experimental procedures were licensed by the UK Home Office (Project licence PPL 60/4176).

2.3 Statistical analyses

To make the analysis of temperature data more manageable, each block of six 5 min temperature records was averaged to produce a mean for each 30 min period. Data on T_b during 24h and testis volume during the study were then assessed in a repeated measures two-way ANOVA (Graph Pad Prism) followed by Tukey's Multiple Comparison Tests. Molt start dates were analysed using one-factor ANOVA followed by Tukey's Multiple Comparison Test.

3. Results

3.1 Body mass

There were significant differences between the treatment groups: $F_{3, 196} = 3.22$, P=0.0386 (Fig. 1). However, there were no consistent differences between any groups. All four groups increased mass significantly between the first two sampling points but there were no subsequent consistent changes. There were two occasions, mid-March and mid-May,

when the cold *ad lib* group had body mass significantly higher than the other three groups, but there were no significant differences between any groups at any other times. Therefore, differences in T_b, testis sizes and molt between the 6 ^{0}C *ad lib* and restricted food groups, and between the 16 ^{0}C *ad lib* and restricted food groups, were not due to under nutrition.

3.2 Daily cycle in body temperature under 12L:12D

There were significant differences between the treatment groups: $F_{3, 1175} = 3.52$, $P=0.0296$. In all groups, T_b increased significantly ($P<0.001$) before dawn and continued to increase after dawn ($P<0.001$). Also, in all four groups, T_b decreased significantly immediately after dusk ($P<0.001$).

Comparing the warm and cold *ad lib* groups (Fig. 2 A), T_b was significantly lower ($P<0.01$) in the cold *ad lib* group during the middle of the night but there were no differences at any other time. In the warm restricted group (Fig. 3A) T_b decreased significantly ($P<0.05$) between access to food being denied (13:00) and 15 min before dusk. T_b in this group was significantly lower than in the warm *ad lib* group 15 min before dusk ($P<0.01$) and 15 min after dusk ($P<0.001$). In the cold restricted group (Fig. 4A) T_b also decreased significantly ($P<0.001$) between access to food being denied and 15 min before dusk. T_b in this group was significantly lower than in the cold *ad lib* group 15 min before dusk ($P<0.001$) and 15 min after dusk ($P<0.001$). The decrease in T_b following access to food being denied was greater at the lower ambient temperature. Between 15:00 and dusk, T_b was lower ($P<0.01$) in the cold restricted group than in the warm restricted group (Figs. 3A and 4A).

Mean body temperature over 24h did not differ significantly between the groups (Fig. 5) although there was a suggestion that mean body temperature was higher in the two groups at 16 ^{0}C.

219

220 *3.3 Changes in testis size*

221 There were significant differences between the treatment groups: $F_{3, 196} = 5.94$,
222 P=0.0029. Ambient temperature had a slight effect during the period of testicular maturation.
223 Between January and April, testicular volume was slightly, but not significantly greater, in
224 the warm *ad lib* group than in the cold *ad lib* group (Fig. 2B). During early March, testicular
225 volume was greater (P<0.01) in the warm restricted group than in the cold restricted group,
226 but not at any other time. In contrast, ambient temperature did have a clear effect on the time
227 of regression in the two *ad lib* groups. Testis volume showed a large decrease (P<0.001)
228 between mid and late April in the warm *ad lib* group, but only a slight decrease (P<0.05) in
229 the cold *ad lib* group. There was a greater decrease in the cold *ad lib* group between late
230 April and mid-May (P<0.001). In both late April and mid-May, testis volume was
231 significantly lower (P<0.001) in the warm *ad lib* group than in the cold *ad lib* group. Lower
232 temperature also delayed regression, although less dramatically, in the food-restricted groups.
233 In mid-May, testis size was lower (P<0.001) in the cold food-restricted group (Fig. 4B) than
234 in the warm food-restricted group (Fig.3 B).

235 Food restriction had no effect on testicular maturation (Figs. 3B and 4B) but it did
236 have an effect on the timing of regression. Testis volume decreased (P<0.001) between mid
237 and late April in the warm *ad lib* group, and between late April and mid-May in the warm
238 restricted group (Fig. 3B). In both late April and mid-May, testis volume was significantly
239 lower (P<0.001) in the warm *ad lib* group than in the warm restricted group. Testicular
240 regression began slightly earlier in the cold *ad lib* group than the cold restricted group (Fig.
241 4B). There was a slight decrease in the cold *ad lib* group between mid and late April (P<0.05)
242 but no decrease until later in the cold restricted group.

243

244 *3.4 Molt*

245 There were significant differences between the treatment groups: $F_{3, 25} = 40.28$,
246 $P<0.0001$. The start of molt closely correlated with the time of testicular regression;
247 treatments affected molt in the same way as regression. Ambient temperature affected the
248 start of molt. Molt started 15 days earlier ($P<0.001$) in the warm *ad lib* group than in the cold
249 *ad lib* group (Fig. 2C) and molt started 16 days earlier ($P<0.001$) in the warm restricted group
250 than in the cold restricted group (Figs. 3C and 4C). Food restriction also affected molt. Molt
251 started 7 days earlier ($P<0.05$) in the warm *ad lib* group than in the warm restricted group
252 (Fig. 3C) and molt started 8 days earlier ($P<0.01$) in the cold *ad lib* group than in the cold
253 restricted group (Fig. 4C).

254

255 **4. Discussion**

256 The first aim of this study was to confirm that low temperature and food restriction
257 have similar effects on gonadal cycles i.e. little effect on maturation but delaying regression.
258 There was indeed little effect of temperature, and no effect of food restriction, on testis size
259 during testicular maturation. In contrast, testicular regression and the start of molt were both
260 significantly delayed by both lower ambient temperature and the restriction of food. The
261 lower ambient temperature (10 degrees lower) delayed molt by 15 days in the food *ad lib*
262 groups and by 16 days in the food restricted groups. Food restriction (to 7h after dawn)
263 delayed molt by 7 days in the 16 ^{0}C groups and by 8 days in the 6 ^{0}C groups. The annual
264 cycle in gonadal maturity is thought to be controlled by two photoperiodic mechanisms
265 acting in tandem throughout the year: a positive drive on GnRH secretion which is
266 predominant during increasing photoperiods and leads to gonadal maturation, and an

inhibitory process which becomes predominant during long photoperiods and leads to gonadal regression (Dawson, 2015). The effects of ambient temperature and food restriction appear to modulate photoperiodic control of the latter process rather than the former. There is a potential ecological explanation why lower temperature should lead to prolonged breeding. In cooler years, while food peak food availability may be less, it is likely to be later, and more prolonged.

However, it is not possible to be categorical from the data here that the effects were restricted to gonadal regression. Lower temperature caused a slight, although non-significant delay in testicular maturation. Food restriction had no effect on maturation. However, the caveat here is that because of the experimental design, there was little food restriction during the early stages of testicular maturation when photoperiod was still short. Nevertheless, ambient temperature and the time of food availability did both clearly have effects on the photo-induced gonadal cycle.

The second aim was to look for a common mechanism through which the two different environmental factors, ambient temperature and food restriction, could operate to impart their similar effects on the gonadal cycle. Obviously both have energetic implications. There is evidence that both, independently, can affect the daily cycle in T_b. In birds with *ad lib* food, the daily cycle in T_b is strictly related to photoperiod; high T_b is normally maintained until dusk and this was true of both *ad lib* food groups in this study. In the food restricted birds, T_b began to decrease after access to food was blocked. Therefore the duration of maximal T_b was less than the photoperiod, and the timing of testicular regression was also as if photoperiod was shorter. In the *ad lib* fed birds, lower ambient temperature induced a greater amplitude in the daily cycle of T_b, with lower T_b during darkness. This too is a characteristic of shorter photoperiods (Dawson, 2017). Thus the timing of testicular

291 regression appears, in some way, to be related to the daily cycle in T_b in addition to
292 prevailing photoperiod.

293 It may be controversial to suggest that the daily cycle in T_b modulates photoperiodic
294 responses in birds since there is a wealth of evidence to show that photoperiod is the major
295 cue used to time gonadal maturation and regression e.g. Dawson (2015), and that light acts
296 directly, in the case of birds, through encephalic photoreceptors (Foster and Follett, 1985;
297 Garcia-Fernandez et al., 2015). However, it is well known in plants that temperature can
298 modulate photoperiodic molecular mechanisms to regulate the timing of flowering (Andres
299 and Coupland, 2012; Song, 2016). Furthermore, the present study led to a subsequent study in
300 which starlings were maintained on ultra-short photoperiods, and gonadal responses related to
301 the daily cycle in T_b much more closely than to photoperiod. Nevertheless, this remains a
302 correlation rather than demonstrating a causal relationship.

303 Thyroid hormones may be an important link between energetics, T_b and photoperiodic
304 responses. They regulate metabolic rate (e.g. hypothyroidism is associated with low T_b) and
305 also play a critical role in photoperiodic responses (Dawson, 1993; Yoshimura, 2006).
306 Experimental treatment with exogenous thyroid hormones can mimic long photoperiods
307 (Dawson, 1989; Follett et al., 1988). Wikelski et al (2008) suggested that energy turnover
308 may determine the duration of circannual cycles in house sparrows (*Passer domesticus*); the
309 lower the rate of energy turnover the longer the cycle length. In the present study, higher
310 mean T_b was associated with shorter gonadal cycle. However, the food-restricted birds were
311 not nutritionally stressed – they maintained their body weight. It was apparently the time of
312 food availability rather than total food intake that was important and in house sparrows
313 periodic food availability can act as a *Zeitgeber* for the whole circadian system (Hau and
314 Gwinner, 1996). Future studies could directly address the question of whether the timing
315 rather than the general availability of food is important.

316 In conclusion, reducing the time of food availability and reducing ambient
317 temperature both modulate photoperiodic responses so that long-photoperiod induced
318 gonadal regression is delayed. Both also affect the daily cycle in T_b to give it characteristics
319 of a shorter photoperiod. This suggests the possibility that the daily cycle in T_b may play a
320 role in photoperiodic responses. Although this is a surprising and tentative conclusion,
321 stronger evidence for this was obtained in a subsequent study.

322

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448 **Figure legends**

449 **Figure 1.**

450 Change in body mass during the study, starting in January and finishing in June. There were
451 four groups of starlings: one group held at 6 ^{0}C with food provided ad lib (Cold ad lib, solid
452 blue n = 7); another group held at 6 ^{0}C with access to food restricted to 7h after dawn (Cold
453 restricted, open blue n = 9); a group held at 16 ^{0}C with food provided ad lib (Warm ad lib,
454 red solid n = 8) and a final group held at 16 ^{0}C with access to food restricted to 7h after dawn
455 (Warm restricted, open red n = 8). Each point represents the mean ± S.E.

456 **Figure 2.**

457 Starlings held under a simulated natural cycle in photoperiod between January and August.
458 Birds were held at 16 ^{0}C (solid red, n= 8) or 6 ^{0}C (solid blue, n=7) and provided with food *ad*
459 *libitum*. Each point represents the mean ± S.E. A. Body temperature (T_b) recorded every 30
460 min for 24 h. Photoperiod was 12L:12D and the duration of darkness is shaded. T_b was lower
461 during the night in birds at 6 ^{0}C but there were no differences during the day. B. Changes in
462 testicular volume between January and June. There were no significant differences during
463 testicular maturation, but the birds held at 16 ^{0}C showed earlier regression. The vertical line
464 represents when photoperiod reached 12:12D when T_b was recorded (A). C. The progress of
465 moult in the two groups. Each point represent the date ± S.E. that each unit of moult score
466 was reached by each bird. Birds held at 16 ^{0}C started to moult sooner.

467 **Figure 3.**

468 Starlings held under a simulated natural cycle in photoperiod between January and August.
469 All birds were held at 16 ^{0}C. One group was provided with food *ad libitum* (solid red, n= 8).
470 For the other group, access to food was prevented 7h after dawn (open red, n=8). Each point

represents the mean ± S.E. A. Body temperature (T_b) recorded every 30 min for 24 h. Photoperiod was 12L:12D and the duration of darkness is shaded. The vertical line represents when access to food was prevented, at 13:00 under 12L:12D. T_b began to decrease after access to food was prevented. B. Changes in testicular volume between January and June. There were no significant differences during testicular maturation, but the birds with *ad libitum* food showed earlier regression. The vertical line represents when photoperiod reached 12:12D when T_b was recorded (A). C. The progress of moult in the two groups. Each point represent the date ± S.E. that each unit of moult score was reached by each bird. Birds with *ad libitum* food started to moult sooner.

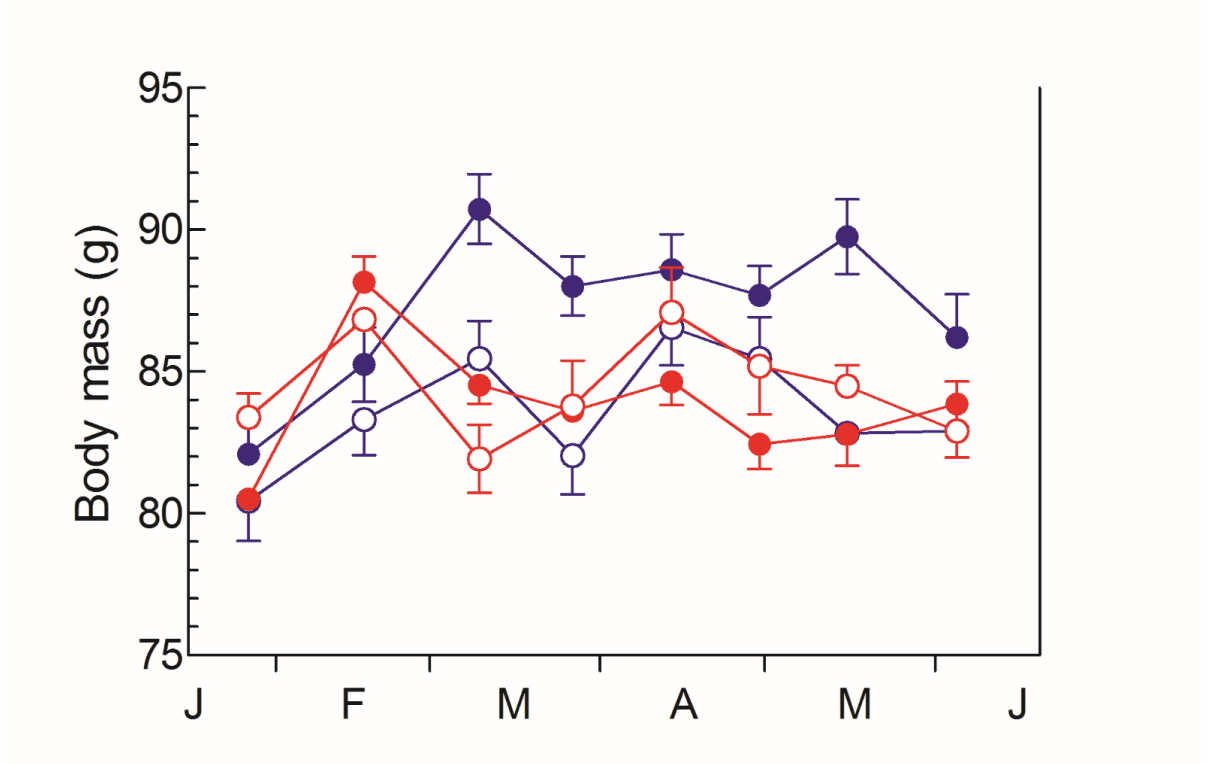
Figure 4.

Starlings held under a simulated natural cycle in photoperiod between January and August. All birds were held at 6 ^{0}C. One group was provided with food *ad libitum* (solid blue, n= 7). For the other group, access to food was prevented 7h after dawn (open blue, n=9). Each point represents the mean ± S.E. A. Body temperature (T_b) recorded every 30 min for 24 h. Photoperiod was 12L:12D and the duration of darkness is shaded. The vertical line represents when access to food was prevented, at 13:00 under 12L:12D. T_b began to decrease after access to food was prevented. B. Changes in testicular volume between January and June. There were no significant differences during testicular maturation, but the birds with *ad libitum* food showed earlier regression. The vertical line represents when photoperiod reached 12:12D when T_b was recorded (A). C. The progress of moult in the two groups. Each point represent the date ± S.E. that each unit of moult score was reached by each bird. Birds with *ad libitum* food started to moult sooner.

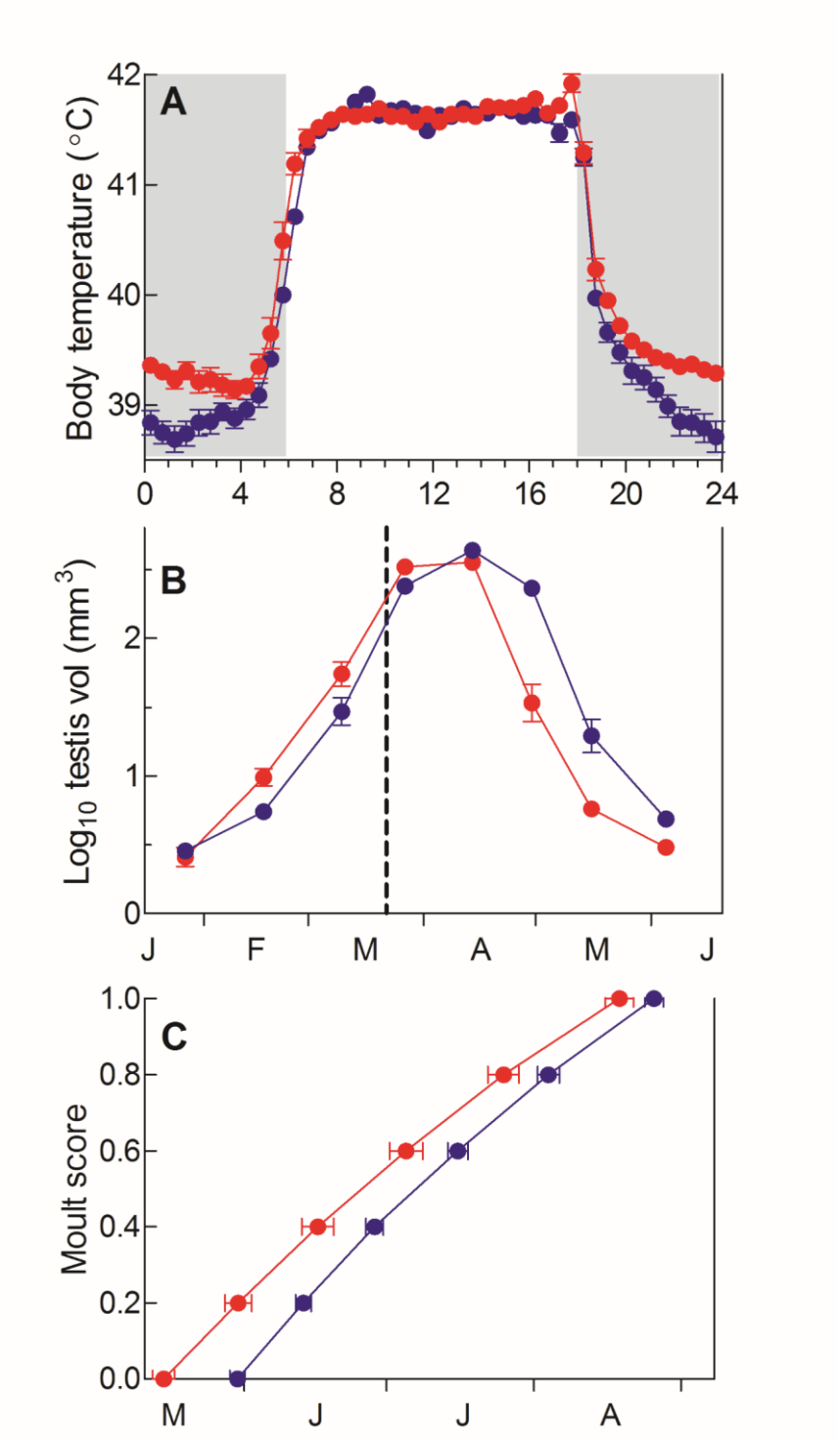
Figure 5.

Mean body temperature in the four groups of starlings when photoperiod was 12L:12D: A Birds held at 6 ^{0}C with food provided ad lib (n = 7); B birds held at 6 ^{0}C with access to food restricted to 7h after dawn (n = 9); birds held at 16 ^{0}C with food provided ad lib (n = 8) and birds held at 16 ^{0}C with access to food restricted to 7h after dawn (n = 8). Each point represents the mean ± S.E. The differences were not significant.

Fig. 1



508 Fig. 2



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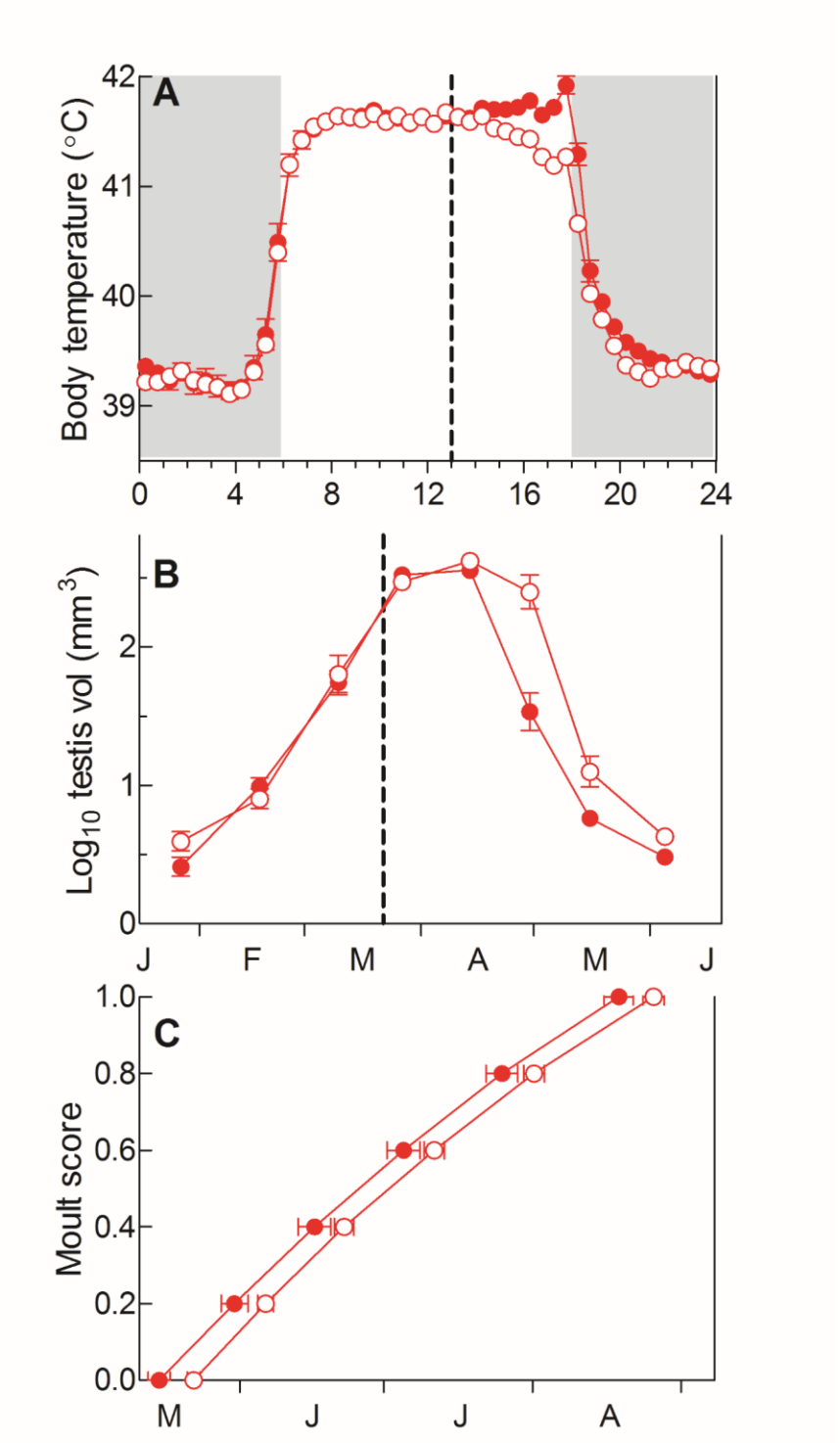
512 Fig. 3



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516 Fig. 4

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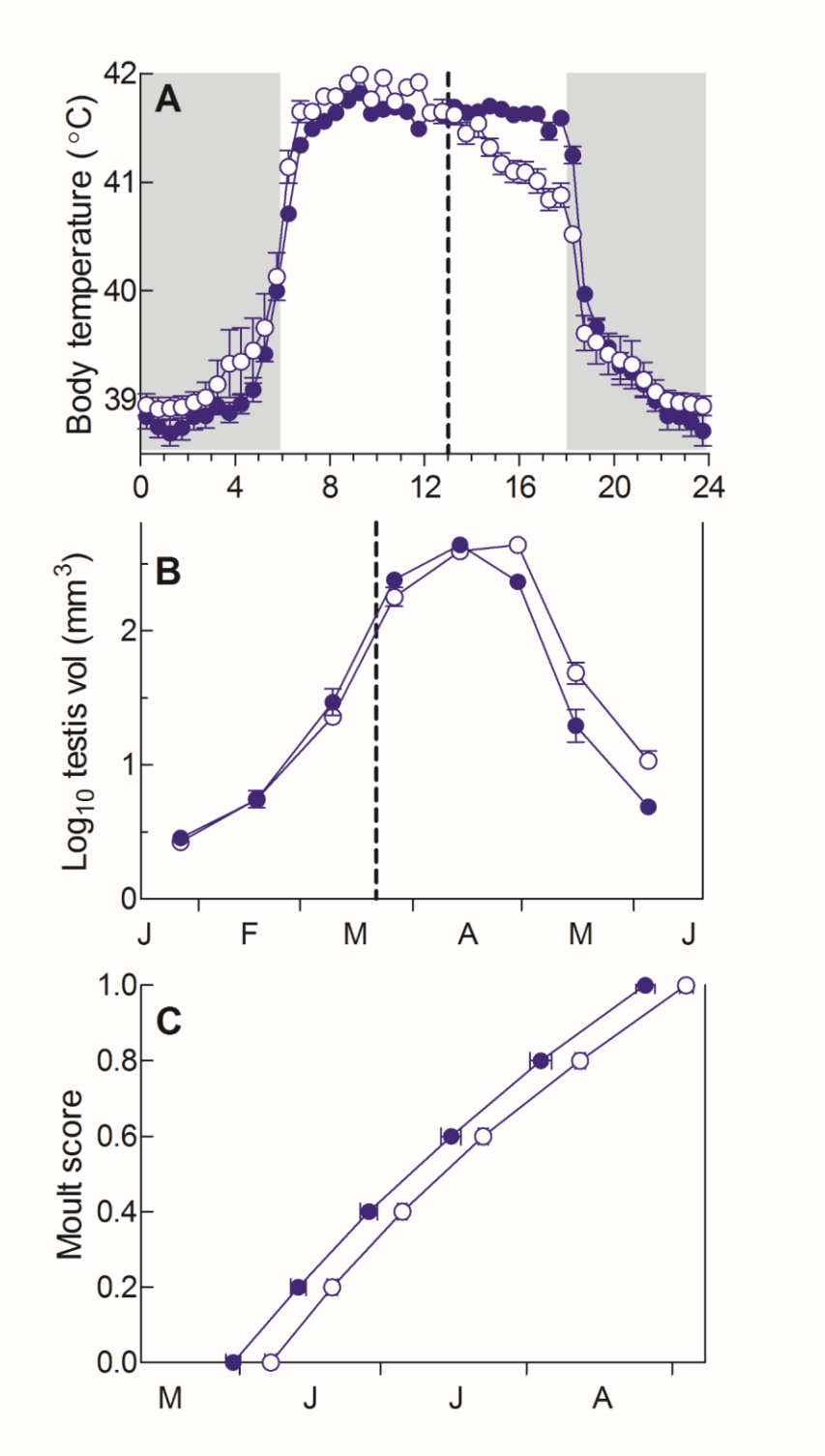

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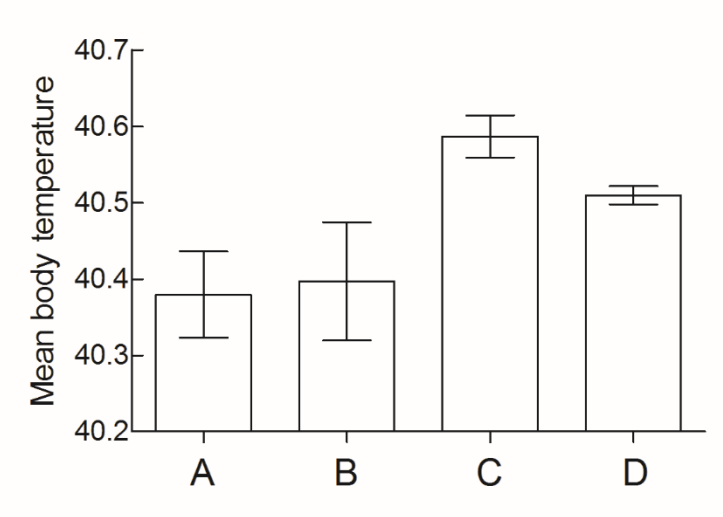
520 Fig. 5



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