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1 **Photoperiodic induction without light-mediated circadian entrainment in a high arctic**
2 **resident bird**
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4
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15 **Key words**: Photoperiodism, circadian, seasonal reproduction, pars tuberalis, eyes absent,
16 deiodinase, Svalbard ptarmigan

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Summary statement

Svalbard ptarmigan show photoperiodic responses when transferred from constant darkness to constant light without circadian entrainment.

Abstract

Organisms use changes in photoperiod to anticipate and exploit favourable conditions in a seasonal environment. While species living at temperate latitudes receive day length information as a year-round input, species living in the Arctic may spend as much as two-thirds of the year without experiencing dawn or dusk. This suggests that specialised mechanisms may be required to maintain seasonal synchrony in polar regions.

Svalbard ptarmigan (*Lagopus muta hyperborea*) are resident at 74-81° north latitude. They spend winter in constant darkness (DD) and summer in constant light (LL); extreme photoperiodic conditions under which they do not display overt circadian rhythms.

Here we explored how arctic adaptation in circadian biology affects photoperiodic time measurement in captive Svalbard ptarmigan. For this purpose, DD-adapted birds, showing no circadian behaviour, either remained in prolonged DD, were transferred into a simulated natural photoperiod (SNP) or were transferred directly into LL. Birds transferred from DD to LL exhibited a strong photoperiodic response in terms of activation of the hypothalamic thyrotropin-mediated photoperiodic response pathway. This was assayed through expression of the *Eya3*, *Tsh*β and deiodinase genes, as well as gonadal development. While transfer to SNP established synchronous diurnal activity patterns, activity in birds transferred from DD to LL showed no evidence of circadian rhythmicity.

These data show that the Svalbard ptarmigan does not require circadian entrainment to develop a photoperiodic response involving conserved molecular elements found in temperate species. Further studies are required to define how exactly arctic adaptation modifies seasonal timer mechanisms.

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43 **Introduction**

44 Animals in temperate and high latitudes use changes in photoperiod (day length) to anticipate
45 upcoming seasons and adjust physiology and behaviour accordingly. The involvement of
46 circadian clocks in this photoperiodic time measurement was first suggested by Erwin
47 Bünning, who proposed a so-called 'external coincidence' mechanism. According to the
48 Bünning hypothesis (Bünning, 1936), organisms express an innate circadian rhythm of photo-
49 inducibility and light exposure coinciding with the photo-inducible phase of this rhythm
50 triggers a photoperiodic response.

51 In order to test the Bünning hypothesis, experimental approaches based on artificial
52 light exposures, such as night break experiments, have been employed (Bünning, 1936; Elliott
53 et al., 1972; Follett and Sharp, 1969; Follett et al., 1992; Gwinner and Eriksson, 1977; Hamner
54 and Enright, 1967; Pittendrigh, 1972). Night break experiments trigger a long day response by
55 combining a short photoperiod with a nocturnal light pulse that occurs in the photo-inducible
56 phase. Positive results of these experiments across diverse taxonomic groups favour a
57 circadian-based photoperiodic readout mechanisms.

58 In birds and mammals, photoperiodic effects on reproduction depend on changes in
59 hypothalamic gonadotrophin releasing hormone (GnRH) secretion at the median eminence,
60 and recent evidence points to a coincidence timer mechanism in the adjacent *Pars tuberalis*
61 (PT) as the key upstream control mechanism (Dardente et al., 2010; Hazlerigg and Loudon,
62 2008; Lincoln et al., 2002; Masumoto et al., 2010; Nakao et al., 2008; Yasuo et al., 2003;
63 Yoshimura et al., 2003).

64 Within the PT, long photoperiods (LP) stimulate the expression of the thyroid
65 stimulating hormone (TSH) β subunit (*Tsh*β) (Nakao et al., 2008). LP induced expression of TSH
66 leads to increased *Dio2* expression in the mediobasal hypothalamus (MBH), through a cAMP
67 dependent pathway in neighbouring ependymal cells known as tanycytes (Bolborea et al.,
68 2015; Hanon et al., 2008; Nakao et al., 2008; Ono et al., 2008). DIO2 locally converts thyroxine
69 (T_4) to the bioactive triiodothyronine (T_3) by outer ring deiodination, thus increasing
70 hypothalamic T_3 concentration under LP. In long day breeding birds and mammals, this in turn
71 increases the release of GnRH in the median eminence, ultimately leading to gonadal
72 activation (Yamamura et al., 2004; Yamamura et al., 2006; Yoshimura et al., 2003). Conversely,
73 under short photoperiod, low levels of TSH in the PT coincide with increased type III
74 iodothyronine deiodinase (*Dio3*) expression in tanycytes, keeping hypothalamic T_3

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75 concentration low and promoting gonadal inactivation (Yasuo et al., 2005). The reciprocal
76 regulation of *Dio2*/ *Dio3* expression and the resulting bioactive T_3 concentration in the MBH is
77 at the core of photoperiodic control of seasonal reproduction and has become a central
78 paradigm in photoperiodic time measurement.

79 Several lines of evidence suggest that this PT-mediated readout system is circadian-
80 based. First, in both birds and mammals so-called 'clock genes' show characteristic rhythmical
81 expression in the PT/ MBH region, consistent with a possible coincidence timer mechanism
82 (Johnston et al., 2005; Lincoln et al., 2002; Tournier et al., 2007; Yasuo et al., 2003; Yasuo et
83 al., 2004). Secondly, in the Japanese quail (*Coturnix japonica*) photoperiodic induction of *Dio2*
84 and downstream physiological responses can be triggered by night break experiments
85 (Yoshimura et al., 2003), implying control through a coincidence timer mechanism.

86 Further evidence for the circadian basis on the hypothalamic long day response derives
87 from research on eyes absent 3 (EYA3). In mammals, EYA3 has been proposed to act as a
88 transcriptional co-activator at the *Tsh*β gene promoter and analysis of the ovine *Eya3*
89 promoter demonstrated that its expression is controlled by circadian clock genes (Dardente
90 et al., 2010; Masumoto et al., 2010).

91 Circadian-based models for photoperiodic time measurement place an emphasis on
92 robust circadian cycles of clock gene expression. This raises the question of what happens in
93 species living at arctic latitudes. Light-dark cycles are absent for extended periods of the year
94 and under such circumstances daily rhythmicity in behaviour and endocrinology breaks down
95 completely (Reierth and Stokkan, 1998; Reierth et al., 1999; Stokkan et al., 1994; van Oort et
96 al., 2005; van Oort et al., 2007). Loss of behavioural and endocrine circadian rhythmicity does
97 not necessarily imply loss of circadian-based photoperiodic response circuits, especially in
98 birds where circadian organisation involves multiple circadian oscillators (Cassone, 2014).
99 Moreover, in temperate bird species, lesioning studies resolve behavioural organisation from
100 photoperiodic sensitivity (Binkley et al., 1972; Menaker and Keatts, 1968; Menaker et al.,
101 1970; Rani et al., 2007; Siopes and Wilson, 1974; Wilson, 1991). Nevertheless adaptation to
102 the Arctic might have had a substantial impact on the entire circadian system, which could
103 also affect circadian-based photoperiodic induction. Fibroblast cultures from reindeer show
104 arrhythmic clock gene expression (Lu et al., 2010) and *in-silico* analysis on clock genes revealed
105 mutations that might impact upon circadian rhythm generation (Lin et al., 2019). If arctic
106 animals cannot sustain circadian rhythmicity in the polar day and polar night, this might limit

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107 photoperiodic responses through coincidence timing to those phases of the year with a robust
108 light-dark cycle.

109 To investigate this, we have performed photoperiod manipulations in captive Svalbard
110 ptarmigan (*Lagopus muta hyperborea* Sundevall, 1845), the northernmost resident
111 herbivorous bird species (Fig. 1). Svalbard ptarmigan are highly seasonal in their breeding
112 physiology (Steen and Unander, 1985; Stokkan et al., 1988; Stokkan et al., 1986) and become
113 behaviourally arrhythmic around the solstices (i.e. during the polar night and the polar day)
114 (Reierth and Stokkan, 1998). Similar dampening of melatonin rhythmicity has also been
115 observed (Reierth et al., 1999).

116 In order to test if a light-dark cycle is necessary to induce a long day response in
117 Svalbard ptarmigan, we transferred birds, acclimated to constant darkness (DD), either into a
118 gradually increasing photoperiod or directly into constant light (LL). The former group
119 therefore received a rhythmic light-dark cycle while the latter did not. The control group
120 remained in DD. We measured gonadal mass and behavioural activity as well as *Eya3*, *Tsh*β,
121 *Dio2* and *Dio3* expression in the PT/ MBH region.

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122 **Material and methods**

123

124 Experimental animals and housing

125 All animals were kept in accordance of the EU directive 201/63/EU under a licence provided
126 by the Norwegian Food Safety authority (Mattilsynet, FOTS 7971). Chicks were hatched from
127 eggs laid by captive adult Svalbard ptarmigan at the University of Tromsø (69° 39´N, 18° 57´E).
128 Hatching took place between June 24th 2017 and August 1st 2017. The chicks were raised either
129 indoors with a photoperiod corresponding to the on- and offset of natural civil twilight in
130 Tromsø or outside on the ground. Upon reaching a body mass of 400 to 500 g, 29 birds (Table
131 S1) were transferred into individual cages (1.5 m x 0.5 m) in light and temperature controlled
132 rooms. All birds were transferred at the end of September 2017. Food (standardised protein
133 food; Norgesfor, Ref. No.:OK 2400 070316) and water were provided *ad libitum* throughout
134 the study. Female and male birds were housed together.

135 Controlled lighting was provided by fluorescent strip lights (Osram L 58 W 830 Lumilux)
136 delivering approximately 1000 lux at floor level. All rooms were further equipped with
137 permanent red illumination (Philips BR125 IR 250 W). During the initial acclimation phase the
138 photoperiod was gradually decreased until reaching DD (red light excepted) on December 22nd
139 2017. Birds in DD were held under red light to allow for husbandry. The birds remained in DD
140 for five weeks prior to experimental light treatments.

141

142 Experimental light treatment and sampling

143 After five weeks of DD five individuals were sampled as an initial control group. This marked
144 the start of the experiment (point 0). Thereafter, the three experimental groups were
145 transferred to their respective light treatments (Fig. 2 and Table S1). Six birds remained in DD
146 until the end of the experiment, nine birds were directly transferred into LL and nine birds
147 were exposed to a simulated natural photoperiod (SNP). The SNP treatment reflected an
148 increase in day length following the progression of civil twilight on- and offset of
149 Longyearbyen, Svalbard (78°13'N 15°38'E; Table S2).

150 Four individuals were sampled after 38 hours in LL. This sampling time was chosen to
151 coincide with acute photoperiodic gene induction as previously reported in the quail MBH and
152 PT (Nakao et al., 2008). Subsequent samplings aimed to investigate chronic changes in gene
153 expression, and were undertaken at single time points on the following days: After five weeks,

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154 four individuals were sampled from the SNP group as they reached LD 12:12. This sampling
155 was performed 3.5 – 4.5 hours after lights on. After ten weeks of light treatment all remaining
156 birds from all groups were sampled. The SNP group had reached LL through a gradual increase
157 in photoperiod four days before the final sampling. All groups were euthanised between 9:00
158 and 15:00 local time. The DD group was euthanised on the day after the LL and SNP group.
159 Samplings of birds in DD was performed under dim red light only.
160 Brains were removed after euthanasia and rapidly transferred onto a cooled metal
161 block until stored at -80 °C. Testes and ovaries were removed and measured *post-mortem*.
162
163 Activity
164 Locomotor activity of all experimental birds was continuously recorded as movement per
165 minute by passive infrared sensors, mounted on the cage doors. Data were collected by an
166 Actimetrics CL200 USB interface coupled to ClockLab data acquisition software (Version 2.61).
167
168 cDNA cloning and *in situ* hybridisation
169 Probe synthesis and *in situ* hybridisation were performed as described in Lomet *et al*. (2018).
170 RNA was extracted from Svalbard ptarmigan brain tissue using TriReagent (Sigma) and
171 converted into cDNA using Omniscript RT kit (Qiagen). The Icelandic rock ptarmigan genome
172 (Kozma et al., 2016) was used to design PCR primers to amplify cDNA fragments for *Tsh*β, *Eya3*,
173 *Dio2* and *Dio3*. PCR was performed with Taq DNA polymerase (Qiagen). PCR products of
174 correct sizes were extracted and cloned into pGEMT easy vectors (Promega). The inserts
175 (Table S3) were sequenced (Eurofins Sequencing services, Germany) and verified against the
176 reference genome.
177 Cloned vectors were stored at -20°C until further use. Prior to hybridisation, vectors
178 were linearised and transcribed using a Promega transcription kit in combination with a ^{35}S-
179 UTP isotope (PerkinElmer) to obtain radioactively labelled complementary riboprobes. The
180 riboprobes were purified with illustra MicroSpin G-50 columns (GE healthcare) and
181 incorporation of ^{35}S-UTP was measured by a liquid scintillation counter (Triathler multilable
182 tester, Hidex).
183 Frozen brains were cryosectioned at 20 µm and sections containing PT and MBH were
184 mounted to pre coated adhesion slides (SuperFrost Plus, VWR). Brain sections were fixed in 4
185 % PFA (0.1 M PB) for 20 minutes at 4 °C and rinsed twice with 0.1 M PB for 5 minutes. Fixed

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186 sections were acetylated with 3.75 % v/v of acetic anhydride in 0.1 M triethanolamine buffer
187 (0.05 N NaOH) and rinsed twice with 0.1 M PB for 5 minutes. Sections were subsequently
188 dehydrated with stepwise increasing ethanol solutions (50 %, 70 %, 96 %, 100 % for 3 minutes
189 each) and dried under vacuum for at least 1 hour.

190 Dried sections were hybridised with 10^6 cpm of riboprobe per slide in hybridisation
191 buffer (50 % deionised formamide, 10 % dextran sulfate, 1 x Denhardt's solution, 300 mM
192 NaCl, 10 mM Tris, 10 mM DTT, 1 mM EDTA, 500 µg/ml tRNA). Hybridisation was performed at
193 56°C overnight. Hybridised sections were washed with 4 x saline sodium citrate (SSC) solutions
194 (3 x 5 minutes) and treated with RNase-A solution (500 mM NaCl, 1 mM Tris, 1 mM EDTA, 20
195 µg/ml) for 30 minutes at 37 °C. Subsequent stringency washes were performed in SSC
196 (supplemented with 1 mM DTT) of decreasing concentration: 2 x SSC (2 x 5 minutes), 1 x SSC
197 (1 x 10 minutes), 0.5 x SSC (1 x 10 minutes), 0.1 x SSC (30 minutes at 60°C), 0.1 x SSC (rinse).
198 Slides were dehydrated afterwards in stepwise increasing ethanol solutions (50 %, 70 %, 96 %,
199 100 % for 3 minutes each) and dried under vacuum. Dried sections were exposed to
200 autoradiographic films (Carestream Kodak BioMax MR film) for 9 to 12 days. Exposed films
201 were developed, fixed and digitalised with an Epson transmission scanner. Optical density
202 (OD) was measured with ImageJ (Version 1.51k, Wayne Rasband).

203

204 Analysis

205 Actograms were produced with the ActogramJ plugin for ImageJ (Schmid et al., 2011) and
206 period length of activity was measured by chi-squared periodograms produced by the same
207 program.

208 Graphs of gene expressions in the PT/ MBH region and gonadal mass were prepared in
209 GraphPad Prism 8 (Version 8.0.2). The results were plotted as each replicate with lines going
210 through the respective mean of each group at each sampling point. Statistical comparisons
211 were made by 1 way ANOVA and Tukey's post hoc tests, performed on log transformed values
212 to ensure homogeneity of variances; the threshold for significance was $p < 0.05$.

213 Individual values for gene expression with the corresponding gender can be found in
214 Table S1.

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215 **Results**

216

217 Activity rhythms

218 Prior to the experimental treatment, all birds in DD exhibited short episodic bouts of activity
219 with no clear periodicity (Figs 3, S1, S2), and for birds continuing on DD the same pattern was
220 maintained. In birds transferred to LL, episodic activity continued, sometimes with ultradian
221 periodicity. Period lengths were typically in the range 3 – 20 h, and highly variable between
222 individuals. Birds transferred to SNP, based on Svalbard civil twilight progression, showed
223 robust daily rhythms with a period of 24h ($p < 0.05$).

224

225 Gonads

226 Testes and ovaries were initially regressed in all groups (Fig. 4), and subsequent development
227 depended on photoperiodic treatment ($p < 0.0001$ by 1 way ANOVA in both cases). Exposure
228 to LL strongly stimulated gonadal maturation for both testes and ovaries, so that after 10
229 weeks masses increased 22-fold and 93-fold, respectively ($p < 0.0001$ by Tukey's post hoc test
230 in both cases). Gonadal maturation in birds maintained in DD and in female birds under SNP,
231 was negligible (DD = 1.4-fold, SNP = 1.1-fold compared to initial values) while male birds
232 transferred to SNP showed a more modest (3.2-fold) but nonetheless statistically significant
233 increase increased testicular mass by the end of the study ($p < 0.001$ by Tukey's post hoc test).

234

235 *Eya3* and *Tsh*β expression

236 The expression of *Tsh*β and *Eya3* over the course of the study was dependent on photoperiod
237 ($p < 0.0001$ by 1 way ANOVA in both cases) (Figs 4, 5). Expression of both genes was below the
238 detection threshold at week 0, and rose dramatically 38 hours after the transfer to LL ($p <$
239 0.001 in both cases by Tukey's post hoc test). Thereafter expression of both genes was
240 maintained at high levels until the end of the study (week 10).

241 In birds exposed to SNP, levels of both genes remained undetectable five weeks after
242 the transfer, when the photoperiod had increased to 12 hours of light. Subsequently, after the
243 photoperiod had progressively increased to LL, expression of both genes increased
244 dramatically to values similar to those in the LL treatment group ($p < 0.001$ compared to initial
245 values by post hoc Tukey's test).

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246 In birds maintained on DD, levels of both genes remained basal throughout the
247 experiment.
248
249 *Dio2* and *Dio3* expression
250 *Dio2* and *Dio3* in the ependymal region of the MBH showed reciprocal changes in expression
251 over the course the study ($p < 0.0001$ by 1 way ANOVA) (Figs 4, 5). Initial *Dio2* expression was
252 relatively weak, while *Dio3* expression was relatively strong (week 0). Transfer to LL increased
253 *Dio2* expression 2.5-fold within 38 hours (week 0 vs 38 hours LL; $p < 0.05$ by post hoc Tukey's
254 test), while over the same period *Dio3* expression was suppressed to background levels (45-
255 fold decrease; $p < 0.01$ by Tukey's post hoc test). Under continued LL exposure, elevated *Dio2*
256 levels and suppressed Dio3 levels were maintained to the end of the experiment.
257 Expression levels of *Dio2* and *Dio3* from birds under SNP gradually increased and
258 decreased respectively over the course the study. In both cases, expression levels after 5
259 weeks under SNP did not differ from initial values, while levels at week 10 were increased 2.3-
260 fold for *Dio2* and decreased 60-fold for *Dio3* ($p < 0.05$ and 0.01, respectively by post hoc
261 Tukey's test).
262 Under constant darkness, no significant changes in either *Dio2* or *Dio3* expression were
263 observed.

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264 **Discussion**

265 In our experiment we transferred DD acclimated Svalbard ptarmigan either into a simulated
266 natural photoperiod or directly into LL. Both photoperiodic treatments caused increased *Eya3*
267 and *Tsh*β expression and changes in the downstream deiodinases expression but birds
268 transferred from DD to LL displayed no circadian behaviour. This absence of circadian
269 rhythmicity in combination with the lack of an external light-dark cycle might question the
270 circadian basis of the long day response in Svalbard ptarmigan.

271 According to theory, a circadian-based rhythm of photo-inducibility triggers a
272 photoperiodic response if light exposure occurs during the photoinducible phase (Bünning,
273 1936). Modern formulations of Bünning's model focus on events in the PT and the MBH,
274 where night-break protocols induce a long day response in local *Tsh*β expression and
275 downstream effects on hypothalamic deiodinase genes (Dardente et al., 2010; Masumoto et
276 al., 2010; Yoshimura et al., 2003). In sheep, promoter analysis of *Eya3*, a co-activator for *Tsh*β,
277 demonstrates transcriptional control through clock genes, further emphasising the circadian
278 basis for photoperiodic time measurement (Dardente et al., 2010).

279 Contrastingly, previous studies on arctic animals report the absence of circadian
280 rhythmicity and suggest this as a possible adaptation to polar latitudes, allowing around the
281 clock foraging in constant arctic light conditions (Lin et al., 2019; Lu et al., 2010; Reierth and
282 Stokkan, 1998; Reierth et al., 1999; van Oort et al., 2005; van Oort et al., 2007). Our study
283 confirms the absence of circadian activity rhythms in DD and LL. In a separate experiment we
284 further found no evidence of circadian body temperature rhythms in DD and LL (Appenroth et
285 al, unpublished).

286 This absence of behavioural and physiological rhythmicity does not exclude the
287 possibility of latent circadian rhythmicity persisting in a coincidence timer mechanism. In non-
288 arctic bird species LL can disrupt circadian activity rhythms but still triggers a photoperiodic
289 response in reproduction (Agarwal et al., 2017; Lumineau and Guyomarc'h, 2003; Simpson
290 and Follett, 1982; Wever, 1980). Moreover, Japanese quail show sustained hypothalamic
291 expression of clock genes in LL, despite behavioural arrhythmicity (Lumineau and Guyomarc'h,
292 2003; Simpson and Follett, 1982; Yasuo et al., 2003). It therefore remains possible that a
293 sustained rhythm of photo-inducibility may also persist within the PT/ MBH region of arctic
294 Svalbard ptarmigan in constant photic conditions. Consequently the DD-to-LL treatment
295 triggers a long day response as light coincides with the photoinducible phase repeatedly after

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296 the transfer (Fig. 6A). Alternatively, the transition from DD to LL might initiate a dampening
297 rhythm of photo-inducibility (Fig. 6B), either by direct induction or by bringing internally
298 desynchronised cellular rhythms into phase (Balsalobre et al., 1998; Nagoshi et al., 2004;
299 Welsh et al., 2004). This scenario would have similar consequences to the persistent
300 rhythmical photo-inducibility described previously and may prove difficult to resolve from one
301 another.

302 Finally, we do not formally exclude that an hour-glass type mechanism operates in
303 these birds. Under this scenario induction relies on the progressive accumulation of a light
304 dependent factor under LL (Fig. 6C). However, we favour a rhythm based model since our
305 molecular characterization of the photoperiodic response shows broad conservation with
306 species known to rely on coincidence timing, like quail (Nakao et al., 2008; Yasuo et al., 2005;
307 Yoshimura et al., 2003) or sheep (Dardente et al., 2010).

308 Similar to Svalbard ptarmigan transferred from DD to LL, birds subjected to a simulated
309 light-dark cycle showed also increased *Eya3* and *Tsh*β expression and changes in the
310 downstream deiodinases expression at the final sampling point in LL but not earlier in the
311 study when the birds were on L:D12:12. This is consistent with other mammals and birds which
312 require a photoperiod between 12.5 to 14 hours for acute changes of photoperiodic genes in
313 PT and MBH (Hanon et al., 2010; Hanon et al., 2008; Król et al., 2012; Nakao et al., 2008; Ono
314 et al., 2008). By the end of the study, birds in the SNP group showed only limited gonadal
315 development. This is in line with earlier reports that wild Svalbard ptarmigan undergo a delay
316 of several weeks in gonadal development even after exposure to long days (Stokkan et al.,
317 1986).

318 In summary, our study showed that a high arctic bird relies on the same molecular
319 photoperiodic factors in the PT and MBH to initiate reproduction as other seasonal mammals
320 and birds. Similar responses were measured in birds going through a SNP and birds directly
321 transferred from DD to LL. The latter observation can reasonably be explained by a variant
322 form of coincidence timer mechanism similar to that seen in temperate species. Further
323 experiments using night break or Nanda Hamner protocols (Saunders, 2005) provide a route
324 to test this hypothesis.

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331

332 **Competing interests**

333 No competing interests declared.

334

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482 **Figure legends**

483

484 **Figure 1. Svalbard ptarmigan (*Lagopus muta hyperborea*) and where to find them.** The
485 picture shows a male in white winter plumage and a female in brown summer plumage
486 (Picture taken by Ida-Helene Sivertsen). The Svalbard ptarmigan is a sub-species of the rock
487 ptarmigan (*Lagopus muta*) and inhabits the high arctic archipelago of Svalbard (74° to 81°
488 north latitude).

489

490 **Figure 2**. **Experimental design**. Constant darkness adapted birds were either transferred into
491 constant light (LL group), simulated natural photoperiod (SNP group) or retained under
492 constant darkness (DD group). Red arrows indicate sampling points.

493

494 **Figure 3. Representative actograms and their respective chi-squared periodograms**. Birds
495 adapted to constant darkness were transferred to their respective light treatments on day 10
496 of the recording (red line) or retained in DD. Actograms are double plotted and grey shadings
497 indicate periods of darkness.
498 Chi squared periodograms were produced for 20 days for the DD group or 10 days before and
499 within experimental photoperiod for the LL and SNP group (upper periodogram: 10d before
500 light treatment (DD), lower periodogram: 10d in light treatment). Q_P values above the red line
501 in the periodogram indicate significant periods ($p<0.05$).

502

503 **Figure 4**. **Gonadal development and gene expression in the MBH measured by *in situ***
504 **hybridisation**. Gonad mass was measured *post-mortem*. Hypothalamic genes were measured
505 before (point 0) and 10 weeks after the transfer into the respective light regime. Additionally
506 gene expression was measured after 38 hour in LL and 5 weeks after the transfer into the
507 simulated natural photoperiod (L:D 12:12).The gene expression is given in optical density (OD)
508 and each replicate is plotted with dotted lines going through the respective mean.

509

510 **Figure 5**. **Representative *in situ* hybridisation radiographs for each gene and each sampling**
511 **point**. Top pictures shows whole brain radiograph for *Dio2* highlighting the region of interest
512 (MBH and PT). Radiographs for the respective sampling points show the PT/ MBH region.

513

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514 **Figure 6**. **Proposed mechanisms of photoperiodic time measurement in the Arctic**. Svalbard
515 ptarmigan show hypothalamic gene expression characteristic for seasonal reproduction when
516 transferred from DD into LL. This process has been proposed to consist of a circadian rhythm
517 of photo-inducibility and coinciding light. Despite absent rhythm in activity a light sensitivity
518 rhythm might be sustained in the PT and MBH throughout constant conditions (A). The rhythm
519 of photo-inducibility might also be initiated by one dawn either by inducing the rhythm or by
520 synchronising individual cells (B). Lastly, the photoperiodic response might be circadian.

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Figure 1.

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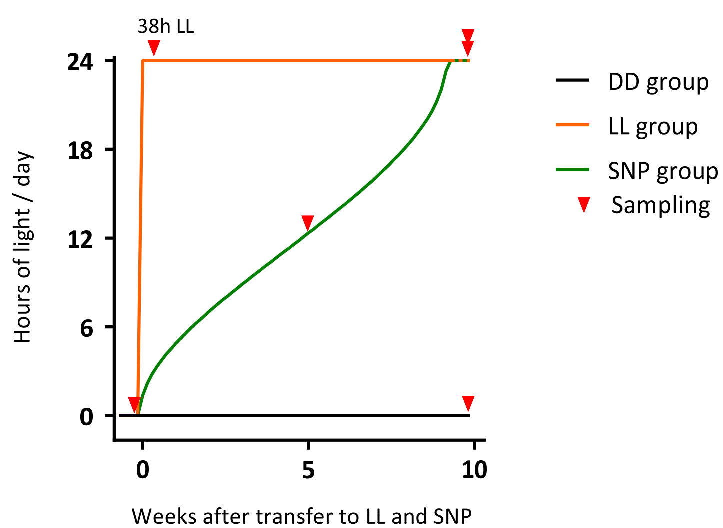

Figure 2.

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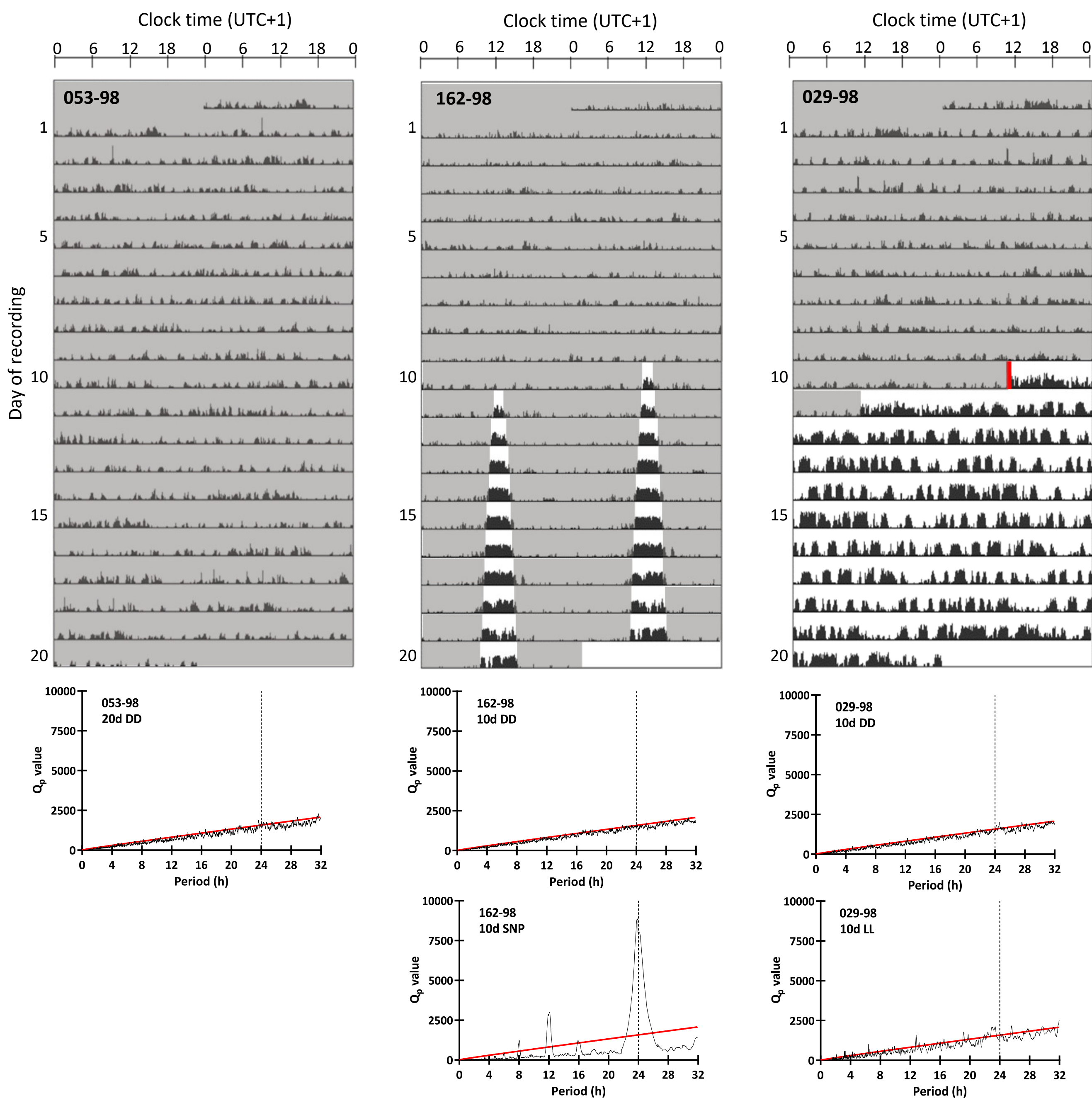

Figure 3.

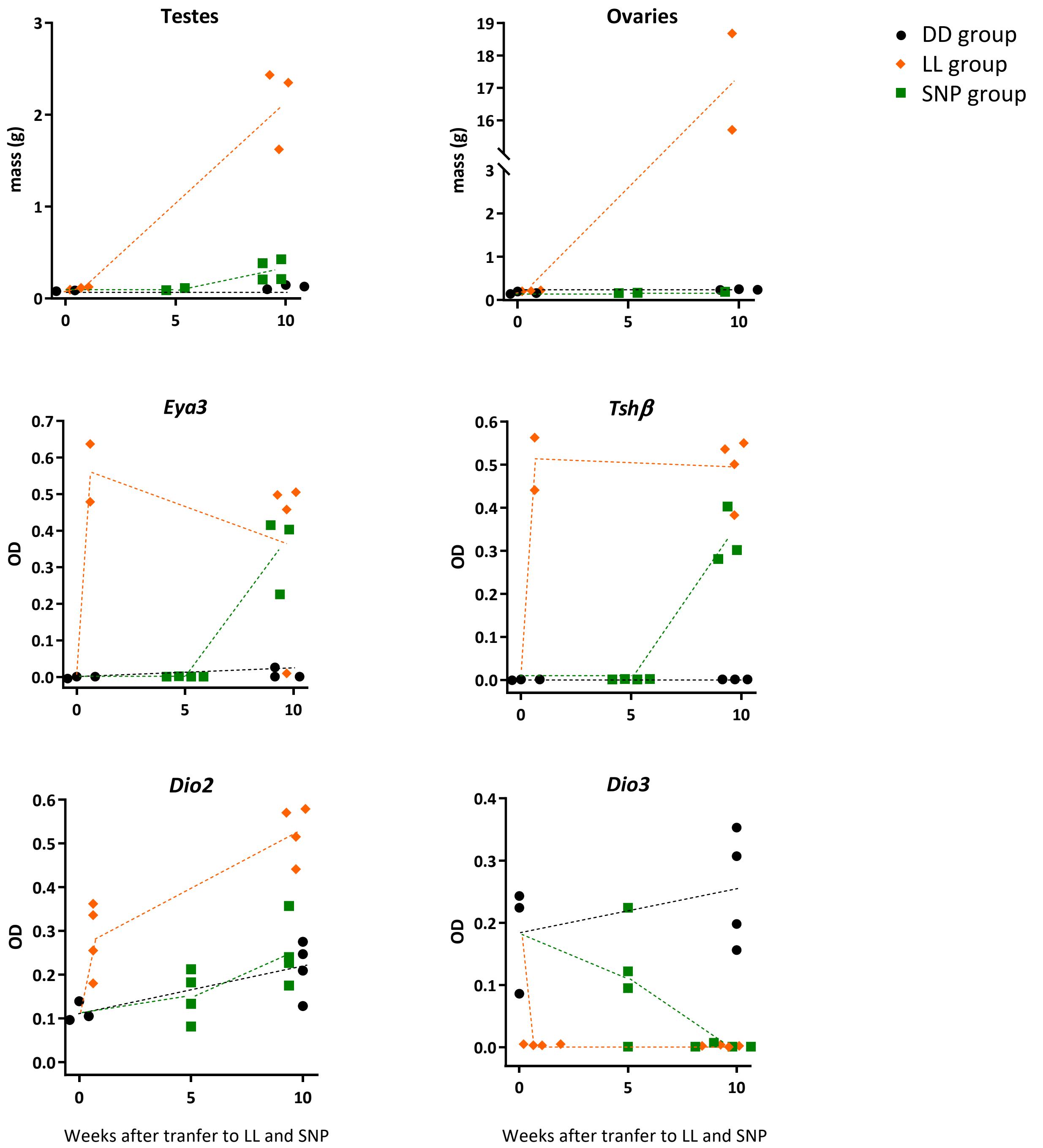

Figure 4.

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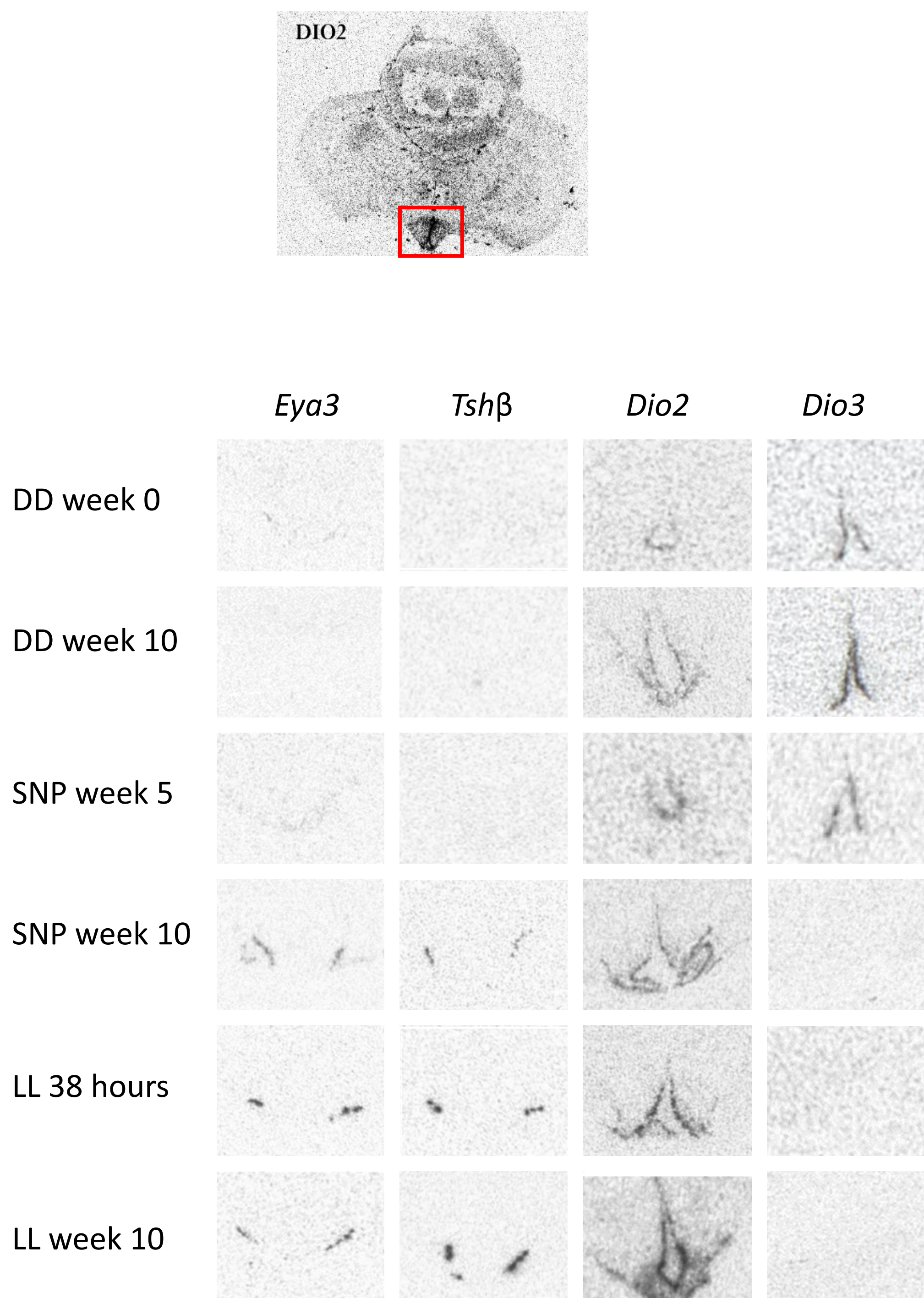

Figure 5.

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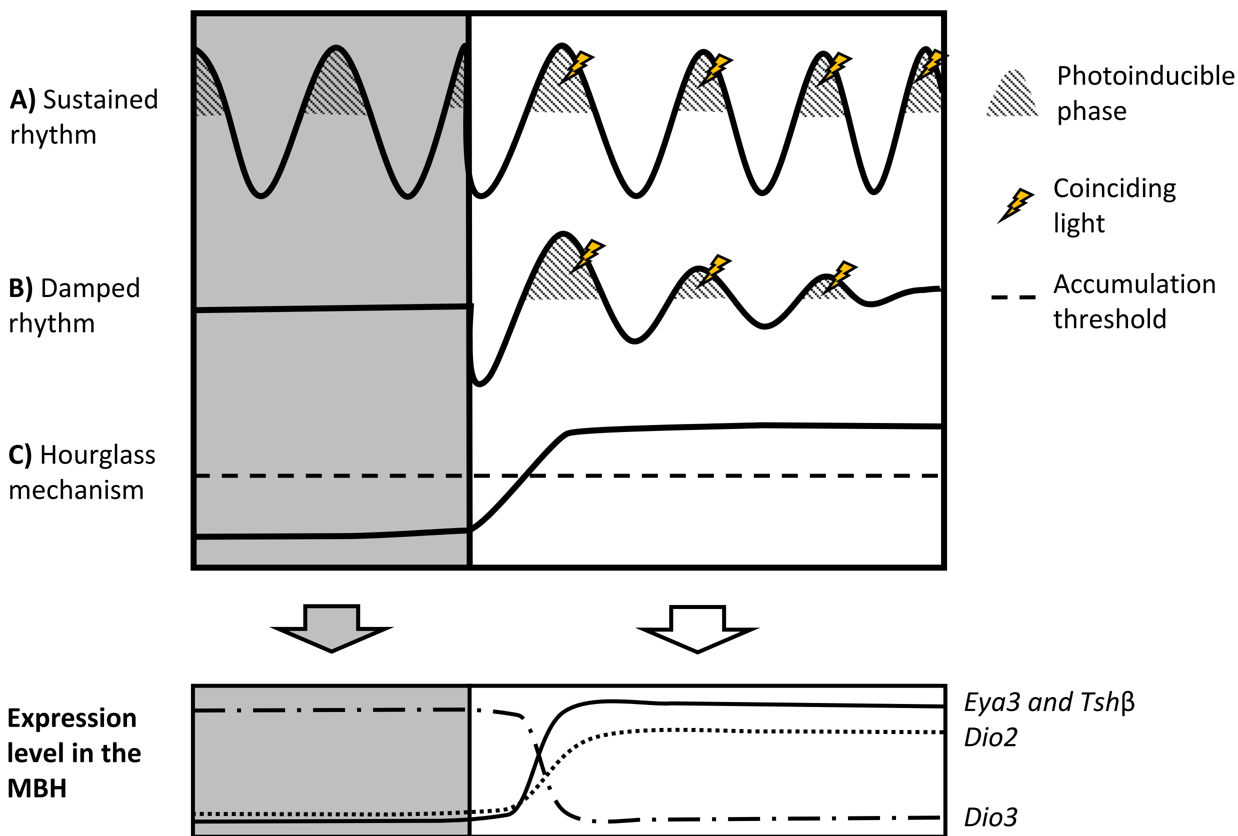

Figure 6.