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RESEARCH ARTICLE

Photoperiod is involved in the regulation of seasonal breeding in male water voles (*Arvicola terrestris*)

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ABSTRACT

Mammals living at temperate latitudes typically display annual cyclicity in their reproductive activity: births are synchronized when environmental conditions are most favorable. In a majority of these species, day length is the main proximate factor used to anticipate seasonal changes and to adapt physiology. The brain integrates this photoperiodic signal through key hypothalamic structures, which regulate the reproductive axis. In this context, our study aimed to characterize regulations that occur along the hypothalamo–pituitary–gonadal (HPG) axis in male fossorial water voles (*Arvicola terrestris*, also known as *Arvicola amphibius*) throughout the year and to further probe the implication of photoperiod in these seasonal regulations. Our monthly field monitoring showed dramatic seasonal changes in the morphology and activity of reproductive organs, as well as in the androgen-dependent lateral scent glands. Moreover, our data uncovered seasonal variations at the hypothalamic level. During the breeding season, kisspeptin expression in the arcuate nucleus (ARC) decreases, while RFRP3 expression in the dorsomedial hypothalamic nucleus (DMH) increases. Our follow-up laboratory study revealed activation of the reproductive axis and confirmed a decrease in kisspeptin expression in males exposed to a long photoperiod (summer condition) compared with those maintained under a short photoperiod (winter condition) that retain all features reminiscent of sexual inhibition. Altogether, our study characterizes neuroendocrine and anatomical markers of seasonal reproductive rhythmicity in male water voles and further suggests that these seasonal changes are strongly impacted by photoperiod.

KEY WORDS: Photoperiodism, Reproduction, Arcuate nucleus, Hypothalamo–pituitary–gonadal axis, Kisspeptin, Rfrp3

INTRODUCTION

Animals living at temperate latitudes are exposed to seasonal variations in their environment, including marked changes in photoperiod, temperature and food availability. Reproduction bears a huge physiological cost, especially with increased energy demand for lactation (Speakman, 2008). To ensure the survival of mothers and juveniles, many species restrict sexual activity to a limited time window of the year, which leads to synchronization of births when environmental conditions are most favorable. Thereby, these species must anticipate seasonal changes to adapt their annual cycle of sexual activity and sexual rest. Most seasonal species use the annual variation of day length (photoperiod) as a predictive environmental factor for synchronizing their reproductive activity (Bronson, 1988; Vasantha, 2015).

In mammals, the photic information is transmitted through a dedicated set of intrinsically photosensitive retinal ganglion cells to the suprachiasmatic nuclei and ultimately to the pineal gland (Berson, 2003). There, photoperiodic information is translated into a hormonal signal via the nocturnal secretion of melatonin. The melatonin production is proportionate to night duration. Thus, its secretion duration increases in winter (short photoperiod) and decreases in summer (long photoperiod). Melatonin then controls the activity of the reproductive axis through a cascade of regulations, which occurs at the level of the medio-basal hypothalamus (Dardente et al., 2019). A long day melatonin signal activates expression of thyroid-stimulating hormone (TSH) in the pars tuberalis of the pituitary, which then oppositely regulates the expression of deiodinases (Dio2 and Dio3) in nearby tanycytes lining the third ventricular wall. In long-day breeders (e.g. hamster species) exposed to long days, Dio2 transcription is activated and the inactive form of thyroid hormone, T4, is converted into its active form, T3. In turn, changes in T3 levels indirectly modulate the expression of two hypothalamic neuropeptides, kisspeptin and RFRP3 (RF-amide-related peptide 3) (Dardente et al., 2019; Klosen et al., 2013; Quignon et al., 2020; Simonneaux et al., 2013). Kisspeptin is the most potent secretagogue of GnRH (de Roux et al., 2003; Seminara et al., 2003). In the arcuate nucleus (ARC), *Kiss1* gene expression is regulated by photoperiod and sex-steroid feedback (Ansel et al., 2010; Revel et al., 2006; Smith et al., 2005). RFRP3 expression in the dorsomedial hypothalamic nucleus (DMH) is also regulated by photoperiod, with higher expression under long days in all species studied so far (Angelopoulou et al., 2019). However, the impact of RFRP3 on reproduction appears to depend on multiple factors and further differs according to species and sex. For instance, central injection of RFRP3 in the male Siberian hamster stimulates luteinizing hormone (LH) release in animals kept under a short photoperiod while it inhibits LH in animals kept under a long photoperiod (Ubuka et al., 2012).

The water vole *Arvicola amphibius* is a small semi-aquatic rodent widespread in Eurasia. A fossorial form of water vole, *Arvicola terrestris*, is more restricted to southwestern Europe (Mahmoudi et al., 2020; Shenbrot and Krasnov, 2005). The fossorial water vole mainly colonizes mid-mountain permanent meadows and orchards, where it digs extensive networks of galleries, which are used for building nests, foraging and storing food (Airoldi, 1976).

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As for the semi-aquatic form, births of fossorial water voles are mainly observed from March to October (Airoldi, 1978; Stoddart, 1971; Ventura and Gosálbez, 1990; Villette et al., 2020). In a previous field study focusing on females, we have shown marked seasonal regulations along the hypothalamo–pituitary–gonadal (HPG) axis, which correlate with reproductive status (Poissenot et al., 2021). In males, seasonal changes have been found in the morphology of testes and testosterone-dependent organs such as the seminal vesicles and lateral scent glands (Stoddart, 1972; Ventura and Gosálbez, 1990). These glands, located on the flank, have a sebaceous activity and may be involved in sex-partner attraction and territoriality (Nagnan-Le Meillour et al., 2019; Saucy, 1988; Stoddart, 1972; Stoddart et al., 1975). Thus, sexual activity of male water voles seems to follow an annual rhythm. However, during particularly mild winters, breeding was observed in fossorial water vole populations located in Switzerland (Meylan and Airoldi, 1975). A continuous breeding was even reported in other fossorial vole populations of the northwest of Spain, where temperatures are mild and food is available in sufficient amounts throughout the year (Somoano et al., 2017). In this context, seasonal regulations of the reproductive axis need further investigation, with special emphasis placed on the importance of photoperiod in these regulations.

This study first aimed to characterize the seasonal regulations of breeding at different levels along the HPG axis in male fossorial water voles in the wild. This was performed through monthly monitoring of wild males trapped in meadows (Auvergne, France) from February 2019 to May 2020. Second, this study directly assessed photoperiodic sensitivity of the HPG axis in male water voles kept under controlled indoor conditions. Males trapped in the field in late autumn, when days are short, were either continually exposed to a short photoperiod or transferred to a long photoperiod, in order to simulate winter or summer light conditions, respectively.

MATERIALS AND METHODS

Ethical statement

This study was carried out in accordance with European directive 2010/60/UE and was approved by an ethical committee for animal experimentation (C2EA-02, project 21994-201907510411944). Animal trapping was authorized in the French department of Puy-de-Dôme by prefectural order 19-00100.

Annual physiological field monitoring

From February 2019 to May 2020, except in December 2019 (due to abundant snow falls), 13 capture sessions (Table 1) were carried out on permanent meadows around the town of Angle-bas (45°42′47″ N, 2°46′12″E, alt. 887 m, department of Puy-de-Dôme, France). A total of 140 wild male fossorial water voles [*Arvicola terrestris*/*Arvicola amphibius* (Linnaeus 1758)] were caught with traps (Topcat®, Andermatt, France) placed into their galleries. Captures were performed during the day and the traps were regularly inspected (~every 15 min). For each animal, body mass and size were recorded, and organs were collected directly in the field. Testes and seminal vesicles were fixed in Bouin's solution for 48 h before being weighed and stored in 70% ethanol. The lateral scent glands were frozen on dry ice and stored at −80°C. Cauda epididymis were collected in NaCl buffer before spermatozoa count. Only males with body mass ≥75 g were included to ensure sexual maturity according to prior guidelines (Airoldi, 1978; Stoddart, 1971).

Exposition to long and short photoperiods

Sixteen sexually mature male fossorial water voles were caught with Sherman traps between 3 and 7 December 2020, on permanent meadows in Angle-bas and Nébouzat (45°42′47″N, 2°46′12″E, alt. 887 m and 45°43′39″N, 2°53′23″E, alt. 813 m; department of Puy-de-Dôme, France). Animals were then brought to the animal facility and housed individually in two ventilated cabinets (BIO-C36, Techniplast, France) at a controlled temperature of 20°C with food and water *ad libitum*. Enough litter was provided to allow animals to build burrows and to hide. The body mass and size of animals were regularly assessed during the experiment (Table 2). Animals were maintained under short photoperiod (SP, 8 h:16 h light:dark, lights on at 06:00 h) for 5 weeks. Animals were then separated into two balanced groups according to their mass and exposed for 7 weeks either to a long day photoperiod (LP, 16 h:8 h light:dark, lights on at 06.00 h, n=8) or maintained under SP (n=8; Fig. 1). Photoperiod cannot be set independently in each cabinet compartment, so one cabinet was used for SP and the other for LP. The cabinets were placed side by side in the same room to minimize potential variations in the environment (noise, vibration, temperature, etc.). A 7-week exposure to the photoperiodic treatment was chosen based on data defining the duration of a full spermatogenetic wave in other voles and hamsters (Grocock and Clarke, 1976; Van Haaster and De Rooij, 1993).

Table 1. Data collection during captures of adult male water voles

Date of capture		Number of voles	IHC and pituitary	Staining of testes and LSG	
2019	26 Feb	16	6		
	3 Apr	22	6		
	30 Apr	10	6		
	5 Jun	6	4	3	2
	11 Jul	16	6	3	4
	21 Aug	18	6		
	18 Sep	11	6		
	19 Oct	10	7		
	26 Nov	10	6		
2020	30 Jan	6		5	
	24 Feb	7			6
	1 Apr	4			
	26 May	4			

Indication of the number of samples used for staining and immunohistochemistry (IHC). LSG, lateral scent glands.

Tissue collection for immunohistochemistry

Fifty-three males caught during the annual field monitoring (Table 1) and five males per group exposed to photoperiodic treatments were used for analysis by immunohistochemistry. These animals were anesthetized with isoflurane, then euthanized by an overdose of pentobarbital and finally intracardially perfused with sodium nitrite followed by 4% paraformaldehyde (PFA) in phosphate buffer. Brains, testes, seminal vesicles, epididymides

Table 2. Body mass and size of male water voles at different times of the photoperiodic treatment experiment

Date		Body mass (g)		Body size (cm)	
		SP	LP	SP	LP
Start of light treatment	8 Jan	83.5±4.2	80.5±4.0	14.6±0.3	14.5±0.3
Mid-term	29 Jan	84.6±5.1	83.1±4.1	14.8±0.3	14.6±0.3
Euthanasia	25 & 26 Feb	74.8±3.8	80.6±3.8	14.9±0.2	14.8±0.3

Data are means±s.e.m.

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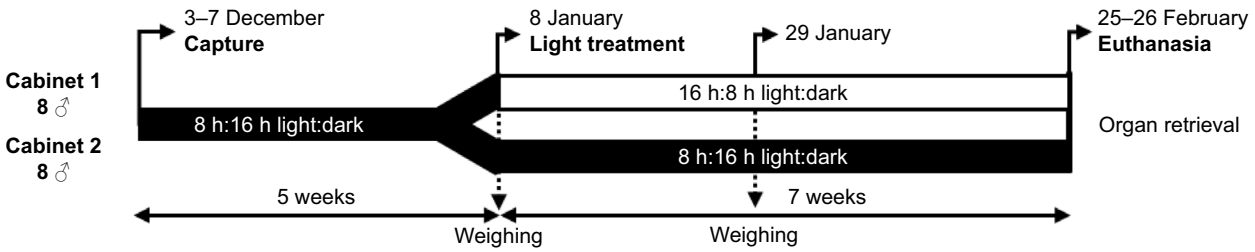

Fig. 1. Diagram of experimental design of photoperiodic treatment of male water voles. Trapped animals were kept 5 weeks in short photoperiod (8 h:16 h light:dark). Animals were then split into two groups: one was exposed to a long photoperiod (16 h:8 h light:dark) while the other remained exposed to the short photoperiod. After 7 weeks of photoperiodic treatment, animals were euthanized to collect brain, pituitary, lateral scent glands, seminal vesicles and testes.

and lateral scent glands were postfixed in 4% PFA for 24–48 h, then weighed before being cryoprotected in 30% sucrose. Pituitary glands were collected in NaCl buffer before being weighed.

Immunohistochemistry

Before immunohistochemistry labeling, brains were frozen at −18°C, sliced in coronal free-floating sections at 30 µm and stored in cryoprotective solution. Brain sections were then washed three times for 5 min in Tris buffer saline (TBS). Sections were incubated for 30 min with 0.3% H_2O_2 in TBS to inhibit endogenous peroxidase activity and for 2 h in TBS containing 0.1% Triton X-100 with 2% normal donkey serum (NDS) to block non-specific binding. Sections were then incubated for 72 h at 4°C in TBST-NDS with a rabbit anti-kisspeptin antibody (1:50,000, AC564; Franceschini et al., 2006) or with a sheep anti-RFRP3 antibody (1:10,000, AC536; Harbid et al., 2013; Poissenot et al., 2019) (Table S1). After three 5-min washes, sections were incubated for 2 h at room temperature (RT) in TBST-NDS with a donkey anti-rabbit or donkey anti-sheep antibody (1:1000 for both, Jackson ImmunoResearch, UK), then sections were washed three times for 5 min and incubated for 1 h at RT with an avidin/biotin system for signal amplification (VECTASTAIN® Elite® ABC HRP Kit, PK-6100, Eurobio, France). Sections were then rinsed three times for 5 min in Tris HCL and revealed with DAB 0.15 mg ml^{-1} dissolved in Tris HCL containing 0.01% H_2O_2 for 15 min. Finally, sections were mounted in TBS, dehydrated and coverslipped in DPX. Specificity of the immunolabeling was checked on consecutive sections of the ARC or DMH by pre-adsorption for 24 h of the primary antibody with the kisspeptin-10 or RFRP3 peptides (Genecust, France) (Fig. S1).

Histological staining

Testes and epididymes were rinsed in phosphate buffer saline (PBS) for 24 h. They were then dehydrated in successive baths of 50%, 70%, 90% and absolute ethanol, followed by one night in butanol at 4°C. Samples were impregnated in paraffin twice at 60°C before final embedding. Once dried, samples were sliced into 5 µm sections and arranged on slides. Before staining, sections were deparaffinized in xylene twice. Sections were then rehydrated with successive baths of absolute, 90%, 70% ethanol and a final bath of tap water before being stained with either Hematoxylin & Eosin or Masson's Trichrome. Finally, sections were dehydrated and coverslipped in DPX.

Lateral scent glands were embedded in OCT (Tissue-Tek, Sakura Finetek, France), frozen in dry ice, and sliced on a cryostat into 10 µm sections. Sections were then dried and stained with Hematoxylin & Eosin with the same protocol as described above.

RNA extraction, RT-PCR and cloning

The partial cDNA for *Npvf* (precursor of RFRP1/RFRP3 peptides) was obtained using hypothalamic mRNA from a single male water vole. All procedures have been described in detail elsewhere (Lomet et al., 2018). RNA was extracted using TriReagent (Sigma, France). The concentration and purity of the sample were determined with a Nanodrop 2000 (ThermoScientific, France), and integrity was checked by standard agarose gel electrophoresis. cDNA was synthesized using the Omniscript RT kit (Qiagen, France) and Oligo-dT primers (Eurofins, Germany). cDNA was cloned using a standard homology procedure whereby multiple rodent *Npvf* sequences were aligned to guide PCR primer design (Table S1). PCR was performed using Platinum Taq High fidelity DNA polymerase (Thermofisher, France). PCR reactions were loaded on a 1.5% agarose gel and migrated at 80–110 V in Tris-EDTA-acetic acid (TEA) buffer for ∼30 min. PCR fragments were extracted on gels and cloned in pGEMT (Promega, France). Eight independent clones were sequenced (Eurofins, France).

Image acquisition and quantifications

Images of sections of brains, testes, epididymes and lateral scent glands were acquired with an AxioScan.Z1 slide scanner (Carl Zeiss, France).

Zen software (Carl Zeiss) was used for the surface measurement of 30 seminiferous tubules on three sections distributed over the entire surface of one testis and for the thickness measurement of the sebaceous gland layer on three sections distributed over the entire surface of one lateral scent gland.

Quantifications of kisspeptin-ir (immunoreactive) fiber density and cell bodies were performed on three sections at the anterior, median and caudal levels of the ARC (corresponding to plates 43, 48 and 51 of the Paxinos and Franklin mouse brain atlas). The counting surface for fiber density was 0.029 mm² on each side of the third ventricle. Images were analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA, ver.1.52p). Images were binarized, the background was subtracted and the area covered by the fibers was measured after thresholding. The kisspeptin-ir fiber density and number of cell bodies were expressed as the mean of the quantifications performed on the three sections of the ARC. Quantification of RFRP3-ir cell bodies was performed for all consecutive sections spaced 180 µm apart that contain the DMH (corresponding to plates 42 to 49 of the Paxinos and Franklin mouse brain atlas).

Statistical analysis

An organ-to-body mass ratio was used to study the relative weight of testes, seminal vesicles, epididymes and lateral scent glands. Lateral scent gland surface was divided by body size to provide a relative surface index. Normality of data distribution was verified with the

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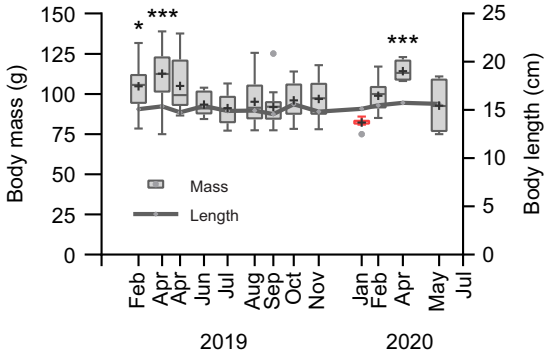

Fig. 2. Seasonal variations in body condition of male wild water voles. For body mass, only significant *post hoc* comparisons with January (in red) are indicated on graphs. Results are shown as Tukey's boxplot with outliers represented by circles and means indicated by a cross. Kruskal–Wallis, followed by Dunn's test, **P*<0.05, ****P*<0.001. No significant difference was observed in body length.

Shapiro–Wilk test. Monthly variations were analyzed by a Kruskal–Wallis test, followed by a Dunn's test. To simplify the interpretation of the data, only *post hoc* comparisons with the month of January are presented. All other pairwise comparisons are available in Tables S2 and S3. Two-group comparisons (season or photoperiodic treatment) were analyzed by an unpaired Student's *t*-test or a Mann–Whitney test. GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was used to perform statistical analyses and draw graphs. Results are shown as Tukey's boxplots with outliers (or each individual) represented by circles and means indicated by a cross.

RESULTS

Annual field monitoring shows seasonal variations along the HPG axis

The average body mass of male water voles caught in the field significantly fluctuated throughout the year (Fig. 2). The maximum average body mass was observed during spring, in April, while the minimum was observed during winter, in January (114 versus 83 g, $P<0.001$, Dunn's test). No difference in body size was detected throughout the year.

The relative mass of testes showed a significant seasonal variation that followed a sinusoidal-like profile (Fig. 3A,B). The relative mass of testes increased progressively from February 2019 to June 2019, then exhibited a sizeable 6-fold decrease until January 2020 (0.3678% of body mass versus 0.0625% of body mass, $P<0.001$, Dunn's test). From January 2020 to May 2020, the relative mass of testes increased again by 6-fold (0.0625% versus 0.3758% of body mass, $P=0.0074$, Dunn's test). A significant difference in the surface of the seminiferous tubules was detected between animals caught in June 2019 and January 2020 ($P<0.001$, Student's *t*-test; Fig. 3D,E). Histological observation of testes sections showed many elongated spermatozoa in the seminiferous tubules in June. In

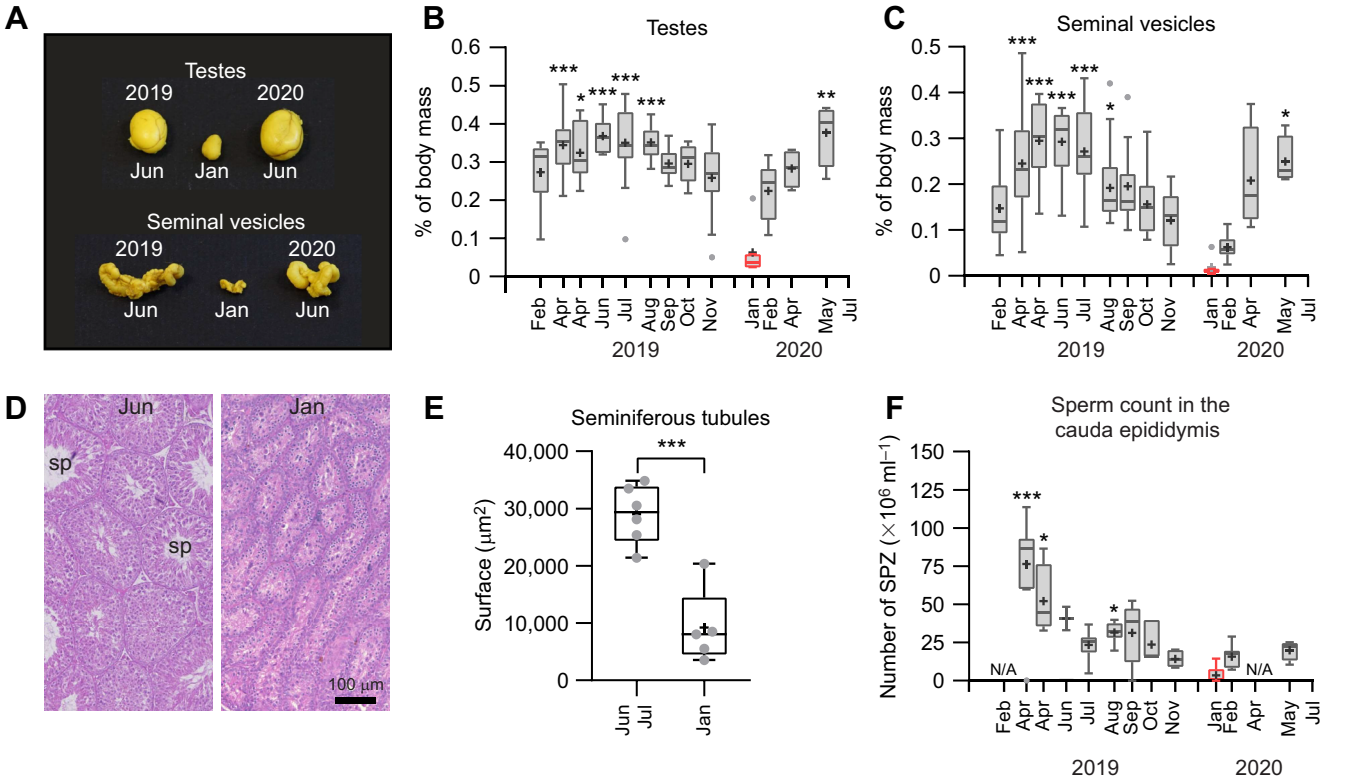

Fig. 3. Marked annual rhythmicity in the reproductive organs of male wild water voles. (A) Representative images of testes and seminal vesicles showing the annual morphological changes in adult male water voles. (B,C) Monthly variations in relative weights of testes and seminal vesicles. (D) Images of stained testes sections showing spermatozoa (Sp) in the lumen of seminiferous tubules in males caught in June. Seminiferous tubules in males trapped in January were regressed, no lumen and spermatozoa were visible. (E) The surface of seminiferous tubules was higher in males caught in summer (June/July, *n*=6) than in those caught in winter (January, *n*=5). (F) Monthly variations in the number of spermatozoa counted in the cauda of the epididymis. Results are shown as Tukey's boxplot with outliers (or each individual) represented by circles and means indicated by a cross. Monthly variations were analyzed by Kruskal–Wallis test, followed by Dunn's test and only significant *post hoc* comparisons with January (in red) are indicated on graphs. Two-group comparisons were analyzed by Student's *t*-test. (**P*<0.05, ***P*<0.01, ****P*<0.001).

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January, only round cells that may correspond to spermatocytes were present in the regressed tubules.

Similarly, the seminal vesicles showed a significant seasonal variation (Fig. 3C). The maximum relative mass of seminal vesicles was reached at the end of April 2019, which was then followed by a dramatic 16-fold decrease until January 2020 (0.2953% of body mass versus 0.0181% of body mass, $P<0.001$, Dunn's test). The relative mass of seminal vesicles significantly increased again from January to May 2020 (0.0181% of body mass versus 0.2501% of body mass, $P=0.021$, Dunn's test). The sperm count in the cauda epididymis significantly differed throughout the year (Fig. 3F). In the cauda epididymis, the maximum number of spermatozoa was found in males caught during spring in April 2019, while the minimum was observed in January 2020 (76.52×10^6 ml^{-1} versus 3.67×10^6 ml^{-1}, $P<0.001$, Dunn's test). Further, only 50% of these January-trapped males had spermatozoa in the cauda epididymis.

The lateral scent glands showed a significant monthly variation in their relative size and mass (Fig. 4A,B). Similar to what was found for testes, a sinusoidal-like profile was observed with a maximal development of lateral scent glands in June 2019 and a significant regression in January 2020 ($P=0.0018$ for size and $P<0.001$ for mass, Dunn's test). The sebaceous gland layer, where the lipidic secretion is produced by the vacuolated cells, was found to be significantly thicker in summer (June/July 2019) than in winter (February 2020) (981.9 µm versus 516.0 µm, Student's *t*-test, $P<0.001$; Fig. 4C,D).

The pituitary mass and the expression of kisspeptin and RFRP3 were monitored between February 2019 and November 2019. The pituitary mass showed a significant monthly variation (Fig. S2). The heaviest pituitaries were found in animals trapped at the end of April 2019, while the lightest were found in animals trapped in November 2019 (4.1 mg versus 2.2 mg, $P=0.0087$, Dunn's test). In the hypothalamic ARC, a significant variation in the density of kisspeptin-ir fibers was observed throughout the year (Fig. 5A,B). The maximum density of kisspeptin-ir fibers was detected in February and November 2019 and the minimum density was observed in May 2019 (1047 and 2098 a.u. versus 53 a.u., $P=0.0077$ and $P<0.001$, Dunn's test). Significant monthly variations were also detected in the number of RFRP3-ir cells in the DMH (Fig. 5C,D). RFRP3-ir cells were only detected in brain sections of animals trapped between June and September 2019, with a maximum number of cells in July.

A partial cDNA for *Npvf* (precursor of RFRP1/RFRP3 peptides) was cloned using hypothalamic mRNA from a single male water vole. The cDNA for *Npvf* has been deposited in GenBank under accession number MT922571. This partial cDNA encompasses sequences predicted to yield the mature peptides RFRP1 and RFRP3 (based on data in hamsters, see Kriegsfeld et al., 2006; Ubuka et al., 2012). The sequences for these two peptides show very high homology with those in other rodents (Fig. 5E).

Photoperiod drives the activation of the HPG axis

Depending on their average body mass, male water voles were equally split into long photoperiod (LP) or short photoperiod (SP) groups. During the photoperiodic treatment, the average body mass of LP-exposed males remained stable. In SP-exposed males, the average body mass decreased from the mid-term to the end of the photoperiodic treatment (84.6 g versus 74.8 g; Table 2). However, no significant difference was found between time points of treatment, or between groups. No difference was detected for body size.

Histological observation of testes and epididymis sections revealed a higher number of spermatozoa in the lumen of seminiferous tubules and in the cauda epididymis of LP-exposed compared with SP-exposed males (Fig. 6A,B). The relative mass of testes in male water voles exposed to LP was significantly higher than in males maintained in SP (0.4418% of body mass versus 0.2613% of body mass, $P=0.0379$, Mann–Whitney; Fig. 6C). Similar results were observed for the relative mass of the epididymis (LP: 0.1022% of body mass versus SP: 0.0748% of body mass, $P=0.0211$, Student's *t*-test; Fig. 6D) and seminal vesicles (LP: 0.1684% of body mass versus SP: 0.0439% of body mass, $P<0.001$, Mann–Whitney; Fig. 6E). LP-exposed animals displayed significantly more developed lateral scent glands than SP-exposed animals (size, LP: 2.285 versus SP: 1.043, $P=0.0343$, Mann–Whitney; mass, LP: 0.0762% of body mass versus SP: 0.02887% of body mass, $P=0.0281$, Mann–Whitney; Fig. 6F,G).

No statistical difference was detected for the pituitary mass between LP- and SP-exposed animals (Fig. 6K). In the hypothalamic ARC, LP-exposed males displayed a significantly lowered number of kisspeptin-ir cells compared with SP-exposed males (20.6 versus 31.2, $P=0.0367$, Student's *t*-test; Fig. 6H,J). A lower density of kisspeptin-ir fibers was also observed in the LP-

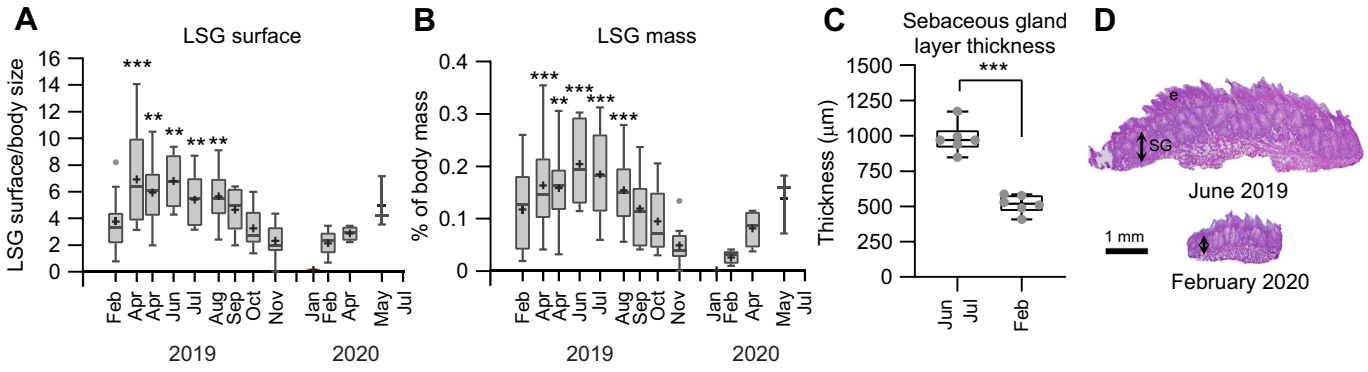

Fig. 4. Marked annual rhythmicity in the lateral scent glands of male wild water voles. (A,B) The lateral scent glands of male water voles showed monthly variations in their surface and mass. (C) The sebaceous gland layer of lateral scent glands was thicker in animals trapped in summer (June and July, $n=6$) compared with the ones trapped in winter (February, $n=6$). (D) Representative images of stained sections of lateral scent glands. The double-headed arrow indicates the thickness of the sebaceous gland layer. LSG, lateral scent gland; SG, sebaceous gland; e, epidermis. Results are shown as Tukey's boxplot with outliers (or each individual) represented by circles and means indicated by a cross. Monthly variations were analyzed by Kruskal–Wallis test, followed by Dunn's test and only significant *post hoc* comparisons with January (in red) are indicated on graphs. Two-group comparisons was analyzed by Student's *t*-test (**$P<0.01$, ***$P<0.001$).

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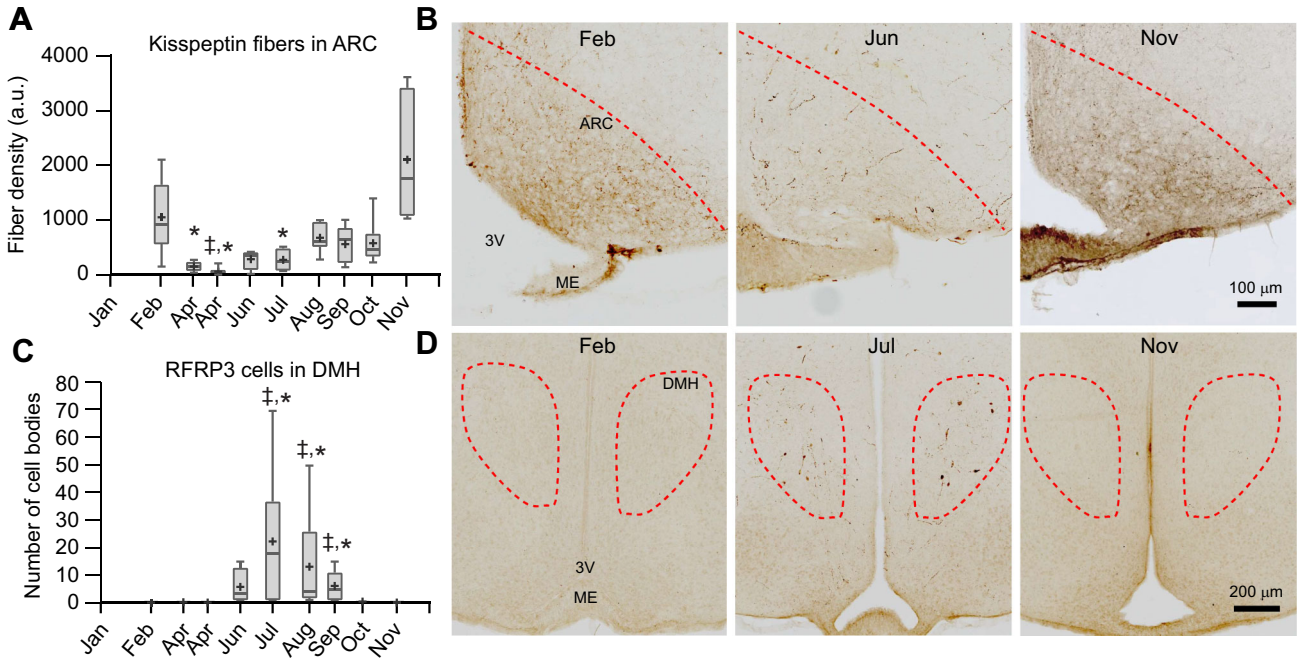

E

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Mus_mus         MEIISLKRFILLTVATSSFLTSNTFCTDEFMMPHFHSKEGDGKYSQLRGIPKGEKERSVSFQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTIDE
Arvi_nilo       MEIISLKRFILLTLATSSFLTSNIFCTDELVMPRFHSKEGYGKYSQLRGIPKGEKERSISPQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTIDE
Gram_surd       MEIISSKCFILLTLAVSSFLTSNIFCTDELVIPHFHSKEGYGKYSQLRGIPKGEKERSVSSQEQKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTIEE
Ratt_norv       MEIISSKRFILLTLATSSFLTSNTLCSDELMMPHFHSKEGYGKYYQLRGIPKGVKERSVTFQELKDWGAKKDIKMSPAPANKVPHSAANLPLRFGRNIED
Micr_ochr       MEIISSKRFILLTLATLSLLTPNIFCTDELMMPHFHSQEKGDKYLQPRGIPKGEKERSVSFQELKDLGAKNVIKMSPAPANKVPHSAANLPLRFGRTAEE
Lasi_bran       MEIISSKRFILLTLATLSLLTPNIFCTEELMMPHFHSQEKDDKYLQPRGIPKGEKERSVSFQALKDLGAKNVIKMSPAPANKVPHSAANLPLRFGRTAEE
Arvi_terr       ------------------------------MPHFHSQEKDDKYLQPRGIPKGEKERSVSFQELKDLGAKNVIKMGPAPANKVPHSAANLPLRFGRTVEE
Pero_mani       MEIISSKQFILLILATSSFLTSNIFCSDELMMPHFHSKENDDKYSQPRGIPEGEKERSVSFQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTTEE
Meso_aura       MEIISSKRFILLTLATSSLLTSNIFCTDELMMPHCHSKEKDDKYSQPRGITKGEKERSVSFQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTLEE
Cric_gris       MEIISSKRFILLTLATSSLLTSNIFCTDELMVPHCHSKEKDGKYSQPRGIPKGEKERSVTFQELKDWGAKNVIKMSPAPANKVPHSAANLPLRFGRTLEE
Meri_ungu       MEIISSKRFILLALVTSSLLTSNIFCTDELMMPHFHSKESEGKYSQPREIPKEEKERSVSFQELKDWGAKNVIKMGPAPANKVPHSAANLPLRFGRTAEE
                                                :*: **:*  .** * * *.:  ****:: *  ** ***: ***.********************. ::

Mus_mus         KRSPAARVNMEAGTRSHFPSLPQRFGRTTAR-SPKTPADLPQKPLHSLGSSELLYVMICQHQEIQSPGGKRTR----------------------------
Arvi_nilo       KRRPTSRAYMEAGTMSHFPSLPQRFGRTTAR-SPKTPADLPQKPLHSLGSSELLYVMICQHQEIQSPGGKRTR-------DDAERKPEK-----------
Gram_surd       KRSPTARANMEAGTMSHFPSLPQRFGRTTAGSILKTQAGLPQKSLHSLASSELLYTMTCQHQEIHSPGQNQPRRRAFMETDDAERKPEN-----------
Ratt_norv       RRSPRARANMEAGTMSHFPSLPQRFGRTTARRITKTLAGLPQKSLHSLASSELLYAMTRQHQEIQSPGQEQPR----------------------------
Micr_ochr       GRSPRAWANVEAGILNHVPSLPQRFGRTTARSIPKTLSRWLQRSLHPLAASELLHAMTCQHQEIQSPGGKQSRRQAFTETEDAEGKHEK-----------
Lasi_bran       GRSPRAWAKVEAGVLNHVPSLPQRFGRTTARSIPKTLRRWLQRSLHPLAASELLHAMTCQHQEIQSPGGKQPRRQAFTETEDAEGKHEKQETWNPSYKML
Arvi_terr       GRSPRAWANMEAGTLNHVPSLPQRFGRTTARSIPKTLSRWLQRSLHPLAASELLHAMTCQHQEIQSPGG----------------------------
Pero_mani       -RSPRAWANMEAGTLSHVPSLPQRFGRTTARSIPKTLSRLLQRSLHSLGASELLYAMTCQHQEIQSHGGKQPWRQALTEADDAEGKQEE-----------
Meso_aura       ERSPRAWSNMEAMILSRVPSLPQRFGRTTARSIPKTPSHLLQRSLHSLATSELLYAMTCQHQEIQSPGGKQPRRQAFVESDDEEGKHEK-----------
Cric_gris       ERSPRARANMEAMTLSRVPSLPQRFGRTTARSIPKTLSHLLQRSLHSLATSELLYAMTCQHQEIQSPDGKQARRQAFMETDDEEGKHEK-----------
Meri_ungu       EKSPRAQANMVAGTVSRVPSLPQRFGR-TARCAPKTMSGSLQRSLYSLAASELLYAMTCQHEEIQGPGQKQPRRQTFSETDDAEGKQEQ-----------
                 : * :   : *   .:.********* *    **     *:.*:.*.:*:**:.*  **:**:. .
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Fig. 5. Male wild water voles display seasonal variations in the expression of kisspeptin and RFRP3. (A,B) Graph and representative images of monthly variations of mean immunoreactive kisspeptin fiber density in the hypothalamic arcuate nucleus. (C,D) Graph and representative images of RFRP3-immunoreactive cell bodies in the dorsomedial hypothalamic nucleus. Results are shown as Tukey's boxplot and means indicated by a cross (Kruskal–Wallis test, followed by Dunn's test; *, significantly different to November; ‡, significantly different to February). ARC, arcuate nucleus; DMH, dorsomedial hypothalamic nucleus; 3V, third ventricle; ME, median eminence. (E) Alignment of RFRP1 and RFRP3 sequences in *Arvicola terrestris* and other selected rodents (*Mus musculus*, *Arvicanthis niloticus*, *Grammomys surdaster*, *Rattus norvegicus*, *Microtus ochrogaster*, *Lasiopodomys brandtii*, *Peromyscus leucopus*, *Mesocricetus auratus*, *Cricetus griseus* and *Meriones unguiculatus*). Yellow: positions unique to *Arvicola terrestris*; bold green: RFRP1 and RFRP3; bold red: dibasic cleavage site+amidation. Asterisks indicate positions that have a single fully conserved residue. Colons indicate conservation between amino acid groups of strongly similar properties. Periods indicate conservation between amino acid groups of weakly similar properties.

exposed males compared with SP-exposed males, but no statistically significant difference was found (10,689 versus 5760 a.u., P=0.0597, Student's t-test; Fig. 6H,I).

DISCUSSION

In the field part of this study, we performed monthly physiological monitoring from February 2019 to May 2020, with the aim to characterize seasonal regulations along the HPG axis in male fossorial water voles.

A seasonal rhythm in body mass was observed: the heaviest males were caught in spring and the lightest males were caught in winter. Similar seasonal changes in body mass have previously been described in other rodent species exhibiting seasonal breeding (Al-Khateeb and Johnson, 1971; Canguilhem and Marx, 1973; Jones et al., 2020; Rowlands, 1936). The lifespan of water voles in the wild was estimated to be between 5 months and 2.5 years, with a marked decrease in the number of older animals in autumn (Stoddart, 1971; Ventura et al., 1991). This seasonal change in body mass might therefore partly reflect the renewal of the population.

A progressive increase in the relative mass of testes was observed from late winter to early summer, followed by a dramatic decrease until winter, consistent with previous observations in water voles

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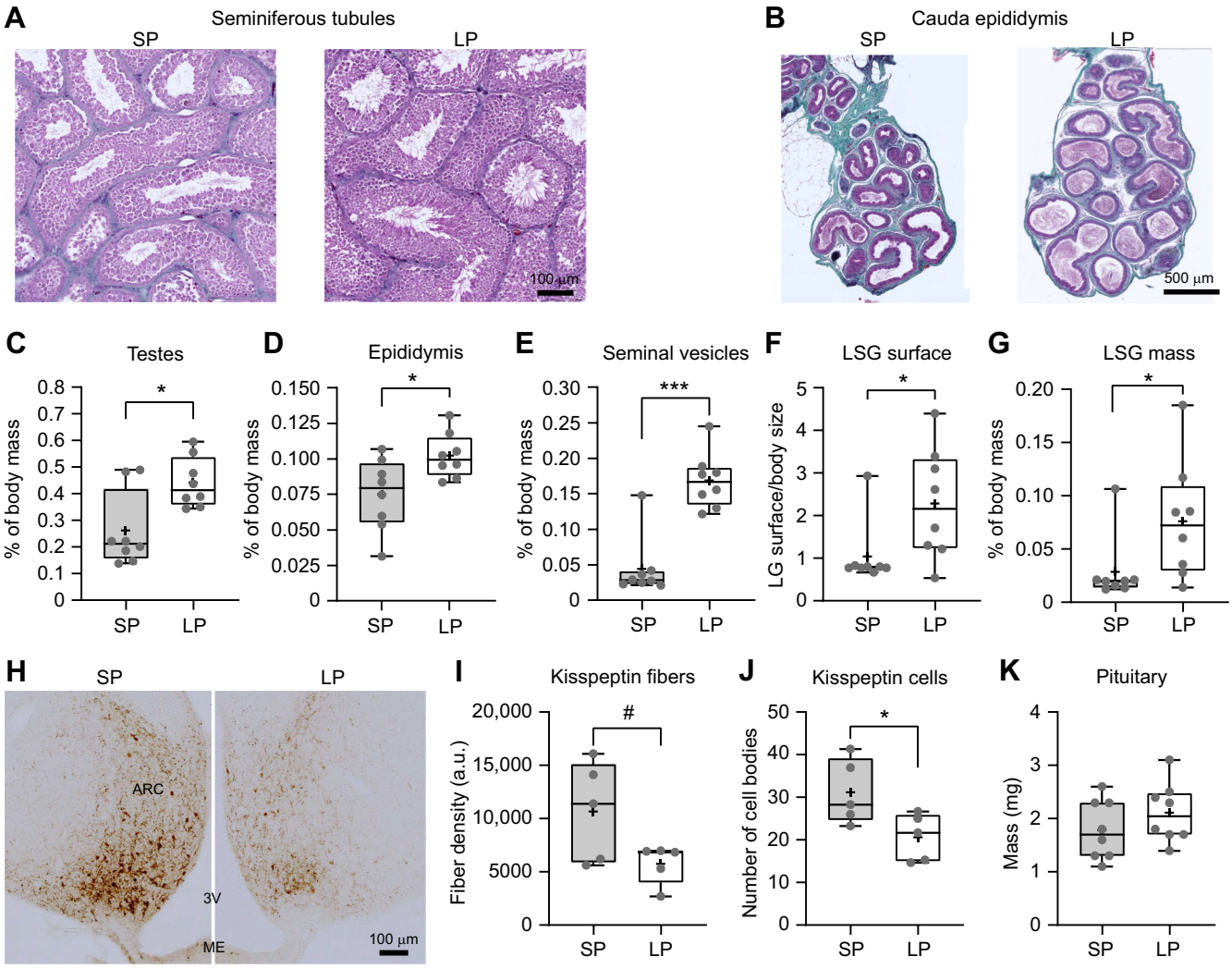

Fig. 6. Effect of 7 weeks of exposure to long photoperiod on the HPG axis in male water voles. (A,B) Images of stained sections of testes and cauda epididymis showed higher number of spermatozoa in male voles exposed to long photoperiod (LP) than to short photoperiod (SP). (C–E) The relative mass of reproductive organs was higher in male voles exposed to long photoperiod. (F,G) Relative surface and mass of lateral scent glands were higher in male voles exposed to long-day photoperiod. (H–J) In the hypothalamic arcuate nucleus, the mean density of immunoreactive kisspeptin fibers and the mean number of cell bodies were lower in males housed in long photoperiod. (K) No difference was observed in pituitary mass. LSG, lateral scent gland; ARC, arcuate nucleus; 3V, third ventricle; ME, median eminence. Results are shown as a boxplot with each individual represented by circles and means indicated by a cross. According to normality, data were analyzed by Mann–Whitney (C,E,F,G) or by Student's *t*-test (D,I,J,K) (**P*<0.05, ****P*<0.001, #*P*=0.0597).

(Stoddart, 1972; Ventura and Gosálbez, 1990) as well as in other vole species and muskrats (Martinet, 1967; Rowlands, 1936; Valentine and Kirkpatrick, 1970; Xie et al., 2019). Histological staining of testes sections and sperm count in the cauda epididymis revealed a seasonal modulation of spermatogenesis. In winter, the seminiferous tubules were completely regressed and contained only spermatogonia. In addition, in the cauda epididymis, few spermatozoa were present, or even completely absent. This result suggests a complete cessation of spermatogenesis in winter as described in the bank vole, hedgehog and muskrat (Massoud et al., 2019; Rowlands, 1936; Xie et al., 2019). A seasonal variation in spermatozoa production was also reported in field voles (Martinet, 1967).

Seminal vesicles and lateral scent glands are known to be androgen dependent (Chai, 1956; Frost et al., 1973; Steadman and Krichesky, 1945; Stoddart, 1972; Thiessen et al., 1968; Vandenbergh, 1973). Therefore, seasonal changes observed in their size and mass likely reflect heightened testosterone production in spring and interruption of testicular steroidogenic activity in winter. Such variations have been observed in many species, including the common pine vole (Al-Khateeb and Johnson, 1971; Martinet, 1967; Stoddart, 1972; Valentine and Kirkpatrick, 1970). The lateral scent glands are described as being involved in territoriality and attraction of sex partners (Stoddart et al., 1975; Xie et al., 2019). They produce lipidic secretions that contain volatile olfactory compounds, for which season-specific signatures were recently identified (Nagnan-Le Meillour et al., 2019).

Testicular activity is under the control of LH and FSH, produced by gonadotropic cells in the anterior pituitary. In male water voles, the pituitary mass varied throughout the year, with an increase during spring, followed by a decrease in summer and autumn. In male field voles, an increase of the pituitary mass has been associated with an increase in pituitary and plasma LH and FSH levels and was accompanied by an increase of the relative mass of testes and seminal vesicles (Al-Khateeb and Johnson, 1971; Craven and Clarke, 1986).

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The release of LH and FSH is regulated by GnRH, which is itself regulated by two distinct neuronal hypothalamic populations expressing the neuropeptides kisspeptin and RFRP3 (Hu et al., 2019; Oakley et al., 2009). In this study, we cloned a partial cDNA of *Npvf* (the precursor of RFRP1/3) in the water vole. The deduced sequence for RFRP3 shows very high homology with the sequence in other rodents, but also with that of the ovine RFRP3 against which our antibody is directed (Harbid et al., 2013). In wild male water voles, RFRP3-ir cells in the DMH were only detected in summer, with a maximum number of cells in July. In contrast, the density of kisspeptin-ir fibers in the ARC was much lower in spring and summer than in autumn and winter. A recent field study in male Brandt's voles has shown seasonal variations in the expression levels of the mRNA encoding these peptides, with an increase in the expression of *Npvf* (RFRP3-encoding gene) and *Kiss1* during the breeding period (Wang et al., 2019). These variations were associated with changes in testes and epididymis relative mass and with a variation of the Dio2/Dio3 ratio synchronized with changes in day length (Wang et al., 2019). The expression of RFRP3 is indeed regulated by photoperiod through melatonin (Mason et al., 2010; Revel et al., 2008; Ubuka et al., 2012). In all seasonal mammals studied so far, a higher number of RFRP3-ir cells in the DMH is consistently found in animals exposed to LP compared with animals exposed to SP (Angelopoulou et al., 2019). Our current data in male water voles are in line with these findings. As shown in Siberian hamsters exposed to LP, a central injection of RFRP3 can inhibit LH release (Ubuka et al., 2012). In male voles, the increase of RFRP3 was concomitant with regression of testes, seminal vesicle and LSG, which suggests a role for RFRP3 in shutting down the HPG axis during summer. However, the role of RFRP3 is complex and, depending on sex and photoperiod, this peptide may also act as a potent activator of the HPG axis (Ancel et al., 2012; Angelopoulou et al., 2019; Ubuka et al., 2012). Therefore, further studies will be warranted to clarify the potential role of RFRP3 in male water voles.

Kisspeptin expression is regulated by photoperiod and sex-steroid feedback. Typically, in seasonal mammals, kisspeptin level in the ARC is positively correlated with the reproductive state, with a stronger labeling of kisspeptin neurons and fibers during the breeding period than during the sexual rest. In the male water voles, the lower density of kisspeptin-ir fibers in the ARC during the breeding season might seem counterintuitive at first glance because kisspeptin stimulates the secretion of GnRH (Messager et al., 2005). However, similar results have already been reported in European and Siberian hamsters (Greives et al., 2007; Rasri-Klosen et al., 2017; Sáenz De Miera et al., 2014), and also in our field study in female water voles (Poissenot et al., 2021). There are two non-mutually exclusive explanations for this finding. First, it could reflect the well-characterized negative feedback exerted by testosterone onto kisspeptin neurons of the ARC during the breeding season (LP), which gets lost under SD, when the testes regress. Second, the higher density of kisspeptin-ir fibers during the non-breeding season might reflect decreased secretion of the peptide at the median eminence. The peptide would then accumulate in these fibers. In contrast, during the breeding season, the peptide is released in the pituitary blood flow as it is synthesized and therefore does not accumulate, as suggested previously (Rasri-Klosen et al., 2017). Further studies will be required to test these two scenarios.

Our field monitoring clearly shows seasonal rhythmicity in morphological and neuroendocrine markers along the HPG axis in male fossorial water voles. Thus, the activity of the reproductive axis is markedly increased in spring and summer, while it is strongly diminished in autumn and winter.

As demonstrated in field voles (Baker and Ranson, 1932a,b, 1933), day length is the main environmental cue for the control of the HPG axis in most seasonal breeders living at temperate latitudes. In the second part of this study, we directly assessed the impact of day length on the HPG axis. To do so, males trapped in late autumn were brought indoors, and exposed to a short-day photoperiod (SP, 8 h of light per day), thereby mimicking the outdoor condition at the time of capture. All these animals were maintained in two independent light-controlled cabinets, which provided the same photoperiodic exposure to all animals housed within a cabinet. Then, after this initial 5-week adaptation period of exposure to short days, animals of one cabinet remained exposed to the same SP while animals of the other cabinet were switched to a long-day photoperiod (LP, 16 h of light per day). These photoperiodic treatments lasted 7 weeks (see Fig. 1). To minimize potential confounding factors (vibrations, temperature or other), both cabinets were installed side-by-side in the same room of the animal facility.

In males exposed to LP, the relative mass of the testes was increased compared with that of SP-exposed males. The relative masses of the epididymes and seminal vesicles displayed quantitatively similar increases in LP-exposed males compared with SP-exposed males. Similar results were previously reported in other vole species exposed to an acute LP treatment (Craven and Clarke, 1986; Martinet and Meunier, 1975; Munley et al., 2020; Nelson, 1985). The LP exposure also affected the development of the androgen-dependent lateral scent glands, as previously reported in Syrian hamsters (Luderschmidt et al., 1984). In SP-exposed males, the lateral scent glands remained almost fully regressed, while they were developed in LP-exposed males, which indicates an increased testicular activity. In the ARC, the number of kisspeptin-ir cells and fiber density were lower in LP-exposed male water voles compared with SP-exposed males. This is fully consistent with our field monitoring results and with data gathered in European and Siberian hamsters (Greives et al., 2007; Rasri-Klosen et al., 2017; Sáenz De Miera et al., 2014).

Our results provide a thorough characterization of the effects of photoperiod on the HPG axis in male water voles. As LP exposure leads to an increase in the reproductive axis activity, these data provide strong support for photoperiodic sensitivity of breeding in water voles. However, we note that SP-exposed males do not present a testicular regression as dramatic as that observed in males caught in the field in January. The relative mass of testes in SP-exposed males is quite similar to that of animals trapped in the field in February 2020. These SP males were captured in early December and kept on SP for 13 weeks. It is therefore possible that the higher-than-expected testes mass reflects the fact that voles no longer respond to the short-day signal and resume activity of the HPG axis. This spontaneous recrudescence of the gonads under prolonged SP, triggered by an endogenous central timing mechanism, is known as 'photorefractoriness', a phenomenon that has been well characterized in multiple species, including the Syrian hamster (for review, see Dardente, 2012; Reiter, 1972). In stark contrast, testes appear fully developed in the LP-exposed males, while seminal vesicles and lateral scent glands present an intermediate development compared with what is found in animals caught in summer during the field study. The photoperiodic treatment might have been too short to induce a complete physiological reactivation of these organs. Moreover, the impact of photoperiod can be modulated by other environmental factors. For example, testicular regression in Syrian hamster kept in SP is slower at 22°C than at 5°C (Larkin et al., 2002), and the fertility of the common vole is directly affected by the quality of the food resource (Martinet et al., 1969).

Finally year-round reproduction of water voles located in the northwest of Spain seems to be enabled by the mild temperatures and food availability (Somoano et al., 2016, 2017). In our study, male voles exposed to either SP and LP were all housed at 20°C and with food *ad libitum*. Therefore, we speculate that variations in temperature and food availability and/or quality in the field might superimpose and modulate the photoperiodic impact to yield full regression or full activation of the HPG axis.

In conclusion, our results show that male water voles are seasonal breeders. Photoperiod appears to be strongly involved in the seasonal regulations of the HPG axis, even though other factors such as temperature and food may have regulatory functions. These other factors could, in particular when day length is intermediate, allow the timing of the beginning and end of the breeding season to be adapted to local conditions (Larkin et al., 2002). Further studies will be required to dissect the relative impact of these environmental cues.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.P., A.C., P.C., A.P., E.B., V.L., H.D., J.D., F.S., M.K.; Methodology: K.P., A.C., A.T., P.C., A.P., E.B., V.L., H.D., J.D., F.S., M.K.; Formal analysis: K.P., M.K.; Investigation: K.P., A.C., C.M., A.T., M.B., D.C., E.R., J.D., F.S., M.K.; Writing - original draft: K.P., M.K.; Writing - review & editing: K.P., A.C., C.M., A.T., M.B., D.C., P.C., E.R., A.P., E.B., V.L., H.D., J.D., F.S., M.K.; Supervision: M.K.; Funding acquisition: M.K.

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References
Airoldi, J.-P. (1976). Le terrier de la forme fouisseuse du campagnol terrestre, *Arvicola terrestris scherman* Shaw (Mammalia, Rodentia). *Rev. suisse Zool.* **41**, 23-42.

Airoldi, J.-P. (1978). Etude par capture et recapture d'une population de campagnols terrestre, *Arvicola terrestris scherman* Shaw (Mammalia, Rodentia). *La Terre la Vie* **32**, 1-45.

Al-Khateeb, A. and Johnson, E. (1971). Seasonal changes of pelage in the vole (*Microtus agrestis*). I. Correlation with changes in the endocrine glands. *Gen. Comp. Endocrinol.* **16**, 217-228. doi:10.1016/0016-6480(71)90033-5

Ancel, C., Bentsen, A. H., Sébert, M. E., Tena-Sempere, M., Mikkelsen, J. D. and Simonneaux, V. (2012). Stimulatory effect of RFRP-3 on the gonadotrophic axis in the male Syrian hamster: the exception proves the rule. *Endocrinology* **153**, 1352-1363. doi:10.1210/en.2011-1622

Angelopoulou, E., Quignon, C., Kriegsfeld, L. J. and Simonneaux, V. (2019). Functional implications of RFRP-3 in the central control of daily and seasonal rhythms in reproduction. *Front. Endocrinol.* **10**, 1-15. doi:10.3389/fendo.2019.00183

Ansel, L., Bolborea, M., Bentsen, A. H., Klosen, P., Mikkelsen, J. D. and Simonneaux, V. (2010). Differential regulation of kiss1 expression by melatonin and gonadal hormones in male and female Syrian hamsters. *J. Biol. Rhythms* **25**, 81-91. doi:10.1177/0748730410361918

Baker, J. R. and Ranson, R. M. (1932a). Factors affecting the breeding of the field mouse (*Microtus agrestis*). Part I.—Light. *Proc. R. Soc. B* **110**, 313-322. doi:10.1098/rspb.1932.0027

Baker, J. R. and Ranson, R. M. (1932b). Factors affecting the breeding of the field mouse (*Microtus agrestis*). Part II.—Temperature and food. *Proc. R. Soc. London. Ser. B* **112**, 39-46. doi:10.1098/rspb.1932.0076

Baker, J. R. and Ranson, R. M. (1933). Factors affecting the breeding of the field mouse (*Microtus agrestis*). Part III.—Locality. *Proc. R. Soc. B* **113**, 486-495. doi:10.1098/rspb.1933.0061

Berson, D. M. (2003). Strange vision: Ganglion cells as circadian photoreceptors. *Trends Neurosci.* **26**, 314-320. doi:10.1016/S0166-2236(03)00130-9

Bronson, F. H. (1988). Mammalian reproductive strategies: genes, photoperiod and latitude. *Reprod. Nutr. Dev.* **28**, 335-347. doi:10.1051/rnd:19880301

Canguilhem, B. and Marx, C. (1973). Regulation of the body weight of the European hamster during the annual cycle. *Pflügers Arch. Eur. J. Physiol.* **338**, 169-175. doi:10.1007/BF00592751

Chai, C. K. (1956). Seminal vesicle growth as a function of androgen stimulation in mice. *Am. J. Physiol.* **186**, 463-467. doi:10.1152/ajplegacy.1956.186.3.463

Craven, R. P. and Clarke, J. R. (1986). Gonadal development and gonadotrophin secretion in the male vole (*Microtus agrestis*) after an abrupt change in photoperiod. *J. Reprod. Fertil.* **76**, 513-518. doi:10.1530/jrf.0.0760513

Dardente, H. (2012). Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. *J. Neuroendocrinol.* **24**, 249-266. doi:10.1111/j.1365-2826.2011.02250.x

Dardente, H., Wood, S., Ebling, F. and Sáenz de Miera, C. (2019). An integrative view of mammalian seasonal neuroendocrinology. *J. Neuroendocrinol.* **31**, 1-17. doi:10.1111/jne.12775

de Roux, N., Genin, E., Carel, J.-C., Matsuda, F., Chaussain, J.-L. and Milgrom, E. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci. USA* **100**, 10972-10976. doi:10.1073/pnas.1834399100

Franceschini, I., Lomet, D., Cateau, M., Delsol, G., Tillet, Y. and Caraty, A. (2006). Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. *Neurosci. Lett.* **401**, 225-230. doi:10.1016/j.neulet.2006.03.039

Frost, P., Giegel, J. L., Weinstein, G. D. and Gomez, E. C. (1973). Biodynamic studies of hamster flank organ growth: hormonal influences. *J. Invest. Dermatol.* **61**, 159-167. doi:10.1111/1523-1747.ep12676217

Greives, T. J., Mason, A. O., Scotti, M. A. L., Levine, J., Ketterson, E. D., Kriegsfeld, L. J. and Demas, G. E. (2007). Environmental control of kisspeptin: implications for seasonal reproduction. *Endocrinology* **148**, 1158-1166. doi:10.1210/en.2006-1249

Grocock, C. A. and Clarke, J. R. (1976). Duration of spermatogenesis in the vole (*Microtus agrestis*) and bank vole (*Clethrionomys glareolus*). *J. Reprod. Fertil.* **47**, 133-135. doi:10.1530/jrf.0.0470133

Harbid, A. A., McLeod, B. J., Caraty, A. and Anderson, G. M. (2013). Seasonal changes in RFamide-related peptide-3 neurons in the hypothalamus of a seasonally breeding marsupial species, the brushtail possum (*Trichosurus vulpecula*). *J. Comp. Neurol.* **521**, 3030-3041. doi:10.1002/cne.23328

Hu, K. L., Chang, H. M., Li, R., Yu, Y. and Qiao, J. (2019). Regulation of LH secretion by RFRP-3 – from the hypothalamus to the pituitary. *Front. Neuroendocrinol.* **52**, 12-21. doi:10.1016/j.yfrne.2018.03.005

Jones, E. J., Poole, K. C., Sollini, J., Town, S. M. and Id, K. B. (2020). Seasonal weight changes in laboratory ferrets. *PLoS ONE* **15**, 1-14. doi:10.1371/journal.pone.0232733

Klosen, P., Sébert, M. E., Rasri, K., Laran-Chich, M. P. and Simonneaux, V. (2013). TSH restores a summer phenotype in photoinhibited mammals via the RF-amides RFRP3 and kisspeptin. *FASEB J.*. **27**, 2677-2686. doi:10.1096/fj.13-229559

Kriegsfeld, L. J., Mei, D. F., Bentley, G. E., Ubuka, T., Mason, A. O., Inoue, K., Ukena, K., Tsutsui, K. and Silver, R. (2006). Identification and characterization of a gonadotropin-inhibitory system in the brains of mammals. *Proc. Natl. Acad. Sci. USA* **103**, 2410-2415. doi:10.1073/pnas.0511003103

Larkin, J. E., Jones, J. and Zucker, I. (2002). Temperature dependence of gonadal regression in Syrian hamsters exposed to short day lengths. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **282**, 744-752. doi:10.1152/ajpregu.00299.2001

Lomet, D., Cognié, J., Chesneau, D., Dubois, E., Hazlerigg, D. and Dardente, H. (2018). The impact of thyroid hormone in seasonal breeding has a restricted transcriptional signature. *Cell. Mol. Life Sci.* **75**, 905-919. doi:10.1007/s00018-017-2667-x

Luderschmidt, C., Hoffmann, K. and Bidlingmaier, F. (1984). Influence of photoperiodism on testicular function and sebaceous glands in Syrian hamster. *J. Invest. Dermatol.* **83**, 157-160. doi:10.1111/1523-1747.ep12263494

Mahmoudi, A., Maul, L. C., Khoshyar, M., Darvish, J., Aliabadian, M. and Kryštufek, B. (2020). Evolutionary history of water voles revisited: confronting a new phylogenetic model from molecular data with the fossil record. *Mammalia* **84**, 171-184. doi:10.1515/mammalia-2018-0178

Martinet, L. (1967). Cycle saisonnier de reproduction du campagnol des champs *Microtus arvalis*. *Ann. Biol. Anim. Biochim. Biophys.* **7**, 245-259. doi:10.1051/rnd:19670302

Martinet, L. and Meunier, M. (1975). Plasma and pituitary levels levels of LH in fields voles, *Microtus arvalis*, reared under two different photoperiods. *J. Physiol. (Paris)* **70**, 539-547.

Martinet, L., Meunier, M. and Lhoste, J.-M. (1969). Influence des variations saisonnières de la luzerne sur la croissance, la mortalité et l'établissement de la maturité sexuelle chez le campagnol des champs (*Microtus arvalis*). *Ann. Biol. Anim. Biochim. Biophys.* **9**, 451-462. doi:10.1051/rnd:19690401

Mason, A. O., Duffy, S., Zhao, S., Ubuka, T., Bentley, G. E., Tsutsui, K., Silver§, R. and Kriegsfeld, L. J. (2010). Photoperiod and reproductive condition are

associated with changes in RFamide-related peptide (RFRP) expression in Syrian hamsters (*Mesocricetus auratus*). *J. Biol. Rhythms* **25**, 176-185. doi:10.1177/0748730410368821

Massoud, D., Lao-Pérez, M., Hurtado, A., Abdo, W., Palomino-Morales, R., Carmona, F. D., Burgos, M., Jiménez, R. and Barrionuevo, F. J. (2019). Germ cell desquamation-based testis regression in a seasonal breeder, the Egyptian long-eared hedgehog, *Hemiechinus auritus*. *PLoS ONE* **13**, 1-16. doi:10.1371/journal.pone.0204851

Messager, S., Chatzidaki, E. E., Ma, D., Hendrick, A. G., Zahn, D., Dixon, J., Thresher, R. R., Malinge, I., Lomet, D., Carlton, M. B. L. et al. (2005). Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc. Natl. Acad. Sci. USA* **102**, 1761-1766. doi:10.1073/pnas.0409330102

Meylan, A. Airoldi, J.-P. (1975). Reproduction hivernale chez *Arvicola terrestris sherman* Shaw (Mammalia, Rodentia). *Rev. Suisse Zool.* **82**, 689-694.

Munley, K. M., Deyoe, J. E., Ren, C. C. and Demas, G. E. (2020). Melatonin mediates seasonal transitions in aggressive behavior and circulating androgen profiles in male Siberian hamsters. *Horm. Behav.* **117**, 104608. doi:10.1016/j.yhbeh.2019.104608

Nagnan-Le Meillour, P., Descamps, A., Le Danvic, C., Grandmougin, M., Saliou, J. M., Klopp, C., Milhes, M., Bompard, C., Chesneau, D., Poissenot, K. et al. (2019). Identification of potential chemosignals in the European water vole *Arvicola terrestris*. *Sci. Rep.* **9**, 1-12. doi:10.1038/s41598-019-54935-z

Nelson, R. J. (1985). Photoperiod influences reproduction in the prairie vole (*Microtus ochrogaster*). *Biol. Reprod.* **33**, 596-602. doi:10.1095/biolreprod33.3.596

Oakley, A. E., Clifton, D. K. and Steiner, R. A. (2009). Kisspeptin signaling in the brain. *Endocr. Rev.* **30**, 713-743. doi:10.1210/er.2009-0005

Poissenot, K., Anger, K., Constantin, P., Cornilleau, F., Lomet, D., Tsutsui, K., Dardente, H., Calandreau, L. and Beltramo, M. (2019). Brain mapping of the gonadotropin-inhibitory hormone-related peptide 2 with a novel antibody suggests a connection with emotional reactivity in the Japanese quail (*Coturnix japonica*, Temminck & Schlegel, 1849). *J. Comp. Neurol.* **527**, 1872-1884. doi:10.1002/cne.24659

Poissenot, K., Moussu, C., Chesneau, D., Ramadier, E., Abi, R., Chorfa, A., Chemineau, P., Michelin, Y., Saez, F., Benoit, E. et al. (2021). Field study reveals morphological and neuroendocrine correlates of seasonal breeding in female water voles, *Arvicola terrestris*. *Gen. Comp. Endocrinol.* **311**, 113853. doi:10.1016/j.ygcen.2021.113853

Quignon, C., Beymer, M., Gauthier, K., Gauer, F. and Simonneaux, V. (2020). Thyroid hormone receptors are required for the melatonin-dependent control of Rfrp gene expression in mice. *FASEB J.* **34**, 12072-12082. doi:10.1096/fj.202000961R

Rasri-Klosen, K., Simonneaux, V. and Klosen, P. (2017). Differential response patterns of kisspeptin and RFamide-related peptide to photoperiod and sex steroid feedback in the Djungarian hamster (*Phodopus sungorus*). *J. Neuroendocrinol.* **29**, 1-13. doi:10.1111/jne.12529

Reiter, R. J. (1972). Evidence for refractoriness of the pituitary–gonadal axis to the pineal gland in golden hamsters and its possible implications in annual reproductive rhythms. *Anat. Rec.* **173**, 365-371. doi:10.1002/ar.1091730311

Revel, F. G., Saboureau, M., Masson-Pévet, M., Pévet, P., Mikkelsen, J. D. D. and Simonneaux, V. (2006). Kisspeptin mediates the photoperiodic control of reproduction in hamsters. *Curr. Biol.* **16**, 1730-1735. doi:10.1016/j.cub.2006.07.025

Revel, F. G., Saboureau, M., Pévet, P., Simonneaux, V. and Mikkelsen, J. D. (2008). RFamide-related peptide gene is a melatonin-driven photoperiodic gene. *Endocrinology* **149**, 902-912. doi:10.1210/en.2007-0848

Rowlands, I. W. (1936). Reproduction of the bank vole II — seasonal changes in the reproductive organs of the male. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **226**, 99-120.

Sáenz De Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M. P., Pévet, P., Hazlerigg, D. G. and Simonneaux, V. (2014). A circannual clock drives expression of genes central for seasonal reproduction. *Curr. Biol.* **24**, 1500-1506. doi:10.1016/j.cub.2014.05.024

Saucy, F. (1988). Dynamique de population, dispersion et organisation sociale de la forme fouisseuse du campagnol terrestre (*Arvicola terrestris scherman* (Shaw), Mammalia, Rodentia). PhD thesis, Université de Neuchatel, Suisse.

Seminara, S. B., Messager, S., Chatzidaki, E. E., Thresher, R. R., Acierno, J. S., Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwinof, K. M., Hendrick, A. G. et al. (2003). The GPR54 gene as a regulator of puberty. *N. Engl. J. Med.* **349**, 1614-1627. doi:10.1056/NEJMoa035322

Shenbrot, G. I. and Krasnov, B. R. (2005). *An Atlas of the Geographic Distribution of the Arvicoline Rodents of the World (Rodentia, Muridae: Arvicolinae)*. Pensoft Publishers.

Simonneaux, V., Ancel, C., Poirel, V. J. and Gauer, F. (2013). Kisspeptins and RFRP-3 act in concert to synchronize rodent reproduction with seasons. *Front. Neurosci.* **7**, 1-11. doi:10.3389/fnins.2013.00022

Smith, J. T., Dungan, H. M., Stoll, E. A., Gottsch, M. L., Braun, R. E., Eacker, S. M., Clifton, D. K. and Steiner, R. A. (2005). Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. *Endocrinology* **146**, 2976-2984. doi:10.1210/en.2005-0323

Somoano, A., Miñarro, M. and Ventura, J. (2016). Reproductive potential of a vole pest (*Arvicola scherman*) in Spanish apple orchards. *Spanish J. Agric. Res.* **14**, e1008. doi:10.5424/sjar/2016144-9870

Somoano, A., Ventura, J. and Miñarro, M. (2017). Continuous breeding of fossorial water voles in northwestern Spain: Potential impact on apple orchards. *Folia Zool.* **66**, 37-49. doi:10.25225/fozo.v66.i1.a6.2017

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos. Trans. R. Soc. B Biol. Sci.* **363**, 375-398. doi:10.1098/rstb.2007.2145

Steadman, F. H. and Krichesky, B. (1945). Prostate and seminal vesicle response to testosterone propionate in intact and castrate rats. *Endocrinology* **37**, 89-95. doi:10.1210/endo-37-2-89

Stoddart, D. M. (1971). Breeding and survival in a population of water voles. *J. Anim. Ecol.* **40**, 487-494. doi:10.2307/3257

Stoddart, D. M. (1972). The lateral scent organs of *Arvicola terrestris* (Rodentia : Microtinae). *J. Zool.* **166**, 49-54. doi:10.1111/j.1469-7998.1972.tb04075.x

Stoddart, D. M., Aplin, R. T. and Wood, M. J. (1975). Evidence for social difference in the flank organ secretion of *Arvicola terrestris* (Rodentia : Microtinae). *J. Zool* **1799**, 529-540. doi:10.1111/j.1469-7998.1975.tb02257.x

Thiessen, D. D., Friend, H. C. and Lindzey, G. (1968). Androgen control of territorial marking in the Mongolian gerbil. *Science (80-.)* **160**, 432-434. doi:10.1126/science.160.3826.432

Ubuka, T., Inoue, K., Fukuda, Y., Mizuno, T., Ukena, K., Kriegsfeld, L. J. and Tsutsui, K. (2012). Identification, expression, and physiological functions of Siberian hamster gonadotropin-inhibitory hormone. *Endocrinology* **153**, 373-385. doi:10.1210/en.2011-1110

Valentine, G. L. and Kirkpatrick, R. L. (1970). Seasonal changes in reproductive and related organs in the pine vole, *Microtus pinetorum*, in southwestern Virginia. *J. Mammal.* **51**, 553-560. doi:10.2307/1378394

Van Haaster, L. H. and De Rooij, D. G. (1993). Cycle of the seminiferous epithelium in the Djungarian hamster (*Phodopus sungorus sungorus*). *Biol. Reprod.* **48**, 515-521. doi:10.1095/biolreprod48.3.515

Vandenbergh, J. G. (1973). Effect of gonadal hormone on the flank gland of the golden hamster. *Horm. Res. Paediatr.* **4**, 28-33. doi:10.1159/000178287

Vasantha, I. (2015). Physiology of seasonal breeding: a review. *J. Vet. Sci. Technol.* **7**, 331. doi:10.4172/2157-7579.1000331

Ventura, J. and Gosálbez, J. (1990). Reproductive cycle of *Arvicola terrestris* (Rodentia, Arvicoidae) in the Aran Valler, Spain. *Rev. suisse Zool.* **55**, 383-391. doi:10.1515/mamm.1991.55.1.85

Ventura, J., Gosálbez, J. and Lopez-Fuster, M. J. (1991). Structure de population d'*Arvicola terrestris* (Linnaeus, 1758) (Rodentia, Arvicolidae) du Nord-Est Ibérique. *Mammalia* **55**, 85-90. doi:10.1515/mamm.1991.55.1.85

Villette, P., Afonso, E., Couval, G., Levret, A., Galan, M., Goydadin, A. C., Cosson, J. F. and Giraudoux, P. (2020). Spatio-temporal trends in richness and persistence of bacterial communities in decline-phase water vole populations. *Sci. Rep.* **10**, 1-19. doi:10.1038/s41598-020-66107-5

Wang, D., Li, N., Tian, L., Ren, F., Li, Z., Chen, Y., Liu, L., Hu, X., Zhang, X., Song, Y. et al. (2019). Dynamic expressions of hypothalamic genes regulate seasonal breeding in a natural rodent population. *Mol. Ecol.* **28**, 3508-3522.

Xie, W., Liu, H., Liu, Q., Gao, Q., Gao, F., Han, Y., Yuan, Z., Zhang, H. and Weng, Q. (2019). Seasonal expressions of prolactin, prolactin receptor and STAT5 in the scented glands of the male muskrats (*Ondatra zibethicus*). *Eur. J. Histochem.* **63**, 2991. doi:10.4081/ejh.2019.2991