Research Article

# Extrinsic and intrinsic factors affecting the daily rhythms of a semiaquatic carnivore in a mediterranean environment

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## Abstract

Field research assessing the effects of both intrinsic and extrinsic factors on animal daily rhythms has been rare, particularly in carnivores and other medium-to-large mammals in hot environments (e.g., Mediterranean regions). This is a crucial knowledge gap, hindering the accurate prediction of how animals might react to widely-anticipated global environmental changes. To assess the behavioral plasticity of Mediterranean semiaquatic mammals in coping with harsh climatic conditions, we investigated the effects of intrinsic and extrinsic factors on the daily rhythms of a Mediterranean population of radio-tracked Eurasian otters (*Lutra lutra*). Multiple components cosinor population models revealed markedly nocturnal activity, affected by seasonality and air temperature. In particular, otters lowered their daylight activity and increased their nighttime activity during the dry season, being generally less active under higher air temperatures. Other extrinsic factors affecting otters' daily rhythms were moon phase, habitat type and wind, whereas differences in activity patterns according to intrinsic factors were mainly related to males' ranging behavior and females' reproductive status. This study provided detailed and population-mean data on *Lutra lutra* daily rhythms, including some insights into inter-individual variation. More importantly, it showed that otters are well adapted to the dry and wet season-cycle typical of Mediterranean regions, while highlighting their potential vulnerability to increasing air temperatures.

## Introduction

The Earth's rotation and orbit around the sun cause noticeable periodicity at certain latitudes, which is manifested by obvious effects on both the light-dark cycle and seasonality. These periodic environmental cues (also called "zeitgebers") lead to the insurgence of endogenous circadian rhythms that synchronize ("entrain") animal activities with factors in the environment (Aschoff, 1981). Understanding the dynamics that make an organism adapt its functions to its surrounding photoperiod, season, or other extrinsic (e.g., moon, rain, air temperature) or intrinsic (e.g., gender, age, reproductive status) factors is a key concept in ecology. It has, in fact, implications in several fields of study that include morphology, physiology, evolution, ethology (Enright, 1970; Daan and Aschoff, 1982; Halle and Stenseth, 2000; Kronfeld-Schor and Dayan, 2003; Hut et al., 2013), and conservation biology (Daan, 1981; Macdonald, 2016). This being said, there is still no comprehensive understanding regarding how intrinsic and extrinsic factors influence animal activity rhythms and their inter- and intra-individual variability (Refinetti, 2012). Field research addressing questions related to animal activity rhythms has been rare, especially pertaining to medium-to-large wild mammals. Existing chronobiology literature instead mostly focuses on laboratory experiments and small mammals, which limits its applicability and predictive power in natural environments (Halle and Stenseth, 2000; Fernández-Duque, 2003).

Particularly little has been investigated, to date, on the influence of seasonality and air temperature on the daily rhythms of free-ranging mammals living in Mediterranean regions (Pita et al., 2011). Research on small mammals by Pita et al. (2011) is a notable exception. However, the publication of similar studies on medium-to-large mammals, and especially carnivores, lags far behind. Moreover, those studies that have been published generally have been limited in sample size, thereby restricting any inferences on individual and seasonal variability (e.g., Beltrán and Delibes, 1994; Palomares and Delibes, 2000). Particularly overlooked so far have been assessments on whether or how air temperature and other climatic variables influence wild mammals' activity and other behaviors in present times (Helm et al., 2013; Bennie et al., 2014). Research addressing climate-related effects on animal ecology, in fact, has generally focused upon modeling the geographic distribution of animals (e.g., Araújo et al., 2011; Cianfrani et al., 2011; Pacifici et al., 2015). Changes in biogeographical distributions, however, arise secondary to individual behavioral responses to varying weather conditions (Helm et al., 2013; Bennie et al., 2014; Macdonald, 2016). Neglecting contemporary thermal constraints on animal behavior thus

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represents a crucial gap, especially in light of anthropogenic climate changes.

Climate change exposes biological timing systems under pressure (Helm et al., 2013). Air temperatures (their variation) may, in fact, have critical roles in (changing) animal rhythms, significantly affecting animal physiology and reproduction (Speakman and Król, 2010). In particular, elevated air temperatures may lead organisms to adopt means of behavioral thermoregulation to prevent hyperthermia (Terrien et al., 2011). Intrinsic physiologic factors — like a species' capacity to dissipate heat — have also been implicated as major constraints affecting an endotherm's energy balance (Speakman and Król, 2010). Indeed, the effects of air temperature variations are strictly interconnected with changes in body composition, thermal conductance and hormonal status induced by seasonality (McNab, 1970; Lovegrove, 2005). It is, therefore, reasonable to expect that the elevated air temperatures, droughts and marked seasonality typical of Mediterranean climates and riverscapes might act synergistically and severely affect the current behaviors of species living within these environments. The virtual absence of research addressing the potential effects of seasonality and climate-related variables on activity behaviors in Mediterranean medium-to-large — and particularly semiaquatic — mammals thus appears both surprising and critical.

We used curve-fitting rhythmometric techniques (Refinetti et al., 2007; Fernández et al., 2009) to assess the effects of extrinsic (season, habitat type, and several climatic/meteorological variables, including air temperature, moon phase, and wind) and intrinsic (gender, age, reproductive status in adult females, and ranging behavior — dispersal vs. residency — in males) factors on daily rhythms in free-ranging Eurasian otters (*Lutra lutra*) radio-tracked in the Mediterranean area of Alentejo, Southern Portugal (Quaglietta, 2011). Eurasian otters, and particularly those living in Mediterranean regions, are a well-suited model species in which to investigate the above-mentioned issues. Due to their unusually-high basal metabolic rate (Kruuk, 2006) and hypothesized limited heat dissipation capacity (Kuhn and Meyer, 2009), Eurasian otters are considered sensitive to ambient and water temperature (Kruuk, 2006, p. 156), theoretically rendering them particularly vulnerable to climate change. This is reinforced by recent research that has forecast a drastic diminution in suitable otter habitats in Southern Europe over the next 50 years due to climate change (Cianfrani et al., 2011). Our study area should therefore be particularly suited to provide baseline information with which to assess the plasticity of freshwater, semiaquatic mammals and their ability to cope with pronounced seasonality and harsh climatic conditions.

In detail, we predicted otter activity to be seasonal, due to changes in photoperiod and environmental conditions; in particular, we expected otters to adjust their activity to limit their exposure to hot temperatures during dry seasons, because of their thermoregulation needs (cf. Kruuk, 2006; Speakman and Król, 2010). We similarly predicted that otters would decrease their activity under higher ambient temperatures (which may easily overcome 45 °C in the study area). In addition, acknowledging the lack of detailed research on Eurasian otters' activity rhythms, we tested the following hypotheses: (i) that otters are largely nocturnal, based on the limited literature available that has mostly focused on temperate zones (Kruuk, 2006); (ii) that otters exhibit higher levels of activity in dams during the dry season, a habitat wherein water (and therefore the otters' aquatic prey) would largely be concentrated in Mediterranean areas (Prenda et al., 2001); iii) that otters increase their (predatory) activity when the moon is more full, due to greater visibility (Kronfeld-Schor et al., 2013; Prugh and Golden, 2014); iv) that lactating females display higher activity levels than females without cubs (Hamel and Côté, 2008), especially during peak lactation, which coincides with the first months of a cub's life, when cubs are inside the den (Kruuk, 2006), because of their increased energy needs (Oftedal and Gittleman, 1989); and (v) that individuals in dispersal have less predictable activity patterns, due to increased exploratory behaviors (Penteriani et al., 2011; Kerk et al., 2015). Eventually, we tested gender, age and wind related-effects on otter activity, as males can be overall more active than females, to increase mating success (Martin

et al., 2010; Pita et al., 2011), subadults can be less predictable and more diurnal than adults (Penteriani et al., 2011; Russell et al., 2015), and strong winds may provoke water turbation, in turn affecting animal behavior (Taylor, 1983; Kuparinen et al., 2010).

## Methods

### Study area

This study was conducted from 2007 to 2010 in Southern Portugal (Alentejo) (Quaglietta, 2011), across an area of approximately 1125 km$^2$ (38°39′–38°27′ N, 8°6.7′–7°43′ W). In this area, the climate is typically Mediterranean. During the study period (15-6-2007/4-10-2010), there was an annual wet season associated with moderate temperatures (December - June; hereafter called the wet season), and an annual hot, dry season (July - November; hereafter called the dry season). The average annual temperature was roughly 15 °C (DS=8), with a high of 42 °C and low of −7 °C during the summer and winter, respectively. The average annual rainfall was 650 mm, the vast majority (≈80%) of the rain falling from October to April, with minimum and maximum monthly rainfall registered for July 2010 (0.2 mm) and December 2009 (175.2 mm), respectively (Centro de Geofisica de Évora, http://www.cge.uevora.pt/).

The study was performed in a water network consisting of eutrophic streams (most of which averaged 5–10 m in width) and several ponds and dams of different sizes (0.01–286.7 ha), encompassing three river watersheds (i.e., Sado, Guadiana, and Tejo). Some of these streams are temporary, either completely drying out during the dry season or becoming restricted to isolated, scattered pools of variable size (mean=116 m$^3$ ±975.5; range: 0.5–35 973 m$^3$). Riparian vegetation — dominated by alders (*Alnus glutinosa*), poplars (*Populus nigra*), willows (*Salix atrocinerea*), and brambles (*Rubus* sp.) — provides refuge for otters (Beja, 1996; L. Quaglietta, *unpublished*), and is mostly well preserved adjacent to streams, while predominantly absent along the shoreline near dams. Elevation ranges from 200 to 370 m. Agriculture and raising livestock are the main human activities. The distribution of otters in the study area is homogeneous, and there is evidence of regular reproduction (Quaglietta, 2011; Quaglietta et al., 2013, 2015). No significant otter predators (with the potential exception of feral dogs and humans) or competitors are thought to exist in this territory. The otters there mostly feed on the abundant, invasive Red Swamp crayfish (*Procambarus clarkii*) and on both invasive and native fish (Quaglietta, 2011; L. Quaglietta, *unpublished*).

### Animal handling

We trapped 51 otters, of which we handled 32 and radio-tracked 16 (9 males and 7 females), constituting the most complete dataset of trapped and radio-tracked Eurasian otters published to date. Our research was approved by the Portuguese Institute for the Conservation of Nature and Forest, who granted us otter capture licenses (permits No. 104/2009 and 105/2010). In the field, after visually estimating their weight, we injected the otters intramuscularly with 10 mg/kg of ketamine and 0.25 mg/kg of midazolam, a combination considered particularly suited for wild, stressed otters (Spelman et al., 1993; Ó Néill et al., 2008). In the lab, we maintained anesthesia using isoflurane gas (1–1.5% in oxygen) as we implanted the otters with a very-high-frequency (VHF) radio transmitter (IMP 300/L 38 g, 8.1×2.3 cm and 400/L 95 g, 9.7×3.3 cm - Telonics Inc., Mesa, Arizona) within their peritoneal cavity (Quaglietta, 2011 provides further details), mostly in accordance with Ó Néill et al. (2008) (the main exception being that we operated on otters in the closed and sterile facilities of the Évora University veterinary hospital). The tags weighed less than 1–2% of the otters' total body weight. After we sutured the incision closed, we administered two antibiotics (15 mg/kg of amoxicillin LA and 8 mg/kg of cefovecin) and an anti-inflammatory drug (0.2 mg/kg of meloxican) subcutaneously. We also directly applied to the incision an ointment with antibiotic activity (polymixin b, neomycin and bacitracin). We minimized the otters' injuries, stress and absence from their territory by using trap-alarms (see

Ó Néill et al., 2007), and by releasing otters immediately after they recovered from anaesthesia into their capture site.

We estimated otter age based on tooth wear, body dimensions, and the development of sexual characteristics, defining subadults (8 months – 2 years) and adults (>2 years) as in (Quaglietta et al., 2013). We determined each animal's gender by direct observation of external anatomy (confirmed by genetic analyses — cf. Quaglietta et al., 2013, 2015), and assessed reproductive status by examining the development of their testes or nipples. One pregnant female (F4) was identified by abdominal palpation and weighing. Since this happened after the animal had already undergone the stress of capture, and when it was already under anaesthesia, we decided to tag her anyway, also considering that pregnant and lactating females of other otter species had already been successfully radio-tracked (Reid et al., 1986). The same reasoning held for four lactating females (F1, F3, F5, and F13). The combination of radio-tracking, direct observations and one picture obtained by camera trapping allowed us to estimate the approximate age of otter cubs raised by tagged females (based on the cubs' body size and the female otters' behavior). We considered females with cubs less than 3 months old — when these still do not venture out of the den (Kruuk, 2006) — to be at peak lactation, while females with cubs between 3 and 6 months old were considered to be in late lactation, and females with cubs more than 6 months were pooled with females with no cubs at all (to increase their sample size). Out of the 7 males, 3 resided stably in their ranges and 4 dispersed from their natal area (cf. Quaglietta et al., 2013).

## Data collection

Otters were radio-tracked by triangulation using a Sika (Biotrack, Ltd., Wareham, United Kingdom) receiver and 3- and 6-element Yagi antennas (Biotrack, Ltd.), from an average distance of roughly 200 m. We inferred activity by sudden changes in the volume of the radio signal (Beja, 1996; Quaglietta, 2011). Radiotracking extended from the 15th of June 2007 to the 4th of October 2010, totaling 1209 days. Over this period, we collected 2225 single radiolocations (hereafter called fixes), collected with a 36 to 37 h delay to homogeneously cover the 24 hours. In addition, we performed 238 sessions of continuous monitoring of the same focal animal (average duration=9.4 hours; SD=5.8), which led to 8220 additional radiolocations, collected in 15 min intervals (hereafter called fixes_cycles). Of the 16 tagged animals, 14 (7 females, of which 2 subadults and 5 adults, and 7 males, of which 5 subadults and 2 adults) provided ⩾49 fixes or radiotracking data for ⩾45 monitoring days, and were subsequently considered for analysis. For these otters, the average number of fixes and continuous monitoring sessions, monitoring days and total tracking period per animal were 156 (SD=66; range: 49–315), 17 (SD=11; range: 3–45), 126 (SD=68), and 401 (SD=244), respectively. We collected fixes with the same frequency during both the night and day, to obtain a homogeneous dataset (we later verified that the fixes were uniformly distributed across the 24 hours: Rayleigh distribution test z=1.04, n=2224, 0.05<p<0.10). Individual otters thus provided repeated samples at fixed time intervals encompassing several 24 hour cycles. This approach, which can be considered a combination of longitudinal and transverse sampling, allows for the summarization of rhythm parameters across sampled individuals to assess daily activity rhythms at a population level (Fernández et al., 2004; Refinetti et al., 2007; Pita et al., 2011). To limit temporal dependence in the data, we sub-sampled the fixes_cycles by randomly selecting one fix per hour (e.g., Rheingantz et al., 2016 and studies cited therein), retaining all fixes plus the randomly selected fixes_cycles for subsequent analyses (N=4097). It was not possible to record data blind because our study involved focal animals in the field.

A meteorological station located centrally within the study area (on the Évora University campus, Pólo da Mitra) provided data on air temperature (°C) every 10 minutes (Évora University Geophysics Centre – http://www.cge.uevora.pt/), thereby allowing their association with both fixes and fixes_cycles with high precision (Tab. S1). In the field, we recorded moon phase (when visible — new, waxing, full, waning) and wind (absent, weak, medium, strong) (Tab. S1). The habitat type used by otters (i.e., stream, pond, dam) was also estimated during field triangulations, and later confirmed using a geographic information system (GIS) (Tab. S1). Moon phase was validated a posteriori via comparisons with data from the Astronomic Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/) (Tab. S1).

## Data analysis

We modeled the influence of intrinsic and extrinsic factors on the probability of Eurasian otters being (located) active using multiple components (MC) cosinor regression models (see Bingham et al., 1982; Fernández et al., 2009). These models have traditionally been applied in medical research (e.g., Fernández et al., 2009), but have recently been used for the analysis of small mammal circadian rhythms (Pita et al., 2011). They are deemed to be similar, but more flexible and powerful than Fourier analysis, and particularly suited for analyzing noisy and sparse time series (i.e., potentially lacking perfect sinusoidality and with data that are not necessarily equidistant) (Fernández et al., 2009). Other advantages of MC models are that they allow for the estimation of parameters that can then be used for biologically-sound comparisons (e.g., Pita et al., 2011). In particular, for each period considered, they provide estimates of: (i) the rhythm-adjusted mean (MESOR — Midline Estimating Statistic Of Rhythm), defined as the average value of the cosine functions fitted to the data, being closely related to 24 h average activity (which differs from the arithmetic mean when the data are not equidistant and/or do not cover an integer number of cycles — cf. Refinetti et al., 2007); (ii) the amplitude, which is half the extent of rhythmic change in a cycle approximated by the fitted cosine functions, measuring the magnitude of the rhythm change; and (iii) the acrophase, corresponding to the time lag at which the fitted cosine functions have their peak (Fernández et al., 2009). Note that when only one period is fitted (i.e., C=1), the model equals a single cosinor model (Fernández et al., 2009).

To choose the multiple components, we started by fitting a global model of activity, including the main components thought to affect otter activity (Beja, 1996; Kruuk, 2006). We thus followed Pita et al. (2011) fitting all together the fundamental circadian 24 h period (CD), the hemi-circadian 12 h period (HCD) to approximate sunrise and sunset timings, and a short-term ultradian period (UD), selected from UD rhythms (2, 3, 4, 5 and 6 h cycles) to approximate presumptive UD feeding or territorial otter rhythms. MC models were fitted as generalized linear mixed-models (GLMMs) with a binomial error distribution (see also (Pita et al., 2011)), using the statistical software package R version 3.2.3 (R Core Team, 2015) and the `glmer` function of the `lme4` package (Bates et al., 2015). We treated individual (cf. Gillies et al., 2006) and year of monitoring (Pita et al., 2011) as random effects, allowing for inferences at the population level (i.e., extrapolating results beyond radio-tracked individuals) and accounting for potential lack of independence in data collected within the same year (Tab. S1).

In accordance with Fernández et al. (2009), models were fitted as:

$$\text{logit}\left(Y\left(\tau\right)\right) = I + MC + FE + RE =$$
$$= M + \sum_{c=1}^{C}\left[\beta_c \cos\left(\frac{2\pi\tau}{T}\right) + \gamma_c \sin\left(\frac{2\pi\tau}{T}\right)\right] + RE + e_\tau$$
(1)

where $Y^\tau$ is the probability of activity at time $\tau$, $I$ is the intercept, $MC$ are the multiple (periodic) components, $FE$ are the fixed effects, $RE$ are the random effects, $C$ is the number of sinusoidal components (here CD, HCD and UD components, thus $C$=3), and $T$ is the time (h) of each fitted period (24, 12 and 2–6 h). Since the response variable is modeled as the odds ratio of activity, MESORs were given as $\frac{\exp I}{1+\exp I}$. Amplitude was denoted by the difference between the peak and the MESOR, while the acrophase was the time (h) corresponding to the activity peak (Fernández et al., 2009; Pita et al., 2011).

Seasonal effects were analyzed by fitting three alternative MC models, one accounting for no seasonal variation in daily rhythms; one accounting for seasonal variations in mean activity but no variations in
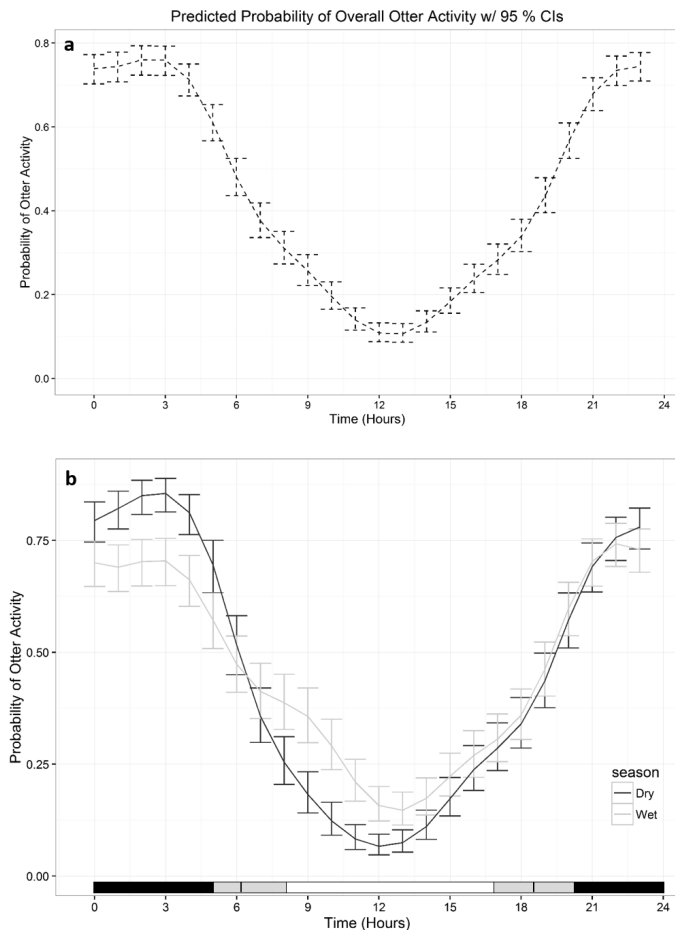
Figure 1 – Daily variation of activity — overall pattern (a) and separated by season (b) — in Eurasian otters (*Lutra lutra*) radio-tracked in a Mediterranean area in Southern Portugal (N=4097 otter radio-locations) according to mixed-effects multiperiodic logistic regression models. Error bars are 95% confidence intervals of predicted probability of activity. Bars in time axis represent night-time (black), daytime (white), minimal and maximal timings of sunrise and sunset (grey) and respective means (vertical lines), considering overall days of activity sampling.

Table 1 – MESORs (and confidence intervals — CIs), amplitudes (and CIs), and acrophases of daily activity rhythms of Eurasian otters (*Lutra lutra*) radio-tracked in Southern Portugal — overall, by season, and by population subgroups — estimated from mixed-effects multiperiodic logistic regression models (MC).

|  | MESOR | 95% CI | Amplitude | 95% CI | Acrophase |
|---|---|---|---|---|---|
| Average | 0.47 | 0.39–0.51 | 0.47 | 0.40–0.53 | 03:00 |
| Dry | 0.45 | 0.35–0.55 | 0.49 | 0.39–0.59 | 03:00 |
| Wet | 0.51 | 0.40–0.61 | 0.37 | 0.27–0.48 | 03:00 |
| | | | | | |
| Resident males | 0.42 | 0.32–0.54 | 0.49 | 0.38–0.59 | 02:00 |
| Males in dispersal | 0.45 | 0.39–0.53 | 0.14 | 0.06–0.20 | 02:00 |
| | | | | | |
| Adults | 0.41 | 0.38–0.46 | 0.38 | 0.34–0.42 | 23:00 |
| Subadults | 0.44 | 0.41–0.48 | 0.30 | 0.26–0.33 | 02:00 |
| | | | | | |
| Gestating females | 0.40 | 0.34–0.56 | 0.46 | 0.30–0.52 | 22:00 |
| Females at lactation peak | 0.34 | 0.23–0.48 | 0.51 | 0.37–0.62 | 03:00 |
| Females in late lactation | 0.40 | 0.33–0.53 | 0.47 | 0.35–0.55 | 03:00 |
| Females with no cubs | 0.45 | 0.36–0.51 | 0.38 | 0.32–0.47 | 00:00 |

steps, somewhat similar to what is described in Zuur et al. (2009). First, we selected the best multiple components model structure, fitting models with no covariates. Then we selected the best covariate structure, fitting the selected best multiple components' structure. We model-averaged when no single model accounted for >90% of the cumulative Akaike weights (Burnham and Anderson, 2002).

We checked explanatory variables for potential collinearity and redundancy by calculating Pearson correlation coefficients (retaining variables with r<0.7) and variable inflation factors (VIFs — retaining variables with VIFs<3), as per Zuur et al. (2009). For all the hypotheses tested, significant effects are depicted by *p*-values <0.05 or confidence intervals (CIs) not including zero. Best model variance — explained by fixed-effects only and by both fixed and random effects (i.e., the entire model), respectively known as "conditional" and "marginal" coefficients of determination (Nakagawa and Schielzeth, 2013) — were computed using the `MuMIn` package (Barton, 2016). We also generated approximate measures of model discrimination ability, calculating the posterior predictive simulation (Gelman and Hill, 2006, 2006 apud Bates et al., 2014) and the area under the receiver operating characteristic (ROC) curve. We obtained the latter using the package `pROC` (Robin et al., 2011). The functions `profile` and `confint` of package `lme4` provided bootstrapped 95% confidence intervals (CIs) for model estimates.

## Results

### Overall otter activity

Otters were active 45% of the time (N=4103). Otter activity was markedly rhythmic, as indicated by a ΔAIC of 320 between the null model (including only random terms) and the best MC model (Likelihood ratio test – LRT – $\chi^2$=332.21, df=6, *p*<0.001). Specifically, otters were mostly nocturnal (Fig. 1a). The overall average probability of otter activity (MESOR) was 0.45 (95% CI: 0.39–0.51), while the amplitude was 0.47 (0.40–0.53) (Fig. 1a; Tab. 1). Otters displayed two peaks of activity: one during the first part of the night (around 23:00) followed a larger peak at approximately 03:00 (Fig. 1a; Tab. 1).

Multiple components model selection resulted in the best model (i.e., lowest AIC) including the CD period, the HCD period, and the 6 h UD period, the interactions between season and the CD and the HCD periods, the residuals' percentiles of the GAM of air temperature modeled as a function of time of day and season, two dummy variables (presence of a strong wind and otters being located in dams), and phase of the moon (Tab. 2; Tab. S2). The 95% candidate set models also included a full model with interaction between the season and UD period and a model with no wind effect (Tab. S2). The model average of the three best models (Tab. S3) yielded very similar coefficient estimates for those variables included in the best ranking model (Tab. 2). The relative magnitudes of the sums of squares of the best model indicate that the CD period explains most of the variation in data, followed by the

daily rhythms; and one accounting for differences between seasons in daily rhythms, as per Pita et al. (2011) (Tab. S1). We also tested the (main) effects of other extrinsic factors (air temperature, moon phase, presence of strong wind, and habitat type). As air temperature is inherently dependent upon the time of day (hour), as well as strongly correlated with season, we modeled air temperature as a function of the interaction between time (hour fitted as a smoothing term) and season, using a GAM and the `mgcv` package (Wood, 2016). The residuals' percentiles of the GAM were therefore included in the model selection procedure, allowing us to assess the effect of extreme air temperature values on otter activity, independently of time of day and season.

Intrinsic factors, i.e., gender and age (Tab. S1), were not significant, thus were not retained in the final (best) model. We nonetheless analyzed data separately by gender, to assess gender-specific effects on otter activity. Namely, we constructed GLMMs using females-only and males-only data separately, to test for potential effects of reproductive status and residency versus dispersal on females' and males' probability of activity, respectively. Eventually, since females F4 and F13 displayed an unusual and rather high proportion of diurnal activity (60 times out of 216, 28%), we created a general linear model (GLM, in this case, the number of random term levels was too low to justify using a GLMM) applied to only diurnal locations of these two females, to explore how their diurnal activity varied along with their reproductive status. We performed these analyses despite the very limited sample size (N=2), considering that these data are extremely rare and might provide potentially interesting information.

Model selection was determined using the Akaike information criterion (AIC; Burnham and Anderson, 2002), being performed in two

**Table 2** – Parameter estimates, standard errors, z- and p- values for the best mixed-effects multiperiodic (MC) logistic regression model of Eurasian otter (*Lutra lutra*) circadian activity rhythms. Res.GAM.T.S = percentiles of residual variance of a generalized additive model (GAM) modeling air temperature as a function of the interaction between hour (fitted as a smoothing term) and season (cf. main text).

| Variable | Estimate | SE | z | *p* |
|---|---|---|---|---|
| Intercept | −0.214 | 0.126 | −1.692 | 0.091 |
| Season (Wet) | 0.058 | 0.082 | 0.702 | 0.483 |
| $\cos(2\pi\,\mathrm{Hour}/24)$ | 1.982 | 0.093 | 21.295 | <0.001 |
| $\sin(2\pi\,\mathrm{Hour}/24)$ | 0.368 | 0.075 | 4.919 | <0.001 |
| $\cos(2\pi\,\mathrm{Hour}/12)$ | −0.164 | 0.083 | −1.974 | 0.048 |
| $\sin(2\pi\,\mathrm{Hour}/12)$ | 0.228 | 0.081 | 2.806 | 0.005 |
| $\cos(2\pi\,\mathrm{Hour}/6)$ | −0.170 | 0.052 | −3.273 | 0.001 |
| $\sin(2\pi\,\mathrm{Hour}/6)$ | −0.092 | 0.052 | −1.746 | 0.081 |
| Res.GAM.T.S | −0.240 | 0.053 | −4.500 | <0.001 |
| Dam | 0.386 | 0.082 | 4.692 | <0.001 |
| Moon (Waxing) | −0.110 | 0.100 | −1.104 | 0.269 |
| Moon (Full) | 0.120 | 0.105 | 1.143 | 0.253 |
| Moon (Waning) | 0.262 | 0.108 | 2.440 | 0.015 |
| Wind | −0.899 | 0.427 | −2.106 | 0.035 |
| Season (Wet) $\times \cos(2\pi\,\mathrm{Hour}/24)$ | −0.718 | 0.118 | −6.099 | <0.001 |
| Season (Wet) $\times \sin(2\pi\,\mathrm{Hour}/24)$ | −0.134 | 0.100 | −1.340 | 0.180 |
| Season (Wet) $\times \cos(2\pi\,\mathrm{Hour}/12)$ | 0.145 | 0.108 | 1.348 | 0.178 |
| Season (Wet) $\times \sin(2\pi\,\mathrm{Hour}/12)$ | −0.390 | 0.107 | −3.647 | <0.001 |

**Table 3** – F statistics corresponding to the sequential decomposition of the contributions of fixed-effects terms for the best mixed-effects multiperiodic (MC) logistic regression model of Eurasian otter (*Lutra lutra*) circadian activity rhythms. Res.GAM.T.S = percentiles of residual variance of a generalized additive model (GAM) modeling air temperature as a function of the interaction between hour (fitted as a smoothing term) and season (cf. main text).

| | df | Sum Sq. | Mean Sq. | F |
|---|---|---|---|---|
| Season | 1 | 0 | 0 | 0.000 |
| $\cos(2\pi\,\mathrm{Hour}/24)$ | 1 | 710.48 | 710.48 | 710.477 |
| $\sin(2\pi\,\mathrm{Hour}/24)$ | 1 | 31.73 | 31.73 | 31.729 |
| $\cos(2\pi\,\mathrm{Hour}/12)$ | 1 | 0.77 | 0.77 | 0.775 |
| $\sin(2\pi\,\mathrm{Hour}/12)$ | 1 | 0.25 | 0.25 | 0.254 |
| $\cos(2\pi\,\mathrm{Hour}/6)$ | 1 | 6.9 | 6.9 | 6.903 |
| $\sin(2\pi\,\mathrm{Hour}/6)$ | 1 | 3.05 | 3.05 | 3.045 |
| Res.GAM.T.S | 1 | 24.5 | 24.5 | 24.504 |
| Dam | 1 | 19.53 | 19.53 | 19.527 |
| Moon (Waxing) | 3 | 11.67 | 3.89 | 3.891 |
| Wind | 1 | 2.97 | 2.97 | 2.966 |
| Season (Wet) $\times \cos(2\pi\,\mathrm{Hour}/24)$ | 1 | 32.87 | 32.87 | 32.871 |
| Season (Wet) $\times \sin(2\pi\,\mathrm{Hour}/24)$ | 1 | 2.45 | 2.45 | 2.447 |
| Season (Wet) $\times \cos(2\pi\,\mathrm{Hour}/12)$ | 1 | 1.73 | 1.73 | 1.732 |
| Season (Wet) $\times \sin(2\pi\,\mathrm{Hour}/12)$ | 1 | 13.3 | 13.3 | 13.300 |

interaction between the season and CD period (Tab. 3). Other relevant environmental predictors were, in order of magnitude, the residual percentiles of the GAM for air temperature (more details on this variable are in the next section), the interaction between the season and HCD period, the presence of a dam (otters in dams were significantly more likely to be active than otters in ponds or streams), and the phase of the moon (a waning moon positively associated with otter activity) (Tab. 3), while the presence of strong winds only explained minor variations, decreasing the probability of locating an active otter ($p$=0.035; Tab. 2; Tab. 3).

Variations between individual otters and sampling years were not significant — i.e., the standard deviations of random variables were ≈0 (they were still low, <0.001, when parameters were estimated using a maximum penalized likelihood approach and an uninformative prior in a Bayesian setting using the `blme` R package — Chung et al., 2013). We nevertheless retained the random effects, considering our study design; and because including the two random effects increased $R^2$ (to 32.7 versus 25.7 with a GLM). The best model's marginal and conditional coefficients of determination were both 33%. The best model had reasonable discriminatory ability, with an area under the curve (AUC) of 0.79, a (one-tailed) posterior predictive $p$ value of 1, and a dispersion parameter of 1.10 (considered only negligibly over-dispersed — cf. Zuur et al., 2009).
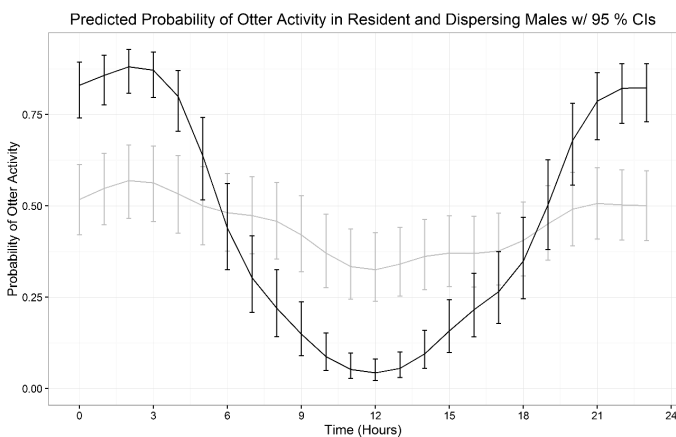


Predicted Probability of Otter Activity in Resident and Dispersing Males w/ 95 % CIs

**Figure 2** – Daily variation of activity in resident, adult males (black) and males in dispersal (grey) of Eurasian otters (*Lutra lutra*) according to mixed-effects multiperiodic logistic regression models. Error bars are 95% confidence intervals for predicted probability of activity.

## Influence of extrinsic factors

Season interacted with the time of day such that, during the dry season, diurnal activity was lower, and nocturnal activity was higher than during the wet season. These differences were particularly marked during the first part of the day (08:00–13:00) and the second part of the night (00:30-04:30) respectively, as indicated by non-overlapping 95% confidence intervals of predicted activity during each season (Fig. 1b). MESOR was greater in the wet season (0.51, 95% CI: 0.40–0.61 vs. 0.45, 0.35–0.55 in the dry season), while amplitude was greater during the dry season (0.49, 0.39–0.59 vs. 0.37, 0.27–0.48 in the wet season) (Fig. 1b; Tab. 1). Higher air temperature negatively affected the probability of otter activity (Tab. 2), with removal of the residual variance of the generalized additive model (GAM) for air temperature leading to a ΔAIC of 18.3 (LRT $\chi^2$=20.4; df=1; $p$<0.001).

## Influence of intrinsic factors

Analyzing data separately by gender, some inter-individual variability in rhythms due to gender-specific effects emerged. In particular, inspection of composite waveforms of the models applied separately only to males (N=7) and females (N=7) data revealed that males in dispersal were almost aphasic, displaying higher levels of diurnal activity than resident males (Fig. 2), and that gestating females were more prone to be active during the central hours of the morning (≈10:00–11:00) and overall slightly less periodic than females in other reproductive phases (but see Discussion for possible drawbacks) (Fig. 3). Also, the GLMM applied to the same female dataset showed that gestating females were overall less active than females in other reproductive phases, with a ΔAIC of 41 between the null model (including only random terms) and a model with a dummy variable discriminating gestating (1) from non-gestating (0) females (LRT $\chi^2$=6.12, df=1, $p$<0.013) (Tab. S4). Finally, the GLM applied only to the diurnal locations of females F4 and F13 indicated that the diurnal activity of these two females decreased while they were lactating 3-to-6 month old cubs ($p$=0.016), while it was relatively higher during gestation and the early phase (first three months) of lactation (Tab. S5). A post hoc Tukey's multiple comparisons test showed that it was when F4 and F13 had 3-to-6 month old cubs ($p$<0.001) or no cubs (or cubs older than 6 months) ($p$=0.072) that these two females significantly decreased the frequency of their diurnal activity relative to all other phases.

Comparisons of MC models' rhythm parameters also highlighted inter-individual variability related to ranging behavior in males and reproductive status in females (Tab. 1), as well as suggested minor variability related to age (Tab. 1). In fact, males in dispersal had much lower amplitudes (0.14, 0.06–0.20) than resident males (0.49; 0.38–0.59), while MESORs were similar in both classes (males in dispersal:
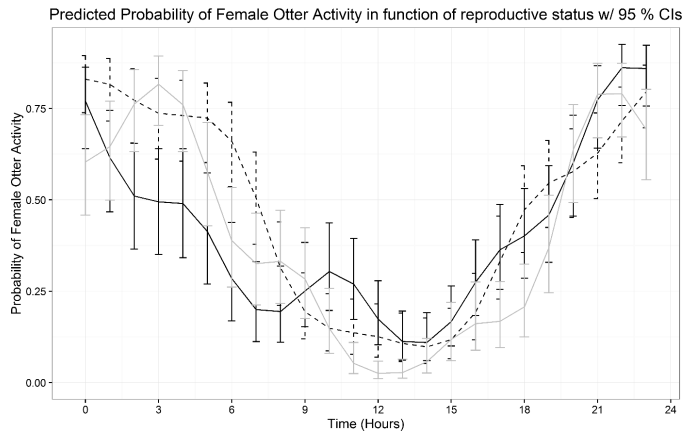
Predicted Probability of Female Otter Activity in function of reproductive status w/ 95 % Cls

**Figure 3** – Daily variation of activity in adult female Eurasian otters (*Lutra lutra*) of different reproductive status (black = gestating, grey = lactation peak, dashed = no cubs or cubs older than 6 months) according to mixed-effects multiperiodic logistic regression models. Error bars are 95% confidence intervals for predicted probability of activity.
**Note**: females that are lactating for cubs between 3 and 6 months old are not shown for sake of clarity (their profile was somehow in the middle between that of females at lactation peak and that of females with no cubs or cubs older than 6 months).

0.45, 0.39–0.53; resident males: 0.42, 0.32–0.54) (Tab. 1). Among females, the lowest MESORs were for females at their peak of lactation (0.34, 0.23–0.48), who also displayed the highest amplitudes (0.51, 0.37–0.62) (Tab. 1). Conversely, females without cubs or with cubs older than 6 months exhibited the highest MESORs (0.45, 0.36–0.51) and lowest amplitudes (0.38, 0.32–0.47) (Tab. 1). Subadult individuals (of either gender) had lower amplitudes (0.30, 0.26–0.33) than adults (0.38, 0.34–0.42) (Tab. 1); while MESORs were 0.44 (0.41–0.48) in subadults and 0.41 (0.38–0.46) in adults (Tab. 1).

## Discussion

This study revealed that both extrinsic and intrinsic factors influence the daily rhythms of the Eurasian otter in a Mediterranean environment. In particular, we report that season and air temperature significantly affect the chronobiology of a Mediterranean semiaquatic carnivore, and provide novel insights into Eurasian otters' inter-individual variation in activity patterns, specifically as a function of male ranging behavior, female reproductive status, and, secondarily, age. These results corroborate previous body of published knowledge on the ecology and conservation of Mediterranean otters (reviewed in Quaglietta, 2011), suggesting potential vulnerability of semiaquatic mammals to elevated air temperatures and the harsh climatic conditions typical of Mediterranean ecosystems.

### Influence of season, air temperature and other extrinsic factors

As expected, otters were mostly nocturnal. Predominantly nocturnal activity is indeed the most common pattern witnessed throughout the species range (cf. Kruuk, 2006), as well as in line with what was observed during five 24 h cycles in two female otters previously radio-tracked along the Southwest coast of Portugal (Beja, 1996). Carnivores are known to synchronize their activities with those of their prey (e.g., Lodé, 1995; Zielinski, 1988; Schmidt et al., 2009). Otters, in particular, are believed to coordinate their activity with when their main sources of prey are more available, i.e., easier to hunt (Kruuk, 2006). Fish and the American Red Swamp crayfish are the main prey otters have in our study area, both abundant and homogeneously spread (Quaglietta, 2011; L. Quaglietta, *unpublished*). Here, crayfish are predominantly nocturnal, often taking refuge in burrows during the day (Ilhéu et al., 2003), while fish are mostly diurnal or crepuscular (Ilhéu et al., 2004). Crayfish appear to be easier to catch than fish, as they are slower, and as suggested by repeated observations of otters hunting and eating several crayfish within a few minutes (L. Quaglietta, *personal observation*). Based on the above considerations, we argue that the night is indeed the best time for otters to forage for both fish (when they are

less mobile) and crayfish (not in hiding). Nocturnal activity could also reflect otters' behavioral thermoregulation strategy (see below), reflecting partial avoidance of the extreme diurnal heat that can occur in Mediterranean climates, as has already been observed amongst other carnivores on the Iberian Peninsula (Beltrán and Delibes, 1994; Palomares and Delibes, 2000). Consistent with this, a recent biogeographical review of time partitioning in mammals revealed that the proportion of nocturnal species is highest in arid regions and lowest at extremely high altitudes (Bennie et al., 2014).

In accordance with our prediction, we found that otter daily rhythms were highly influenced by the strong seasonality that is typical of Mediterranean-type climates. In particular, otters further diminished their daylight activity during the dry season, which was when mean activity was also generally lower, and rhythm amplitude higher. Moreover, also agreeing with our predictions, higher air temperatures had an inhibitory effect on overall otter activity. Most likely, otters adjusted their activity to seasonal changes using photoperiodic information for seasonal phasing, a phenomenon, known as "photoperiodism", which has been documented to occur across a variety of vertebrates (Heldmaier et al., 1989; Refinetti, 2012). We think that the observed influence of seasonality and air temperature on otter daily rhythms could be the result of some adaptation to conserve energy (cf. Moen, 1978), molded by the hot climate and resource unpredictability typical of Mediterranean environments. It is thus quite possible that otters, like other mammals (Lovegrove, 2005; Terrien et al., 2011) and vertebrates (Heldmaier et al., 1989; Speakman and Król, 2010), could exhibit behavioral thermoregulation to cope with the extremely hot, ambient summer temperatures within our study area. Reducing activity during the dry season and the central hours of the day would be a strategy to avoid hyperthermia, as well as a consequence of reduced energy costs (i.e., needs for caloric intake) induced by heat exposure, in accordance with the recently-proposed "circadian thermoenergetics" theory (Hut et al., 2012; van der Vinne et al., 2014). Species capacity to dissipate heat is indeed considered a major constraint on the energy balance of endotherms (Speakman and Król, 2010). Although, on one hand, one might expect semiaquatic animals like otters to be less sensitive to warming, given that the high conductivity of water (>23-fold that of air) helps to dissipate heat (Speakman and Król, 2010), on the other hand, as carnivores, otters have relatively elevated basal metabolic rates (BMR) (McNab, 1989), up to 38–48% higher than those of terrestrial mammals of the same size (Kruuk, 2006). Higher BMRs generate heat (Naya et al., 2013). Thus, organisms with higher BMRs and a limited capacity to dissipate heat have a significantly increased risk of hyperthermia. This may be especially true when air temperatures are very high, as in our study area (where they are often >40 °C during the dry season), as ambient temperatures are the most important factor controlling species' thermal conductance (Naya et al., 2013). Significantly, preliminary infrared thermography research suggests that Eurasian otters could be a less heat-tolerant species with limited dissipation capacity, due to the insulating properties of their thick fur (Kuhn and Meyer, 2009). It therefore appears likely that freshwater, semiaquatic mammals living in Mediterranean environments — such as the Eurasian otters we studied — are actually quite sensitive to elevated air temperatures. This, in turn, potentially render them particularly vulnerable to predicted environmental changes imposed, for instance, by global warming.

Previous studies that have attempted to address photoperiodic, air temperature-related or seasonal effects on mammalian activity have mostly concentrated on the colder temperatures and winters that are typical of temperate zones (e.g., Moen, 1978; Garshelis and Pelton, 1980; Heldmaier et al., 1989; Zalewski, 2001; Kowalczyk et al., 2003; Kolbe and Squires, 2007; Hamel and Côté, 2008; Zub et al., 2009; Martin et al., 2010; Russell et al., 2015; Brivio et al., 2016), while our findings add to the few previously available for warmer environments and seasons, characteristic of Mediterranean regions. The few examples include certain studies (e.g., Beltrán and Delibes, 1994; Palomares and Delibes, 2000) which allowed for limited to no population-level inferences. Very similar to our results, Pita et al. (2011) found that Cabrera

voles were less active (displayed lower mean activity, but higher amplitudes) during the dry season, and concentrated their activity at dawn and dusk, while preferring diurnal hours during the wet season. Also consistent with our findings, the quantity of time that Iberian lynxes were found to be active was negatively correlated with summer temperatures in Spain (Beltrán and Delibes, 1994), while Egyptian mongooses appeared to take afternoon "siestas" during the summer, possibly to avoid the heat (Palomares and Delibes, 2000). Limited activity during times of elevated air temperature in carnivores has been observed in a limited number of other species, which include wolves (Theuerkauf et al., 2003), badgers (Kowalczyk et al., 2003), and weasels (Zub et al., 2013).

Other effects related to extrinsic factors that we observed pertained to habitat, moon phase, and wind speed. In particular, otters were more likely to be active in dams than in ponds or streams, irrespective of the season. We primarily attribute this finding to two factors. First, whereas water (and hence the otters' prey) was always present at dams, droughts often depleted water in streams, to the point where several portions of stream beds commonly became dry. When streams lacked water, there was no prey for otters. This could explain, at least in part, why otters were more active around dams (with otters possibly intent on foraging). Second, contrary to streams and, to a lesser extent, ponds, dams were mostly devoid of riparian vegetation cover, and therefore had fewer suitable resting sites for otters (Quaglietta, 2011; L. Quaglietta, *unpublished*). Therefore, the otters located around dams would be more likely to be observed as active, than in the other two habitat types (where they often rested, although they also frequently foraged in these habitats — Quaglietta, 2011; L. Quaglietta, *unpublished*). Otter activity increased when the moon was waning, contrary to our expectation that otters would be more predatory during full moons, because of the inherent increased visibility (cf. Kotler et al., 2010). Our unanticipated finding adds to the growing evidence that more complex responses to moon phases might exist among free-ranging carnivores (e.g., Beltrán and Delibes, 1994; Penteriani et al., 2013) or, more generally, predators (Penteriani et al., 2011). This may result from the interplay between a prey's tendency to minimize their predation risk and increase their foraging success (Penteriani et al., 2011, 2013; Kronfeld-Schor et al., 2013), being also related to their main sensory system, phylogeny and habitat type (Prugh and Golden, 2014). Similar to our observation, Kotler et al. (2010) identified higher levels of activity in gerbils during waning moons, and considered the latter as the more intense of the moon phases with respect to the predator–prey (foxes versus gerbils) foraging game. Accordingly, otters' higher levels of activity during waning moons could reflect an interplay between fish and red crayfish strategies to minimize predation risk by otters and increase their foraging success and otters' hunting behavior and other needs/constraints (e.g., mating, marking). Finally, the presence of strong winds was negatively associated with otter activity (though this appeared to be but a minor effect). Consistent with our results, negative effects of wind on fish-eating birds' foraging behaviors have been reported (Taylor, 1983). Water turbulence provoked by wind reduces water clarity and may increase fish activity (Kuparinen et al., 2010). Our finding could thus reflect the wind's direct (positive) and indirect (negative) effects on otter prey (i.e., fish) and hunting success, respectively.

## Influence of intrinsic factors

Individual variations in otter daily rhythms were negligible (as indicated by the very low value calculated for the "individual" random effect). Analyses performed separately by gender nonetheless highlighted some individual variability. In particular, and in agreement with our a priori hypothesis, males in dispersal were more diurnal and less nocturnal (and overall less periodic) than resident males. By being more active during the day, when resident males mostly rested, and less active during the first part of the night, when resident males were instead rather active, males in dispersal seem thus to occupy a suboptimal position on the time axis of the niche, possibly to reduce intra-species competition (Daan, 1981). Although documentation on rhythm changes caused by competition is rare (Kronfeld-Schor and

Dayan, 2003; Pita et al., 2011), temporal niche-segregation to reduce intra-species competition has been demonstrated (Russell et al., 2015) or thought (Harrington et al., 2009) to occur in other carnivores. Dominance hierarchy may be a likely explanation for the observed temporal segregation among resident and dispersing male otters, particularly considering the expected higher encounter rates — and therefore augmented competition — among individuals of the same species living in a linear landscape (see Quaglietta et al., 2015). Otters in dispersal, which are commonly younger than resident individuals (Quaglietta et al., 2013), could also be more frequently active (less periodic) than resident (adult) otters because of increased need of dispersers to explore new territories (Kerk et al., 2015) and increased time spent foraging due to less-developed hunting skills of younger individuals (Kruuk, 2006; Russell et al., 2015). Consistent with our findings, the activity patterns of dispersing eagle-owls appear to be less predictable than those of residents of the same species (Penteriani et al., 2011).

Regarding females' behavior, we predicted that females at the peak of lactation (having cubs less than three months old) would display more aphasic waveforms, including a certain degree of diurnal activity, relative to females in other reproductive statuses, based on the greater energy demands generally associated with this reproductive phase in female otters (Kruuk, 2006) and other carnivores (Oftedal and Gittleman, 1989). Our findings partly confirmed our prediction, while revealing more complex patterns. Females at their peak of lactation, in fact, showed the highest amplitudes but the lowest MESORs, being primarily active during specific and shorter periods. These females might be less frequently active as they have to spend a significant amount of time feeding and parenting their cubs, with limited spare time to invest in prolonged activities of any other kind (e.g., feeding themselves). They might also have less need to do so, having already satisfied any such necessity before and during their pregnancy (see also Hamel and Côté, 2008). On the other hand, gestating females were overall less active than females in other reproductive phases (as well as active, on average, earlier in the day, data not shown — L. Quaglietta, *unpublished*), possibly because they have to invest time resting and preparing the future natal den. These females also seemed to be slightly more diurnal during the central hours of the morning and more aphasic than females in other reproductive phases (although partially overlapping 95% confidence intervals in Fig. 3 suggest caution in the interpretation of these patterns). Analyses restricted to the diurnal locations of femalesF4 and F13 revealed that it was when these two females stopped lactating for their cubs that they were less diurnal. Somehow similar to our observations, nursing Iriomote cat females (*Prionailurus iriomotensis*) have been found to be more diurnal and aphasic than females with independent kittens (Schmidt et al., 2009). In other studies on the activity patterns of reproductive females of other carnivore species, breeding wolf females have been observed to be the least active and mobile individuals (Theuerkauf et al., 2003), while a female Pine marten (*Martes martes*) showed some diurnal activity during her pregnancy (Zalewski, 2001), and a panther female spent more time resting while caring for her kittens than when not (Kerk et al., 2015).

In the absence of experimental research, and notwithstanding our limited sample sizes relative to the number of individuals in the different reproductive phases and ranging behaviors, the evidence we have gathered overall reveals subtler differences between Eurasian otter females in different reproductive states, providing further evidence that gestation and (the first phase of) lactation are highly challenging phases for the energetics of carnivore females (Oftedal and Gittleman, 1989), as well as adds novel insights into variation in males' activity patterns according to their ranging behavior.

## Conclusions

Mediterranean otters' daily rhythms are influenced by both endogenous and exogenous factors, seemingly primarily shaped by seasonality, thermoregulatory behavioral strategies, hunting behaviors, males' ranging behaviors, females' cub-rearing, and, secondarily, by moon phase and wind. These findings add to the limited existing information on the chronobiology of wild carnivores, particularly semiaquatic

ones, in non-temperate zones. In particular, our results demonstrate that otter current behavior, i.e., rhythmometry, is affected by seasonality, higher air temperature, and other climate-related effects. This corroborates the concern regarding the vulnerability of otters — and generally Mediterranean semiaquatic mammals — to climate change, so far mostly assessed with distributional data (Cianfrani et al., 2011). Research addressing climate change has indeed mostly focused on species distributions, typically failing to consider behavioral and other species' traits (see also Helm et al., 2013; Bennie et al., 2014; McCain and King, 2014; Macdonald, 2016). Further studies evaluating thermal constraints on current animal behaviors are required to assess the ability of semiaquatic mammals to cope with the harsh climatic conditions typical of Mediterranean regions (or other hot, unpredictable environments), or to track and adapt to anthropogenic changes, such as predicted climate change. Especially needed is future research at multiple time scales and with enough statistical resolution to address intra- and inter-individual variations (Refinetti, 2012), interfacing with thermal physiology (Naya et al., 2013) and along latitudinal gradients (Hut et al., 2013).

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## Supplemental information

Additional Supplemental Information may be found in the online version of this article:

**Supplemental Table S1** Description of the 12 variables used in multicosinor analyses.

**Supplemental Table S2** Summary results of MC model selection of Eurasian otter (*Lutra lutra*) daily activity rhythms.

**Supplemental Table S3** Model-averaged parameter estimates, standard errors, z and p values for the 3 best MC models representing Eurasian otter (*Lutra lutra*) daily activity rhythms.

**Supplemental Table S4** Parameter estimates, standard errors, z and p values for a binomial GLMM testing the influence of gestation on the probability of activity in adult Eurasian otter (*Lutra lutra*) females.

**Supplemental Table S5** Parameter estimates, standard errors, z and p values for a binomial GLM testing the influence of reproductive status on the probability of diurnal activity in adult Eurasian otter (*Lutra lutra*) females.