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Fine scale behaviour and time-budget in the cryptic ectotherm European pond turtle *Emys orbicularis*

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Keywords Accelerometry, VeDBA, temperature, cloud coverage, nesting behaviour, *Emys orbicularis*

Summary statement

Female European pond turtles monitored with animal-borne accelerometers adjust their fine scale behaviour and energy expenses according to ambient temperature, cloud coverage and time relative to nesting.

Abstract

For ectotherms, behaviour and associated energetic costs are directly related to thermal conditions. In the present context of global change, estimating time-budget for these species is relevant to assess and predict their capacity to adapt to near future. We tested the hypothesis that in ectotherms where reproduction is highly energy consuming, energy expenditure should vary throughout the breeding season with a maximum around nesting events. To test this hypothesis, we assessed the fine-scale behaviour, time-budget and estimated energetic costs in eight adult female European pond turtles *Emys orbicularis* equipped with data-loggers recording ambient temperature, pressure, light and the animals'

3-axis acceleration. Deployments occurred over four months throughout the nesting season 2017 in semi-natural captive conditions in Alsace, France. All study turtles showed a clear daily pattern over the 24h cycle, with four distinct phases (referred to as Night, Morning, Midday and Evening), associated with different behaviours and activity levels. Before oviposition, turtles were mostly active during Morning, and activity was positively driven by ambient temperature. Activity levels doubled during the nesting period, mostly due to the increased activity in the Evening, when nesting events occurred. Throughout the active season, basking occurrence at Midday was related to air temperature but cloud coverage was an even more important factor. Our results are a first step in predicting the seasonal time and energy budgets of the European pond turtle, and demonstrate the usefulness of animal-borne accelerometers to study free living freshwater turtles over extended periods of time.

1 Introduction

2 Human induced rapid environmental changes [1] include climate change, habitat
3 fragmentation and loss, pollution, species invasions and extinctions. These changes and
4 especially global warming are expected to have a more direct impact on ectotherms than on
5 endotherms, as their physiology acutely depends on ambient temperature [2]. Temperature
6 changes have been reported to have diverse effects on both behaviour and physiology of
7 insects, with contrasted consequences on populations, depending on the species and
8 conditions (e.g. [3-4]). Consequences for fish have been most investigated, with changes in
9 water temperature affecting heart rate, activity and thermoregulation (e.g. [5]). In this
10 context, Sauropsids (reptiles) have been less studied (but see [6-7]) and more particularly
11 marine and freshwater turtles. This latter gap in freshwater turtles may be partly due to the
12 fact that terrapins are challenging to monitor because of their cryptic ecology and their small
13 body size that limits the use of animal-borne automatic devices.

14 Estimating time-budget and energy expenditure in free living animals is a key step to
15 understand the physiology and ecology of species, their function within their ecosystem and,
16 ultimately, their capacity to adapt to the present and future environmental changes [8]. To
17 achieve these goals, animal-borne data-loggers have proven to be appropriate tools because
18 they can record biological and environmental parameters at frequencies compatible with
19 animals' behaviour. Accelerometers can provide high frequency, high resolution information
20 on individual posture, behaviour, and activity patterns of animals [8-9]. Furthermore, activity
21 metrics recorded with these devices (e.g. ODBA/PDBA) can serve as a proxy of animal energy
22 expenditure associated to specific behaviours [10-12].

23 In turtles, reproduction is energetically costly, as adult females come ashore to lay clutches of
24 numerous eggs that fill the entire maternal body cavity at maturation [13-15]. Because of such
25 volume constraints, female turtles are most likely to fast during the nesting season [16]. The
26 European pond turtle, *Emys orbicularis* (Linnaeus, 1758), is a small-sized freshwater turtle that
27 occurs throughout Europe, even reaching the Middle East [17]. It has been reported to show
28 a latitudinal gradient in its reproductive effort [18], with potential double clutches under the
29 warm Mediterranean climate [19]. Its basic behaviour and activity have been extensively
30 investigated (mainly basking, during which animals exit water to increase body temperature
31 via solar radiation [20-22], and displacements [23-24]). Yet, the fine scale daily and seasonal

32 behaviour patterns are almost unknown in the European pond turtle as in most freshwater
33 turtles.

34 Given the above-mentioned ectothermic physiology and constraints related to reproduction
35 both in terms of time and energy, the European pond turtle has been shown to adjust its
36 behaviour and time-budget according to thermal conditions. Yet the actual changes in
37 behaviour and energy expenditures throughout long periods of time, such as the entire
38 nesting season, have not been reported yet. Using accelerometers combined with sensors
39 measuring ambient temperature, pressure, and light, we investigated the fine scale behaviour
40 and associated activity patterns of adult female European pond turtles living freely in semi-
41 natural captive conditions in relation to meteorological conditions throughout the nesting
42 season in Alsace, NE France. We predicted that activity patterns will be lower during the night,
43 when compared with the day, due to diurnal fluctuations in air temperature, rainfall and/or
44 cloud coverage, yet with maximum daily activity patterns around oviposition.

45

46 **Abbreviations:** VeDBA (Vectorial Dynamic Body Acceleration), T_{AirField} (Temperature of
47 air, recorded on the field), $T_{\text{WaterSurface}}$, $T_{\text{Water20cm}}$, $T_{\text{WaterBottom}}$ (Water Temperature recorded at
48 the surface, 20 cm below the surface, and at the bottom of the pond, respectively), $T_{\text{AirMulhouse}}$
49 (Temperature of air recorded in Mulhouse airport, by Météo France), T_{WACU} , P_{WACU} , L_{WACU}
50 (Temperature, Pressure and Light recorded by dataloggers attached on the carapace of
51 turtles; these are not internal measurements), g (grams), m (meters), °C (Celsius degrees), min
52 (minutes), s (seconds), sd (standard deviation).

53

54 **Materials and methods**

55 **Study area**

56 The study was carried out in the conservatory captive breeding facility located at the research
57 station of Petite Camargue Alsacienne (St Louis, Alsace, France; 47.63°N | 7.54°E), between
58 April 13 and August 29, 2017. The facility consists of a 1200m² outdoor enclosure, which
59 includes one 250m² artificial pond (< 2m depth) connected to the underground water table
60 and surrounded by natural vegetation. It also contains an artificial mound facing South, where
61 females can crawl for egg deposition. There, 22 (15 female and 7 male) pond turtles (*Emys*
62 *orbicularis orbicularis*, captured in 2004 as adults of unknown age from a natural population

63 in La Brenne, France) are kept as part of a conservation program, aiming at reintroducing the
64 species in Alsace, NE of France [25]. Therefore, nesting activity is monitored from May to July
65 by daily observations (from 6pm to 11pm) for recording individuals laying (or attempting to
66 lay) eggs [26].

67

68 **Subjects and tools**

69 This study focused on eight adult females European pond turtles (body mass: 749 ± 171 g
70 [mean \pm standard deviation], carapace length 155 ± 14 mm) equipped with dataloggers
71 (hereafter referred as WACU) developed at IPHC (<https://iphc.cnrs.fr>). WACUs are
72 miniaturized autonomous recorders ($21 \times 13 \times 4$ mm, 7 g including batteries and potting)
73 measuring temperature (T_{WACU}), pressure (P_{WACU}) and light (L_{WACU}) at 1 Hz and 3D acceleration
74 at 10 Hz over 4 to 6 months (see general specifications for WACU on [http://iphc.cnrs.fr/-MIBE-](http://iphc.cnrs.fr/-MIBE-.html)
75 [.html](http://iphc.cnrs.fr/-MIBE-.html)). The mass ratio between dataloggers and turtles (<1%) suggests that such deployments
76 cause limited, if any, disturbance to the turtles.

77 WACUs were fixed directly on the top of the turtle carapace using fast-setting epoxy (Araldite)
78 after epibiontes (organisms living on the carapace) and dead tissues were removed by
79 successive and alternating applications of acetone and sand paper (sand grain 80). The entire
80 attachment procedure lasted between 10 and 20 minutes depending on glue setting. Turtles
81 were released ~ 1 hour after WACU attachment. Before attachment to a turtle and after
82 retrieval, each WACU was rotated over all angles at exact GPS times, used as time stamps, to
83 assess and correct the potential drift of their internal Real Time Clock (RTC) throughout the
84 duration of deployment. All times are given as UTC (Universal Time Coordinated), while local
85 time was UTC+2.

86 In addition to data recorded by the WACUs, air and water temperatures were recorded at the
87 study site using Tinytags data-loggers (Gemini Data Loggers, Chichester, UK,
88 <https://www.gemini-dataloggers.com/fr/>). These loggers recorded temperature every 10
89 minutes: (1) in the air ($T_{AirField}$), (2) at three different depths within the water column of the
90 pond, using three devices that were suspended at a vertical rope: (a) at the surface
91 ($T_{WaterSurface}$), (b) at 20 cm depth ($T_{Water20cm}$), and (c) at the bottom of the pond ($T_{WaterBottom}$).
92 Finally, meteorological records from the weather station of the Bale-Mulhouse Airport
93 (Météo-France station 07299, GPS position: $47.61^\circ N$ | $7.51^\circ E$, 263m above sea level, 3km

94 West from the field site) were obtained from the national weather service
95 (<https://www.meteofrance.fr/>): this concerned (3) air temperature ($T_{\text{AirMulhouse}}$), cloud cover,
96 and rain fall, recorded every 3 hours. There was a significant positive correlation between air
97 temperature at the study site and at the airport ($T_{\text{AirField}} [^{\circ}\text{C}] = 0.053[\pm 0.135] + 1.014[\pm 0.007]$
98 $\times T_{\text{AirMulhouse}} [^{\circ}\text{C}]$, $R^2 = 0.865$, $p < 10^{-12}$, $n = 3324$ hourly records). Sunrise and sunset times for the
99 nearby city of Mulhouse (Alsace, NE France) were downloaded from
100 <https://www.timeanddate.com>.

101

102 Animal handling was approved by the French Ministry for National Education, Higher
103 Education and Research and by the Ethical Committee for Animal Experimentation
104 (CREMEAS, CEEA 35, Strasbourg, APAFIS#649-201505121120811_v1).

105

106 **Data handling**

107 Data were handled, pre-treated, visualized and analysed using Matlab (version 8.6.0.267246
108 [R2015b] August 20, 2015). Real Time Clock (RTC) drift was 21.30 ± 2.72 min over 141 days of
109 functioning, range [18.40 – 27.10 min, $n = 8$ WACUs]. Recorded pressure (P_{WACU}) was linearly
110 transformed to indicate the depth under water surface (i.e. 0 at the surface, 0.1 bar at 1m
111 depth). The 3-axis acceleration signal was decomposed using a low-pass filter (filtfilt Matlab
112 function) following [8]. We used a 2 second window for the low pass filter to minimize variance
113 of the vectorial sum of the static components. For each axis, the low-pass filter extracted the
114 static (due to gravity) and dynamic (due to a change in velocity) components of the
115 acceleration signal [8]. The Vectorial Dynamic Body Acceleration (VeDBA, in $\text{m}\cdot\text{s}^{-2}$) was
116 calculated as the vectorial sum of the absolute values of the dynamic component of the three
117 axes: in other words, VeDBA is the total acceleration due to changes in animal velocity. When
118 VeDBA is integrated over a certain time window, the result (in $\text{m}\cdot\text{s}^{-1}$) can be used as a proxy of
119 the animal's locomotor activity. Angles of the anterior-posterior (pitch) and left-right (roll)
120 axes in relation to the horizontal axis were computed from the static component of
121 acceleration.

122

123 **Identification and analysis of daily patterns**

124 As a first step, T_{WACU} , P_{WACU} and L_{WACU} were used to investigate potential changes in associated
125 patterns across the 24-hour cycle. This permitted to divide a 24h cycle in four phases (Fig 1):
126 (1) “Night” was characterized by low light values ($\text{Median}[L_{WACU}] < 1000$ Lux calculated over a
127 1 min window) and highly consistent temperature and pressure readings ($\text{sd}[T_{WACU}] < 1$ &
128 $\text{sd}[P_{WACU}] < 5e^{-3}$ computed over a 1 min window). During Night, “Breathing” bouts could be
129 identified by sudden changes in P_{WACU} . Pressure spikes within Breathing bouts were defined
130 as “Inhalation” events (Fig 1B).
131 (2) “Midday” was characterized by high light values ($\text{Mean}[L_{WACU}] > 20000$ Lux over a 1 min
132 window) and increased temperature values, i.e. several degrees higher than the lowest
133 temperature of the 24-h-period. During Midday, “Basking” events were defined as periods
134 where T_{WACU} continuously increased over time. Sudden drops in T_{WACU} or sudden increases in
135 pressure were interpreted as “Diving” events.
136 (3) “Morning” was defined as the period between Night and Midday.
137 (4) “Evening” was defined as the period between Midday and Night.

138

139 **Fig 1. Example of a 24-h record collected by a WACU deployed on an adult female European**
140 **pond turtle *Emys orbicularis* (Individual 8, on May 31, 2017, UTC time) at Petite Camargue**
141 **Alsacienne, Alsace, France, from which daily schedule could be estimated as Morning,**
142 **Midday, Evening and Night bouts.** From top to bottom: (A) Temperature: $T_{\text{WaterSurface}}$ in pink
143 and T_{WACU} in black for Morning and Evening, in red for Midday including basking, in blue for
144 Night and in green for breath bouts during Night. (B) Pressure (reversed vertical axis) showing
145 a breath bout in details, with blue circles corresponding to inhalation events. (C) Ambient Light
146 (see T_{WACU} for colours). (D) Angles (in degrees) extracted from static components of
147 acceleration from which egg-laying events can be inferred. (E) $V_e\text{DBA}$ from dynamic
148 component of acceleration computed on a 1 min window.

149

150 To detect egg-laying events, we constructed an algorithm that could detect all laying events
151 visually observed in the field. This algorithm was based on the angles extracted from the static
152 component of acceleration. Egg-laying events were characterized by $\text{variance}[\text{roll}] < 50$ and
153 $\text{variance}[\text{pitch}] > 120$ or $\text{variance}[\text{roll}] < 10$ and $\text{variance}[\text{pitch}] > 40$ computed over a 2 min
154 window that fitted with the movements of rear legs of turtles digging their nest.

155 Apart from Night phase, P_{WACU} could not be used in all cases for depth assessment because of
156 erratic pressure signal during active behaviours occurring the rest of the day (see Discussion
157 below). To assess the depth of the animals in the water column (above, at the surface, at 20cm
158 or more below the surface), we thus used correlations of T_{WACU} with all four temperatures
159 recorded in the field with Tinytags. We reasoned that the best correlation between T_{WACU} and
160 the temperature of the surrounding environment (Intercept ≈ 0 , slope ≈ 1 , $R^2 \approx 1$) would
161 indicate at best the vertical position (in air or water) of the turtle (Fig 1A).

162 Statistical models were constructed in R (R version 3.2.4 [2016-03-10]), using the packages
163 *nlme* for linear mixed models, and *lme4* for GLMMs (Generalized Linear Mixed Models, [27-
164 28]). To model the probability of basking behaviour (i.e. during Midday), we ran a GLMM with
165 a binomial distribution of Midday phases as variable. Fixed factors were environmental
166 parameters (temperature, rain fall, cloud coverage, daylength at Mulhouse weather station)
167 that were preliminarily centred and reduced (mean = 0 and variance = 1). Turtle ID was used
168 as a random variable to deal with pseudo replication in the dataset [29-31]. Using a stepwise
169 backward analysis based on AIC, we selected the most parsimonious model. The slope was
170 considered as a proxy for the weight of the effect.

171

172 **Results**

173 All eight WACUs provided complete records during their deployment, leading to 137 days of
174 data for each individual, i.e. 1096 complete days in total.

175

176 **Daily activity patterns**

177 (1) Over the entire records, Night phases lasted on average 9.3 ± 3.4 hours. Each Night phase
178 contained on average 23.2 ± 14.3 Breathing bouts. One single Breathing bout lasted on
179 average 162 ± 103 s and consisted of 9.6 ± 7.2 inhalation events (i.e. 222.0 ± 158.9 inhalation
180 events per Night) (Table 1). Time elapsed between two successive Breathing bouts was $22.5 \pm$
181 76.9 min (90%-quantile = 40.5 min, $n = 25550$).

182 We manually checked three series of 24 continuous hours for four individuals (i.e. 12 complete
183 24-h cycles), chosen at random, to estimate the efficacy of our algorithm. In total, 262
184 Breathing bouts were correctly detected by the algorithm, 38 were false positives (12.7%) and
185 31 were false negatives (10.6%). 2795 Inhalation events were correctly detected by the

186 algorithm, 484 were false positives (14.8%) and 425 were false negatives (13.2%). For both
187 events, the number of false positives was close to number of false negatives. Therefore, we
188 are confident that our algorithm estimated the actual number of Breathing bouts fairly
189 accurately. Yet, the time required to manually analyse these 12 random 24-h cycles was
190 20,000 times greater than that needed when using the algorithm.

191 Mean P_{WACU} [during Night phase] was 0.037 ± 0.016 bar, giving a mean depth at the top of the
192 carapace of 37 ± 16 cm below the surface at night. Yet the temperature on contact with turtles
193 (T_{WACU}) during Night was best correlated with water temperature at the surface ($T_{WaterSurface}$ [during
194 Night bouts] = $0.53[\pm 10^{-3}] + 0.99[\pm < 10^{-3}] \times T_{WaterSurface}$ [during Night bouts], $R^2 = 0.97$, $P < 10^{-12}$,
195 $n = 37e^6$), suggesting that turtles spent their night submerged close to the surface (see Fig 1A
196 for example). This is confirmed by the calculation of the mean difference between the
197 pressure recorded just before a breathing event, and the minimum pressure during a
198 breathing event, that was 0.0044 ± 0.003 bar, indicating that at night, turtles rose on average
199 4.4 ± 3.1 cm out of the water when breathing.

200 (2) Midday phases did not occur every time: over the 1096 recorded 24h-cycles (one for each
201 day and each turtle), 142 showed no Midday phase (Table 1). 78 of these events were common
202 to at least seven of the eight turtles and concerned 10 given days. GLMM analysis showed
203 that the probability of Midday phase to occur was best explained by cloud coverage (slope = -
204 $2.52[\pm 0.28]$, z-test = -8.9, $p < 2e-16$), before DayLength (slope = $0.75[\pm 0.12]$, z-test = 6.1, $p =$
205 $1.e-9$) and rain fall (slope = $-0.35[\pm 0.08]$, z-test = -4.3, $p = 1.e-5$).

206 (3) Morning phases were characterized by a specific moment of the day, when T_{WACU} was close
207 to $T_{WaterSurface}$ ($R^2 = 0.925$), yet with highly variable Pressure readings (unlike Nights) (Fig 1).
208 This indicates that turtles were spending their Morning submerged close to the surface, while
209 being relatively active (as confirmed by the acceleration data, see below, Table 1).

210 (4) Evening phases were associated with slow linear decrease in T_{WACU} , yet, with variable P_{WACU}
211 (Table 1).

212

213 **Table 1: Summary of individual biometrics, nesting event characteristics, daily bout**
214 **durations and associated VeDBA in 8 adult female European pond turtles, *Emys orbicularis*,**
215 **monitored at Petite Camargue Alsacienne, Alsace, France, from 14 April to 28 August, 2017.**
216 **Values are means \pm sd.; n represents the number of events for each variable.**

217

218 **Changes in activity levels throughout the nesting period**

219 VeDBA was used to assess activity levels and showed three main periods relative to the egg-
220 laying date. Daily VeDBA showed a large increase around the egg-laying date (Fig 2A), mostly
221 due to increased Evening activity (Fig 2D), when egg-laying occurred. Before the egg-laying
222 date, daily VeDBA was best explained by VeDBA computed during Morning phase ($R^2 = 0.61$,
223 $p < 2e-16$) compared to the other phases. More precisely, daily VeDBA was best correlated with
224 hourly VeDBA computed within the [4am-10am, UTC] window of the day ($R^2 = 0.76$, $p < 2e-16$).
225 Before the egg-laying date, daily VeDBA was also positively correlated to T_{AirField} ($R^2 = 0.33$, p
226 $< 2e-16$) (Fig 2A), whereas this relation was weaker afterwards ($R^2 = 0.06$, $p = 1e-10$). VeDBA
227 during Morning was also positively correlated to $T_{\text{WaterSurface}}$ before the egg-laying date ($R^2 =$
228 0.35 , $p < 2e-16$), but the relationship was weak for the rest of the season ($R^2 = 0.087$, $p = 5e-$
229 16). Hourly VeDBA was clearly influenced by air temperature through a non-linear pattern:
230 maximal hourly VeDBA increased with air temperature until around 25°C but then decreased
231 at higher air temperatures.

232

233 **Fig 2. Time-budget of 8 adult females European pond turtle *Emys orbicularis* at Petite**
234 **Camargue Alsacienne, Alsace, France from 14 April to 29 August, 2017.** For each day and
235 each individual, the sum of VeDBA computed over 24h is represented with a single circle. On
236 all graphs, black asterisks on the x-axis represent egg-laying events detected by accelerometry
237 signal. (A) Thick and thin lines are respectively the daily mean \pm standard deviation computed
238 for all 8 individuals. The red line is a linear transformation of air temperature recorded on the
239 field (T_{AirField}). Parameters were estimated by plotting T_{AirField} against daily VeDBA over
240 the period preceding egg-laying date. Note that this linear transformation fits well with mean
241 VeDBA over this period (left from the vertical dotted line), but not after egg-laying date. (B)
242 Hours of start and end of each daily bout, with means shown as solid lines. Hours of start of
243 Morning, Midday, Evening and Night bouts are respectively in green, red, cyan and blue. (C)
244 Duration of each bout, with black solid line for the daily mean. (D) Hourly VeDBA for each
245 bout, with black solid line for the daily mean. At the far-right bottom graph, note the huge
246 increase in hourly VeDBA during the evening bout when egg-laying occurred.

247

248 **Identification of nesting events**

249 Nocturnal field patrolling (from 6pm to 11 pm from 15 May to 5 July, 2017) permitted to
250 observe all but one (Turtle#5) of the eight turtles crawling on the mount for nesting. During
251 digging nest, before a turtle deposits its eggs, the angles extracted from the static component
252 of acceleration showed a very specific pattern, associated with regular, repetitive leg-
253 movements (Fig 1D). This acceleration signal was sufficiently stereotypical to allow detection
254 of nest digging by a computer algorithm (see Methods). Our customised algorithm detected
255 all nesting observed in the field, but also digging that did not led to successful oviposition
256 (nesting attempts), plus three additional nesting events and attempts for the Turtle#5 that
257 had not been directly observed nesting in the field. All acceleration-derived nest digging and
258 nesting events occurred between 29 May and 25 June, 2017, during the Evening. Only Turtle#5
259 that was not observed during nocturnal patrols nested during the Morning. On average, nest
260 digging lasted 73 ± 38 min and was associated with a mean VeDBA of $0.31 \pm 0.06 \text{ m}\cdot\text{s}^{-2}$ (Table
261 1). As a comparison, mean VeDBA computed over the entire dataset was $0.16 \pm 0.12 \text{ m}\cdot\text{s}^{-2}$, i.e.
262 half that observed during nest digging. Accordingly, nesting (walking, nest digging, laying eggs,
263 nest covering) appears as a highly energy-consuming activity.

264

265 **Discussion**

266 This study shows that 3-axis accelerometry associated with simple temperature, pressure,
267 light sensors provide unrevealed fine-scale behaviours and time-budget in free living
268 freshwater turtles throughout the nesting season. We show that in Alsace, NE of France, adult
269 females behave according to four distinguishable phases over the circadian cycle, referred as
270 Night, Morning, Midday, and Evening. Each of the four phases were associated with particular
271 behaviours and activity levels. Additionally, the high resolution and high frequency data
272 collected with these devices permitted to assess breathing events, timing of nesting events
273 and energetic proxies (VeDBA) derived from acceleration. Importantly, we show that the
274 nesting events appear to be a turning point in terms of individual behaviour, energy
275 expenditure, and environmental drivers: before nesting occurs, the daily activity is highly
276 related to ambient temperature, whereas it shows more stochastic patterns and changes
277 afterward (Figs 2A and 2D).

278

279 **Automatic detection of daily patterns**

280 The algorithm we developed to identify daily patterns has proven a powerful, fast and
281 customisable tool. First, it allowed to work on extensive datasets (one 3-axis accelerometer
282 operating at 10Hz over 140 days provides 130 million lines of [Time,X,Y,Z], i.e. ~4.5 GB of data),
283 without subjectivity in pattern cutting. Second, despite automatic detection based on
284 threshold values that may introduce biases (e.g. a point just above a threshold value is
285 automatically removed whereas a visual/manual treatment may have led to take it into
286 account), visual inspection of 12 randomly chosen days revealed that the algorithm is highly
287 robust and consistent in terms of number of events.

288

289 **Foraging occurs in the morning**

290 In the morning, turtles experienced temperatures (T_{WACU}) closest to $T_{WaterSurface}$ (Fig 1A),
291 indicating that turtles mostly remained close to the surface, as confirmed by direct field
292 observations. The subsurface layer has been proposed as the preferred environment for
293 foraging in pond turtles [32-33]. However, actual behaviour of WACU-equipped individuals
294 monitored in our study was hard to deduce from recorded data only. There was neither an
295 informative signal in the temperature records, nor a clear pattern in pressure and angle data.
296 Yet, the pressure signal was very erratic, suggesting that turtles repeatedly banged into
297 obstacles with the logger attached to the carapace. Such erratic pressure signal constrained
298 us to use water temperature as a proxy of the water depth where turtles actually behaved.
299 We suggest that erratic pressure signals could be associated with motivated intrusions of
300 turtles in reeds where they may actively seek for food. Putative foraging during Morning is
301 supported by high VeDBA but most importantly by the significant effect of ambient
302 temperature between 4am and 10am on daily VeDBA: the warmest the water, the most active
303 turtles were foraging. Previous studies reported feeding mostly occurs in morning in other
304 Emydidae [34]. At the opposite, it is very unlikely that feeding occurs during Night because
305 VeDBA was minimal at that time of the day and light limited for turtles visually foraging. In
306 short, it is most likely that feeding occurred early in the day, before basking, with the latter
307 facilitating digestion during warmer periods in the middle of the day. In the future, underwater
308 or animal-borne cameras should be used to test our hypothesis that Morning is the most
309 prone phase of the day for foraging in pond turtles.

310

311 **Nesting is a nightly energy-consuming activity**

312 25 of the 26 detected nesting events and attempts occurred between May 29 and June 11,
313 2017, and all but two occurred during the Evening. These nesting dates and their timing are in
314 line with the literature for France where 76% (n=21) of *Emys* turtles nest after sunset, with
315 the remaining nesting in the morning [24]. *Emys* turtles have been reported to nest between
316 the end of May and the end of June in France [35] and in Turkey [36], and throughout June in
317 Italy [37]. Interestingly, one of our eight individuals (ID#5, Table 1) laid two successive clutches
318 22 days apart. Double (and even triple) clutches have been reported in *Emys orbicularis* [19]
319 but is thought to be quite rare in France [35]. Temperature but also sunshine duration have
320 been reported to be important factors influencing nesting: in Slovakia first nesting attempts
321 occur after a 13-day period when temperature is 17.7°C [38]. We found similar values, with
322 mean T_{AirField} being 18.6°C during the same 13-day period.

323 Our results show that in our captive facility where the nesting mount was only a few meters
324 from the pond, nesting events and attempts are highly energy-consuming activities, about
325 twice more expensive in terms of VeDBA than any other activity recorded in the present study
326 (Table 1). Importantly, all eight studied individuals made 3.25 ± 1.75 attempts before they
327 successfully laid eggs (eventually laying twice, as reported for ID#5), increasing by three times
328 the actual energy and time devoted to nesting. In natural conditions where *Emys* turtles can
329 travel 150 to 1000 m to their nesting ground [39], actual energetic expenses are most likely to
330 be even more extended. Deployment of accelerometers on free ranging turtles are required
331 to estimate such costs in the wild.

332 In addition, nesting appears as a turning point in the season. Before it occurs, turtles are
333 mainly active in the morning (Fig 2D), probably foraging. The level of activity is then closely
334 linked to the ambient temperature (Fig 2A). During the nesting period, daily activity doubles
335 (Fig 2A), due to the intense nest digging activity happening in the evening (Fig 2D). After then,
336 turtles are more active during the evening and the midday time (Fig 2D), and activity is no
337 more linearly linked to ambient temperature (Fig 2A).

338

339 **Temperature has a dual effect on the level of activity**

340 We noticed an interesting non-linear effect of ambient temperature on the hourly activity
341 level (VeDBA), with a turning point around 25°C. VeDBA having a Gamma-like distribution, it

342 is convenient to focus on maximal VeDBA values: for temperatures between 7 and 25°C,
343 maximal VeDBA increased with temperature experienced by the turtles, whereas maximal
344 VeDBA decreased when temperature exceeded 25°C (until the maximal air temperature
345 recorded in the study, i.e. 47°C). These results suggest that for temperatures lower than 25°C,
346 European pond turtle activity is positively related to temperature. Above this temperature
347 threshold, which typically occurs during the early afternoon, individuals are most likely to bask
348 or rest, leading to lower activity: the warmer the air, the more turtles lay immobile emerged.
349 Turtles were least active during the Night. However, we did not detect any bimodal activity
350 patterns during daytime (as described in [40]).
351 Air temperature and sunlight have been previously reported to drive basking behaviour in
352 freshwater turtles [24, 41]. Our study showed that in Alsace, NE France, cloud coverage rather
353 than temperature most strongly affected the basking behaviour of turtles during Midday. For
354 instance, a cloud coverage > 90% dramatically reduced the number of basking events, if they
355 occurred at all. We propose that when the sun is not visible, individuals rather stay
356 submerged, since basking would be ineffective, unnecessarily elevating potential predation
357 risks. Recording the level of solar radiation experienced by the turtles directly might even
358 better explain observed basking behaviour, as clouds are not uniformly obstructing the sun.

359

360 **Conclusion**

361 Accelerometers deployed on gravid *Emys* turtles permit to assess fine scale behaviour, time
362 budget and energetic expenditure, and most interestingly to quantify nesting events, which
363 are likely underestimated in the field, because they mostly occur during the night. The nesting
364 period appears to be the most intense activity in terms of energy expenditure that needs to
365 be estimated in natural conditions. Nesting also happens as a turning point for seasonal
366 activity patterns: before nesting, daily activity is mainly due to foraging-like, temperature-
367 driven activity in the Morning, whereas after nesting, individual activity is more complex. In
368 the near future, video-assisted monitoring of turtle behaviour and calibration of actual energy
369 expenditure with accelerometry-derived VeDBA, coupled with spatial telemetry should permit
370 the extension of time budgets to quantitative energy budgets.

371 Our study may have some limitations, mainly due to the fact that all individuals inhabited one
372 single, relatively small pond, where all individuals encountered the same environmental

373 constraints. One may, thus, stress that each individual's behaviour may have impacted that of
374 the rest of the group. For instance, some turtles (*Pseudemys nelsoni*) have been reported to
375 present social learning by observing conspecifics [42]. In the future, social structure should be
376 investigated for assessing such potential inter-individual interactions.

377

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385

386 **Data availability**

387 After publication data will be archived in Dryad repository.

388

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502

503 **Table 2 : Summary of individual biometrics, nesting event characteristics, daily bout durations and associated VeDBA in 8 adult female**
 504 **European pond turtles, *Emys orbicularis*, monitored at Petite Camargue Alsacienne, Alsace, France, from 14 April to 28 August, 2017. Values**
 505 **are means \pm sd.; n represents the number of events for each variable.**

Turtle ID #		1	2	3	4	5	6	7	8	Total
Body mass (g)		549	551	670	730	726	790	962	1015	749\pm171
Carapace length (mm)		135	138	150	155	161	155	169	177	155\pm14
Nb of nesting attempts/events detected by accelerometry		6	5	1	4	3	3	1	3	3.25\pm1.75 (n=26)
Dates of nesting *detected by accelerometry		08 June	11 June	31 May	02 June	3 June* and 25 June*	02 June	02 June	31 May	[31 May-25 June]
Egg-laying events	Mean Duration (h)	1.72	1.11	1.00	0.98	1.41	0.70	1.00	1.20	1.22\pm0.63
	Mean Hourly VeDBA (m/s)	980.7	1098.3	914.2	1203.9	1251.5	1473.6	1118.2	833.7	1111.7\pm224.6
Morning	Duration (h)	6.14 \pm 3.52	6.63 \pm 4.35	5.80 \pm 3.96	7.41 \pm 4.06	6.30 \pm 4.02	6.94 \pm 4.46	6.59 \pm 4.17	6.08 \pm 3.34	6.49\pm4.02
	Hourly VeDBA (m/s)	607.1 \pm 142.0	645.8 \pm 143.7	698.3 \pm 135.1	635.4 \pm 155.1	669.3 \pm 138.8	600.4 \pm 122.6	577.3 \pm 99.5	555.7 \pm 87.4	623.8\pm137.0
	n	136	137	137	136	136	137	135	136	1090
Midday	Duration (h)	4.99 \pm 2.42	5.33 \pm 2.14	5.83 \pm 2.57	5.17 \pm 2.41	5.74 \pm 2.58	4.34 \pm 2.61	5.14 \pm 2.58	4.33 \pm 2.56	5.11\pm2.53
	Hourly VeDBA (m/s)	535.5 \pm 100.5	577.9 \pm 135.4	549.4 \pm 105.1	562.7 \pm 140.7	562.4 \pm 127.1	527.6 \pm 100.4	509.5 \pm 84.3	526.0 \pm 85.3	543.6\pm113.1

	n	117	118	121	114	119	115	125	120	949
Evening	Duration (h)	4.07±2.22	3.91±1.67	3.96±2.05	4.35±1.71	4.10±2.16	4.69±2.35	5.57±3.52	4.01±2.03	4.34±2.34
	Hourly VeDBA (m/s)	578.0±168.7	635.3±176.2	595.2±135.0	603.1±149.2	591.7±141.4	547.9±133.3	541.6±106.2	527.5±113.6	577.2±145.4
	n	117	118	121	114	119	115	124	120	948
Night	Duration (h)	10.09±3.47	9.33±3.15	9.49±3.32	8.66±2.96	9.14±3.47	9.47±3.52	7.77±3.42	10.58±3.4	9.32±3.43
	Hourly VeDBA (m/s)	417.6±6.3	412.1±8.6	444.7±8.7	408.8±19.4	412.0±9.1	417.3±7.1	430.6±11.5	430.0±7.1	421.6±15.6
	n	138	138	138	138	138	137	137	138	1102
Basking	Duration (h)	1.63±0.86	1.78±0.89	1.83±0.89	1.74±0.96	1.73±0.88	1.50±1.04	1.63±0.89	1.53±0.97	1.67±0.93
	n	117	118	121	114	119	115	125	120	949

506