

1 Title: Seasonal weight changes in laboratory ferrets

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20 Abstract

21 Ferrets (*Mustela putorius furo*) are a valuable animal model used in biomedical research.
22 Ferrets undergo significant variation in body weight seasonally, affected by photoperiod,
23 and these variations make it difficult to use weight as an indicator of health status. To
24 overcome this requires a better understanding of these seasonal weight changes. We
25 provide a normative weight data set for the female ferret accounting for seasonal
26 changes, and also investigate the effect of fluid regulation on weight change. Female
27 ferrets (n=39) underwent behavioural testing from May 2017 to August 2019 and were
28 weighed daily while housed in an animal care facility with controlled light exposure. In the
29 winter (October to March), animals experienced 10 hours of light and 14 hours of dark,
30 while in summer (March to October), this contingency was reversed. Individual animals
31 varied in their body weight from approximately 700 to 1200 g. However, weights fluctuated
32 with light cycle, with animals losing weight in summer, and gaining weight in winter such
33 that they fluctuated between approximately 80% and 120% of their long term average
34 weight. Ferrets were weighed as part of their health assessment while experiencing water
35 regulation for behavioural training. Water regulation superimposed additional weight
36 changes on these seasonal fluctuations, with weight loss during the 5 day water
37 regulation period being greater in summer than winter. These data establish a normative
38 benchmark for seasonal weight variation in female ferrets that can be incorporated into
39 the health assessment of an animal's condition.

40 Introduction

41 Domesticated ferrets (*Mustela putorius furo*) are valuable animal models for a wide range
42 of biomedical research areas, including: neuroscience [1–6], drug development [7] and
43 respiratory diseases such as Influenza and Severe Acute Respiratory Syndrome (SARS)
44 [8,9] including the new coronavirus strain, SARS-CoV-2 [10]. In laboratory animals
45 exposed to scientific procedures, a standard approach to monitoring health status is to
46 measure body weight. Weight loss is a key indicator of health problems, and therefore
47 understanding the factors that contribute to natural variation in body weight is critical for
48 correctly monitoring an animal's condition. Ferrets undergo significant variation in their
49 body weight seasonally; however, there is currently no normative data available to provide
50 a benchmark for the expected seasonal weight changes. Seasonal variations may mask
51 or exaggerate changes in body weight due to an experimental procedure or change in
52 health status and thus must be integrated into assessments of a ferret's health status.

53 Seasonal weight changes have been demonstrated in multiple species
54 independent of diurnality, including monkeys [11,12], raccoons [13], hamsters [14] and
55 rodents [15]. There are a range of potential factors that elicit seasonal weight changes,
56 but temperature and day length are key triggers, which are ultimately crucial for survival.

57 Ferrets are members of the mustelid family and have been domesticated from
58 European polecats, a species which was native to western Euroasia. Seasonal weight
59 changes have been observed in polecats and other closely related species such as mink.
60 These weight changes are seen as adaptations to the differing energy intake and
61 expenditure requirements of winter and summer [16,17]. In animal care facilities, daylight

62 hours can be easily regulated and are often set at a 12-hour light cycle (12-hours ON, 12-
63 hours OFF) or synchronised with the external environment; for example, varying from a
64 minimum 8-hour cycle in winter (8-hours ON, 16-hours OFF) to maximum 16-hour cycle
65 in summer (16-hours ON, 8-hours OFF) [18–21]. Variation in the photoperiod can change
66 factors such as eating habits, coat thickness, sleep and activity levels - all of which may
67 contribute to normal and possible abnormal weight changes. Previous research has
68 demonstrated that ferret weights increase as hours of daylight decrease, leading to
69 sinusoidal weight fluctuations with annual light cycle [22,23]. Contrastingly, in another
70 study where the sleep habits of two male ferrets were tracked, light/dark schedule was
71 shown to have no effect on their weight [24].

72 In addition to body weight, changes in photoperiod have also been linked to the
73 timing of the oestrus cycle, which occurs once per year in female ferrets [20,22,25,26].
74 One of the first studies showed that sexual activity in ferrets increased when light duration
75 or intensity increased [27]. Since then, further research has described ferret oestrus as
76 seasonal and photoperiod activated [28]. The relationship between photoperiod, oestrus
77 and body weight is unknown, but Donovan (1986) concluded that while there was not a
78 critical weight to trigger oestrus, oestrus does require a minimum weight of around 420g.

79 The aim of this study is to provide data on the normative weights of female ferrets,
80 accounting for seasonal changes over multiple years. In addition, we document changes
81 in weight that occur due to water regulation. We hypothesized that controlled light
82 exposure in animal care facilities would induce naturalistic fluctuations in the ferrets' body
83 weight.

84 **Methods**

85 **Ethics Statement**

86 All the animals in this study were maintained for the purpose of investigating the neural
87 basis of hearing, undergoing experimental procedures that were approved by local ethical
88 review committees (Animal Welfare and Ethical Review Board) at University College
89 London and The Royal Veterinary College, University of London and performed under
90 license from the UK Home Office (Project License 70/8987) and in accordance with the
91 Animals (Scientific Procedures) Act 1986.

92 **Animals**

93 The data from 39 healthy female pigmented ferrets (0.5 – 4 years) were used for this
94 study. All animals underwent behavioural testing in psychoacoustic tasks that required
95 regulated access to water. Water was available during twice-daily testing sessions, with
96 supplementary wet food and/or water provided to ensure animals received a minimum of
97 60 ml/kg of water. Testing took place from Monday to Friday in, roughly, a three-weeks
98 on and one week off schedule. This ensured that ferrets did not experience water
99 regulation more than 50% of the time. When not participating in behavioural testing,
100 animals had free access to water. During testing periods, each animal was weighed daily
101 using digital scales (Salter, UK) prior to their morning testing session. Data was obtained
102 from all available animals between May 2017 and August 2019 (months of participation,
103 mean \pm SD: 11 months \pm 4.1).

104 Animals were housed at 15-24°C in social groups (n = 2 to 8 ferrets) and had free
105 access to high-protein food pellets. Animals lived in enriched cages and freely exercised
106 during daily cage cleans, with the opportunity to interact with humans, other ferrets and a
107 variety of enrichment facilities (e.g. tunnels and balls). Our colony was comprised
108 exclusively of female ferrets and typically contained between 25 and 30 animals.

109 The light cycle was changed in accordance with UK daylight savings: during
110 ‘winter’ (October to March) ferrets were exposed to 10 hours of light and 14 hours of dark;
111 during ‘summer’ (March to October) this was reversed to 14 hours of light and 10 hours
112 of dark. The animal facility in which the animals were housed was windowless, and thus
113 animals did not have access to natural light. The transition between ‘seasons’ was
114 staggered such that timings were changed one hour per week over 4 weeks, centred on
115 clock change for UK daylight saving time (Figure 1A).

116 The age of each ferret was calculated from the approximate date of birth provided
117 by the supplier (Highgate Farms, UK). Also available for each animal was the oestrus
118 time, which was estimated from the record of each animal’s yearly hormone injection
119 (0.5ml s.c. Proligestone, Delvosteron, Intervet). Hormone injections were given within 24-
120 72 hours of animals exhibiting visible signs of oestrus, in order to suppress oestrus until
121 the following spring and thus prevent life-threatening anaemia experienced by females in
122 sustained oestrus [29].

123 **Data Analysis**

124 Data was recorded and analysed in Matlab (version R2018a, MathWorks Inc, MA, USA)
125 using custom written scripts. Weight measurements were either examined in absolute

126 terms or relative to each animal's long-term average (calculated from all available data).
127 To examine day-light triggered weight changes, data from summer and winter were
128 considered independently in terms of weeks from the transition to shorter/longer days.
129 ANOVAs were performed in SPSS (IBM) using Greenhouse-Geisser corrections for
130 violations of sphericity where appropriate. Generalised linear models (GLMs) were
131 performed using MATLAB's 'fitglm' functions using a step-wise approach to fit models of
132 increasing complexity. Model parameters were retained where an F-test indicated a
133 significant drop in deviance upon inclusion of the term.

134 Results

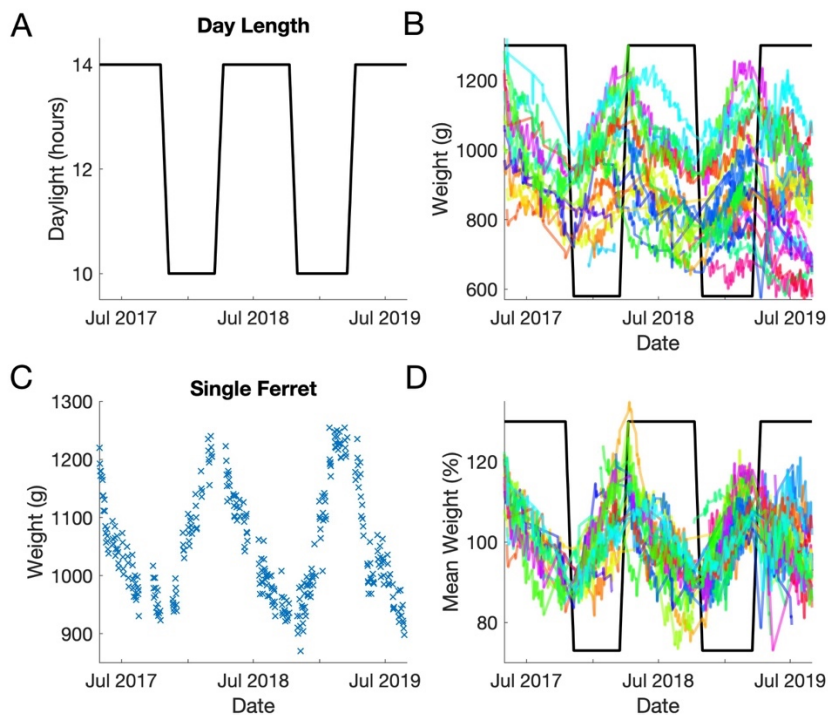
135 The weights of 39 female ferrets were recorded as part of their daily health monitoring.
136 Weight values ranged from 553g-1350g. There was considerable variation across
137 animals, with average weights spanning 693 to 1195g, with a population mean (\pm SD) of
138 864.8 (\pm 119.0g). There was substantial weight variation within each animal. For example,
139 the animal shown in Fig. 1C weighed 1240g on 5th March 2018, and 870g on the 9th of
140 November that year, a change of 370g over nearly 7 months. The average standard
141 deviation across all measurements was 64.9 (\pm 30.3g), or equivalently, 7.45% \pm 3.25% of
142 each animals' mean weight. We next explored how variation in body weight was linked to
143 seasonal changes and fluid regulation during behavioural testing.

144 Female ferrets show significant seasonal weight variations

145 When weights are considered over time, cycles emerge that correlate with the seasonal
146 light changes (Fig. 1A). The pattern of weight change for one ferret over the collection

147 period is shown in Figure 1B. All ferrets conformed to a similar seasonal pattern of weight
148 change with weight greatest in April (when lights were altered to their summer day length)
149 and lowest in October (when the light cycle was switched to winter day lengths, Fig. 1C).
150 The observed decreases in weight during the summer period and increases in weight
151 over the winter months, resulted in sinusoidal weight fluctuations over the two-year
152 measurement period.

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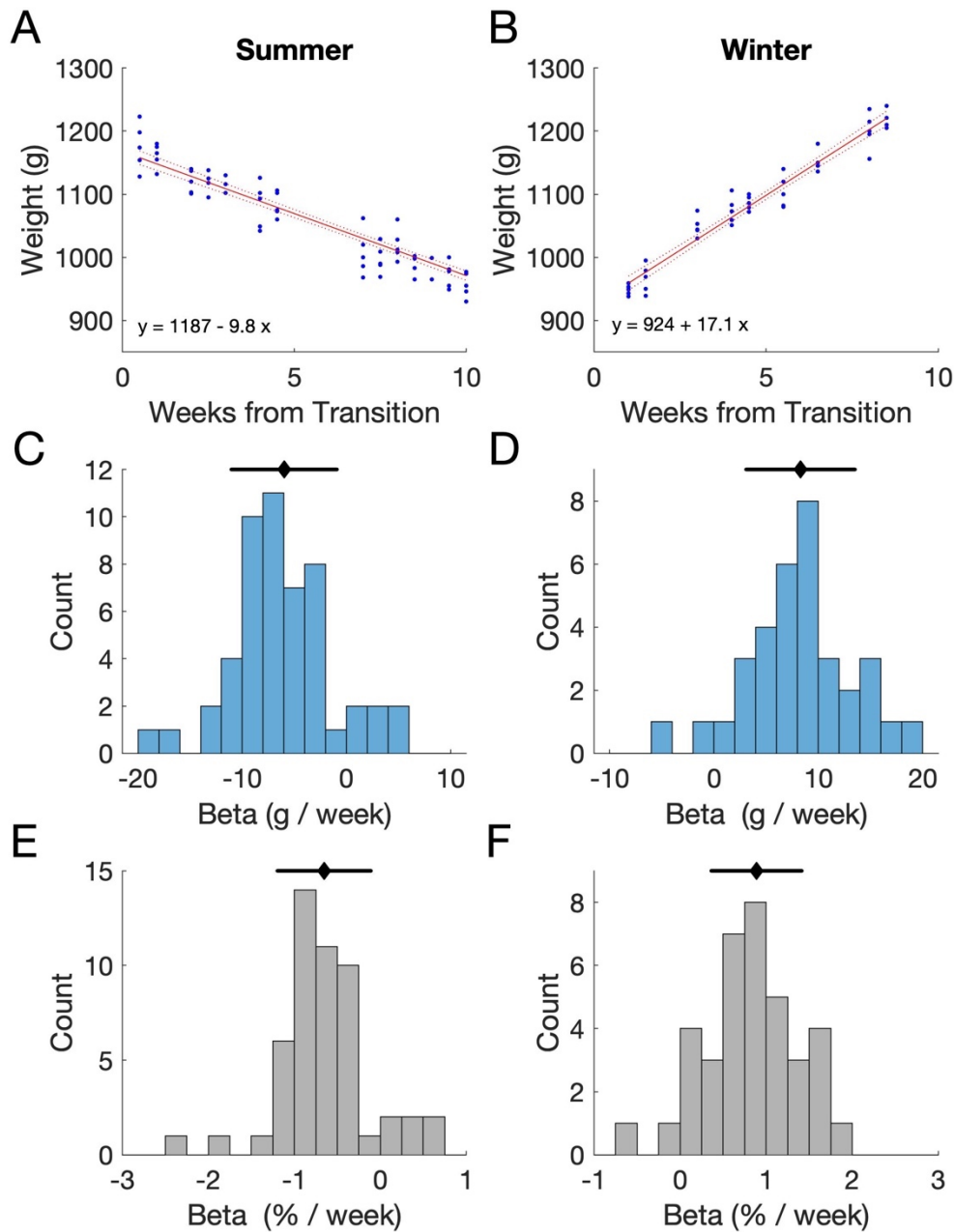
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155 **Figure 1: Seasonal fluctuations in body weight**

156 **A.** Relative light hours and clock change transition periods ferrets were exposed to during the
157 28-month period. **B.** Weight change for a single ferret (F1606) between May 2017 and August
158 2019. **C.** Absolute weight of all ferrets (n=39) between May 2017 and August 2019. **D.** Seasonal
159 variation in weight expressed as a percentage change from each ferret's average bodyweight.

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161 To quantify the observed changes in weight with season, we divided weight
162 measurements into 'summer' and 'winter' periods according to day length (summer = 14
163 hours daylight, winter = 8 hours daylight), considering time as the number of weeks since
164 the transition to longer/shorter days. For each animal and season for which we had at
165 least 8 weeks of data, we performed a linear regression to determine the relationship
166 between time (in weeks) and body weight (Fig. 2A-B). In summer, there was a statistically
167 significant relationship between time and body weight during the summer period for all
168 animals (51 animal x transition combinations, 33 unique animals measured across one or
169 more seasonal transition; R^2 (mean; min to max) = 0.59; 0.07 to 0.96, $p < 0.05$ (49/51
170 $p < 0.001$). In winter, there was a significant relationship between week and body weight
171 for 33/37 animal-transitions (28 unique animals; R^2 (mean; min to max), 0.74; 0.10 to 0.97
172 $p < 0.05$, 31/33 $p < 0.001$). We, therefore, used the resulting regression coefficients (β) to
173 determine the predicted weight change per week. We expressed weights in grams (Fig.
174 2C-D) and also relative to their starting weight (Fig. 2E-F). Measuring weight changes in
175 this way allowed us to see a highly stereotyped pattern of weight loss/ gain. Weight
176 changes were negative in summer (-6.0 g/week \pm 5.1g/week; -0.65 % \pm 0.55%) and
177 positive in winter (+8.3 g/week \pm 5.2 g/week; +0.89% \pm 0.53%), consistent with a pattern
178 of weight changes observed across the year.



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180 Figure 2: Seasonal weight changes

181 **A-B** Data from one animal (F1606) during summer 2018 (**A**) and winter 2017/18 (**B**).
182 Symbols indicate individual weight measurements; plotted according to the number of
183 weeks since the transition to summer light cycles. Line indicates the regression fit (and
184 confidence bounds). Regression coefficients, Summer: $\beta = -9.84$ g/week or -0.94% per

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185 week ($t=-26.8$, $p<0.001$); Winter: $\beta=17.01$ g/week or 1.66% per week ($t=27.8$, $p<0.001$).
186 **C-D** Regression coefficients for all unique animal-transition combinations between 2017-
187 2019 during summer (**C**, $n=51$ ferret x transition combinations, 33 unique animals) and
188 winter (**D**, $n=37$ ferret x transition combinations, 28 unique animals). Black lines indicate
189 the mean and standard deviation. **E-F** Regression coefficients from C-D expressed as
190 percentage of long-term mean body weight.

191 [Water regulation is associated with predictable weight variation](#)

192 We next consider the impact of fluid regulation on body weight. The ferret is a popular
193 model for neuroscience research as animals can be readily trained in a variety of complex
194 behavioural tasks using water as a positive reward [30–32]. The animals that formed this
195 dataset performed psychoacoustic tasks and were weighed as part of their daily health
196 monitoring while on water regulation. Water regulation typically took place over a 5-day
197 cycle, with water being removed from the home cage from Sunday night until Friday
198 afternoon. We sought to quantify the impact of regulation on body weight, and whether
199 there was any interaction with season changes reported above.

200 We divided data into summer and winter periods and compared day of water
201 regulation to bodyweight in absolute terms (Fig. 3A) or relative to each animal's long-term
202 mean summer or winter weight (Fig. 3B). Both metrics show that weight declined with day
203 on water regulation, although the trends differed between summer and winter. In the
204 winter weight loss reached a stable baseline, whereas in the summer weight loss
205 appeared more linear. These results were confirmed statistically using a two-way
206 repeated measures ANOVA to analyse absolute body weight, where we found a main
207 effect of day ($F_{4, 116} = 59.4$, $p<0.001$) and interaction between day and season ($F_{4, 116} =$
208 3.51 , $p = 0.023$). Similar results were also found when analysing relative body weight,

209 where again both main effect of day ($F_{4, 116} = 59.1, p < 0.001$) and interaction between
210 day and season ($F_{4, 116} = 3.93, p = 0.014$) were significant. In neither analysis was the
211 effect of season significant by itself.

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225 Modelling the contribution of season and fluid regulation to body weight

226 To determine the relative contribution of season and water regulation duration on body
227 weight, we fitted General Linear Models to weight data, using the following predictors:
228 starting weight (the first measurement of weight made after the light transition, this data
229 point was excluded from the modelling), the number of weeks since light transition, and
230 the day of water regulation. We again considered summer and winter data separately,

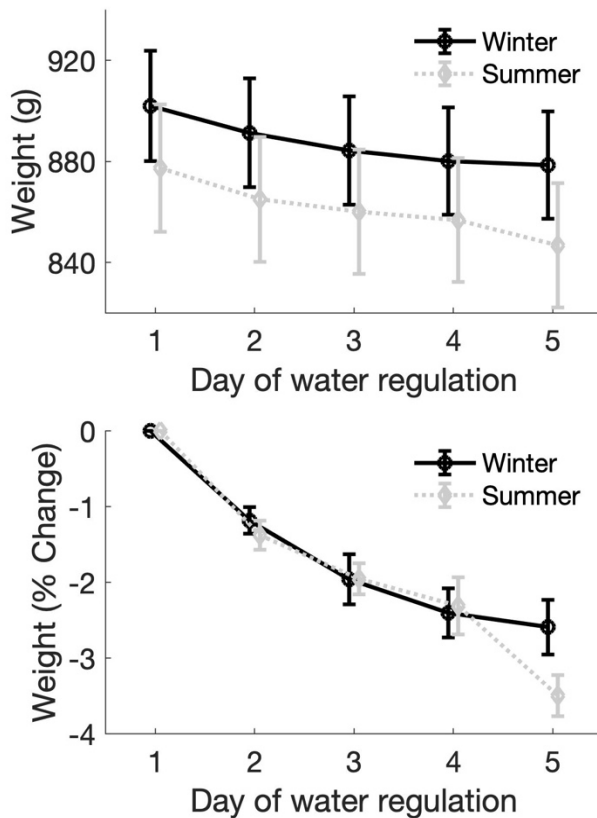
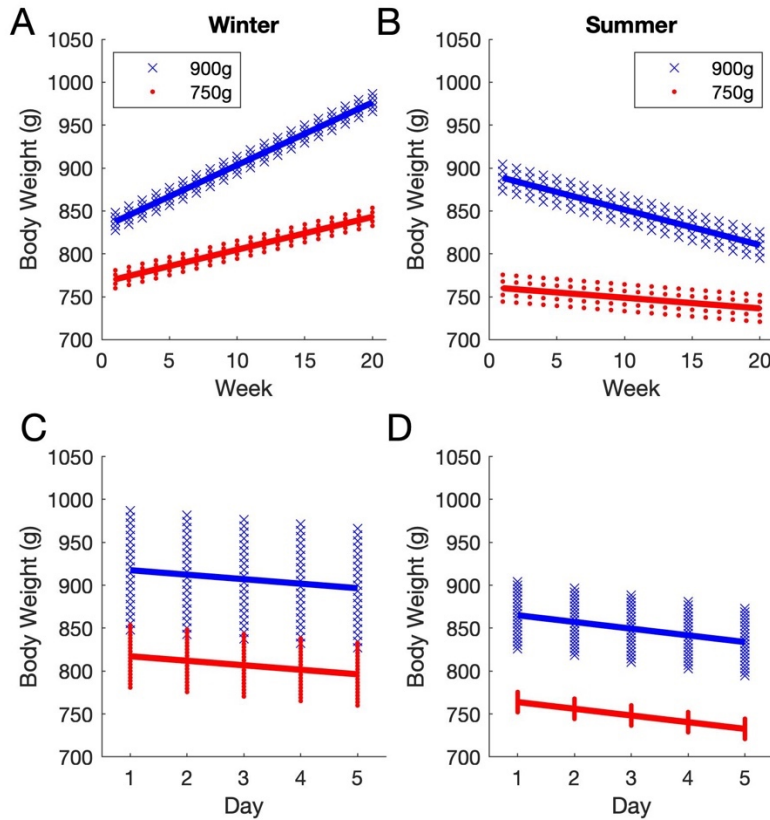


Figure 3: Weight changes resulting from water regulation:

A-B Water regulation related weight changes for 30 animals in which both summer and winter data were available. **A**; mean \pm SEM weight in g for each day of water regulation for summer and winter periods. **B** mean \pm SEM weight change expressed as a percentage change from the weight measurement made on a Monday morning (day 1).

231 and for each, found the best fitting model using a significant drop in deviance as the
232 criterion for including parameters (using the F statistic to compare models, $p < 0.05$). In
233 each case the best fitting mode retained each of the three main effects (week since
234 transition, day of water regulation and starting weight) as well as the two-way interaction
235 between the week and starting weight (see table 1). To illustrate the key features of these
236 models, we used the fitted models to simulate the changes in weight that would occur
237 over a 20-week period in summer and winter for animals of 750g and 900g (Fig. 4).
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241 Figure 4: Predicted weight changes

242 **A-B**, predicted weight changes over a 20-week period for two simulated ferrets of 750g (red)
 243 and 900g (blue) in winter (**A**) and summer (**B**). Predictions were generated for each simulated
 244 animal for 20 weeks at each light duration, for 5 days of water regulation within each week (i.e.
 245 100 values per season). **C-D** Predicted within-week changes during winter (**C**) and summer (**D**).

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Winter			
	<i>Beta</i>	<i>t-statistic</i>	<i>p-value</i>
Intercept	460.51	18.454	4.57E-71
Transition week	-13.667	-6.5697	6.20E-11

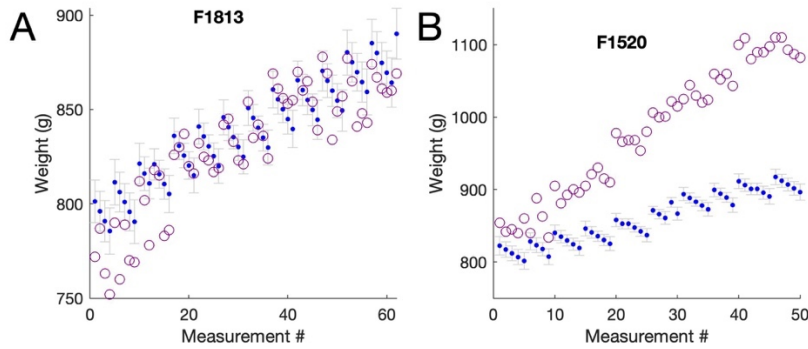
Day of water reg.	-5.1302	-3.9662	7.52E-05
Starting weight	0.42788	16.407	2.53E-57
Week x start weight	0.023295	10.343	1.52E-24

Summer	Beta	t-statistic	p-value
Intercept	121.07	7.4993	7.97E-14
Transition week	12.739	10.864	4.34E-27
Day of water reg.	-7.6443	-8.3847	7.12E-17
Starting weight	0.88069	58.105	<1E-99
Week x start weight	-0.018721	-16.623	6.50E-60

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248 **Table 1:** GLM results for summer and winter data. Only significant predictors for which there was a
249 significant reduction in model deviance are shown. For both summer and winter weights the resulting
250 model retained the starting weight of the animal, the week of transition, and the day of water regulation,
251 as well as a significant start weight * day of regulation interaction.

252 While our models could recapitulate key trends in the data, and for some animals
253 the predicted and observed weights were closely aligned (Figure 5A), others were much
254 less well predicted (e.g. Figure 5B). Moreover, to be useful as a diagnostic measure of
255 healthy or abnormal weight changes, the model should be able to estimate an expected
256 weight of an animal given factors such as the season and its starting weight. To test this,
257 and determine whether this model had any utility as a diagnostic measure, we applied the
258 regression model obtained by fitting data to all animals (above) to data from all but one
259 animal, excluding each animal in turn. We used the resulting model to predict weight
260 measurements (and their 99% confidence intervals) for the left-out animal's data and then
261 compare predicted and actual weight measurements.



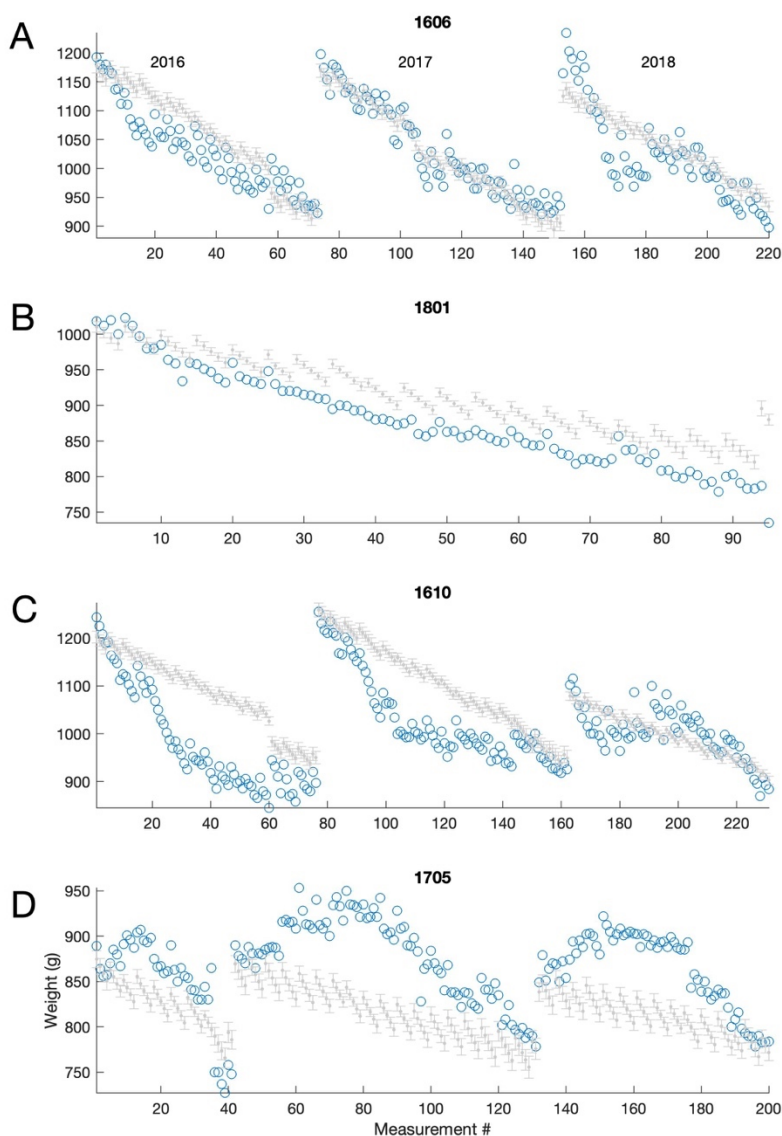
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263 Figure 5: Comparisons of observed and predicted values for two ferrets (A, F1830, winter, B, F1520,
264 winter) selected as two examples of animals whose observed weight values were relatively well fit (A) and
265 poorly fit (B) by data predicted from a GLM modelling expected weight values based on starting weight,
266 number of weeks since light transition and day of water regulation. Open data points show the observed
267 weight values, dots and errorbars show the 99% confidence intervals of the model predictions.

268 We found that that although predicted and observed weights were correlated
269 (significant correlations were found for 29/30 animals in summer with r values (mean \pm SD)
270 $r=0.69\pm 0.19$, 24/32 animals in winter; $r=0.72\pm 0.33$), generally the ability to predict 'held-
271 out' animals was poor. Very few of our observed weight measurements fell within the
272 predicted confidence limits ($\sim 10\%$ of all data). Examination of the observed and predicted
273 values revealed that while the models predicted the key trends, they poorly captured the
274 extent of the variability occurring within individual animals over time. In Figure 6, four
275 example animals are shown – the first two animals show what is typical of most of our
276 animals which is that the model captures the trend but not the details, whereas the second
277 two animals show very different patterns of weight loss which, while quite different from
278 most animals is highly consistent within that individual.

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282 Figure 6: Comparisons of observed (open symbols) and predicted values (gray error bars indicating the
283 99% confidence interval of the fit) for four animals. For each animal all available data is shown, which
284 includes data from each of three summers 2016-2018, for animals in A,C,D, and a single year (2018) in
285 animal F1801, shown in B. The animals in A and B show the typical near-linear pattern of weight loss
286 over time, the shape of which is well captured by the predictions. However, rarely are the observed

287 values within the predicted range by the model. In animal A, the fluctuation in weight due to water
288 regulation is underestimated, and in one year weight loss was more rapid than the model predicted, while
289 in B, the animal varies very little while on water regulation and therefore the fluctuations are over
290 estimated. For this animal the model also underestimates the gradient of weight loss through the summer.
291 Most animals looked like those in A and B, however a few animals had substantially different patterns
292 which were highly consistent within each animal from year to year. Both animals in C and D show a
293 pattern of weight loss which is not linear (and therefore unsurprisingly poorly modelled). Animal F1610 (C)
294 shows a rapid weight loss that stabilises at a baseline, whereas Animal F1705 continues to gain weight
295 after the weight change, before beginning to lose weight for the remainder of the season.

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297 Given the observation that weight loss/gain with season could be fit as a linear
298 model (Figure 2E,F), but that there was significant inter-animal variation we adapted our
299 modelling approach to address three questions about the changes in body weight
300 between weeks that occurred with water regulation that were common across animals:
301 First, was the change in weight between weeks constant, or did it vary through the
302 season? Second, does the change in weight between weeks, expressed as a proportion
303 of the animal's weight, depend on the size of the animal? Third, does some of the
304 additional variability we observed relate to whether animals have been on water
305 regulation the previous week? To address this, we first considered the change in weight
306 that occurred from each week (week i) to the next (week $i+1$) (using only the body
307 weight measurements from the first day on water regulation, i.e. Monday mornings),
308 expressing this as a % change in weight relative to bodyweight measured in week i . We
309 then fitted a GLM to these % values, using three predictors: (1) the number of weeks
310 since seasonal transition (i.e. summer to winter), (2) the animal's mean weight, and (3)

311 whether the animal had been on water regulation the previous week (week $i-1$). As
312 before, data for summer and winter were modelled separately.
313 When modelling weight changes in the winter the intercept was significant (beta = 1.38,
314 table 2), suggesting that typically animals gained 1.38% of their body weight weekly in
315 winter, and the week was also a significant predictor (beta = -0.065) indicating that
316 animals initially gained weight more rapidly. In summer the intercept was again significant
317 (beta = -0.73) and the only other significant predictor was whether the animal had been
318 on regulation the previous week (beta = -0.82, indicating that animals who had access to
319 free water in the previous week lost more weight than those who had not had access to
320 free water). In summary therefore, it is possible to estimate typical patterns of weight loss
321 / gain that should occur over the course of a week but data such as that shown in Figure
322 6C,D demonstrate that each animal must really be assessed individually (ideally in
323 comparison to its own historic data) in the context of other factors.

Winter			
	Beta	t-statistic	p-value
Intercept	1.3778	6.3158	6.63E-10
Free water previous week	-0.065161	-3.3247	9.60E-04

Summer			
	Beta	t-statistic	p-value
Intercept	-0.73	-9.8469	1.65E-21
Free water previous week	-0.82439	4.1028	4.56E-05

324
325 Table 2
326 Final models for estimating the % change in body weight from week n to $n+1$. The number of weeks since
327 transition, the animals mean body weight and whether the animal had been water regulated in week $n-1$

328 were considered as factors, with only the number of weeks since transition being predictive for the
329 summer data, and whether the animal had been on water regulation or not the previous week influencing
330 the summer data.

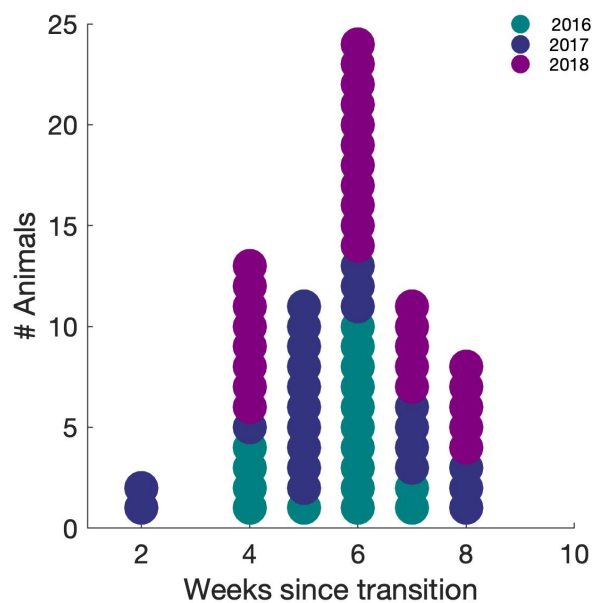
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332 In addition to body weight data, we also had the timing of oestrus for each animal
333 (69 measurements, 39 unique animals). Oestrus varied from 2 to 8 weeks after the first
334 change in light cycle length in the spring. Timing did not vary significantly across the three
335 years (Kruskalwallis test, $p=0.54$) with the average value being 5.7 ± 1.4 weeks after the
336 first light change in the spring (Figure 7). Unfortunately, the number of missing weight
337 values obtained on the week of light change and the week of oestrus precluded
338 meaningful statistical analysis of the relationship between weight change and oestrus.

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343 Figure 7

344 Timing of oestrus relative to daylight change. Dot histogram with each dot representing the timing of
345 oestrus in a single animal in 2016, 2017 and 2018.

346 Discussion

347 Here we provide a normative weight dataset for the healthy female ferret and demonstrate
348 that ferrets show predictable and stereotypical seasonal fluctuations in weight, with most
349 animals gaining around 0.89% of their average body weight per week in winter and losing
350 around 0.65% of their weight per week in summer. Superimposed upon these seasonal
351 fluctuations, water regulation also causes highly stereotyped changes in body weight.

352 The observed seasonal changes in weight imposed large fluctuations in body
353 weight with animals typically being roughly 15% heavier in winter, and 15% lighter in
354 summer (i.e. a variation of as much as 30% of their mean weight). This pattern of seasonal
355 weight change demonstrated by our ferrets follows previously observed changes [22].
356 The range of mean weights that we observed across our population were in keeping with
357 previously reported data [20,29,33]. There are many possible physiological contributing
358 factors to this seasonal weight loss including coat changes [22,26], fat storage, hormone
359 levels such as melatonin [26] and activity levels which in wild animals are critical for
360 survival [34].

361 Water regulation imposed an additional pattern of weight changes on animals;
362 weight was lost over the week in both summer and winter. On average, between Monday
363 morning (when water bottles had been removed the previous evening) and Friday
364 morning animals lost around 3% of their body weight in winter, and 4% in summer.

365 Depending on diet, water consumption for a ferret can be up to 100ml/day [29] and we
366 ensured ferrets received 60ml/kg of water each day of water restriction (which is the
367 amount that animals maintained on laboratory ferret diet, with free access to water,
368 typically consume in a 24 hour period). Since the key contributor to weight loss in water
369 regulated animals is thought to be reluctance to eat dry food (rather than dehydration per
370 se) providing animals with water combined with ground pellet diet to form a mash [8]
371 appears to be successful at ensuring weight loss does not exceed more than a few
372 percent. Food and water restriction are common methods used as motivation to train
373 many laboratory animals including ferrets, rats and mice in tasks for research [35] and
374 weight loss is a key marker of health status. Understanding how seasonal fluctuations
375 interact with these effects is therefore important to refine health assessment and ensure
376 the highest standards of animal welfare.

377 In addition to body weight data, we also had the timing of oestrus for each animal
378 (69 measurements, 39 unique animals). Oestrus varied from 2 to 8 weeks after the first
379 change in light cycle length in the spring with an average value of 5.7 ± 1.4 weeks. Previous
380 research has observed that the cycle of changes in light duration were responsible for
381 initiating oestrus and weight changes but whether there is a causal relationship between
382 the two remains unknown [20]. Further research is required to directly assess whether it
383 is the day light change itself, weight changes induced by day light change, or an
384 interaction between the two factors that induce oestrus.

385 The animals used in this study were all classed as young to middle aged [29] with
386 the oldest animal being 4 years old. Ferret weights also vary across the lifespan, which
387 is on average between 6-8 years [29]. At birth, female ferrets weigh 6-12g and grow

388 rapidly to 550-700g at 10-12 weeks and 600-950g at approximately 16 weeks (i.e.
389 adulthood) [8,20,23,29]. Ferrets are defined as old after the age of 3-4 years and are at
390 greater risk of geriatric diseases but also natural weight loss [29,36].

391 We do not have sufficient repeated data from older animals to determine how
392 ageing interacts with seasonal weight changes. Greater weight loss is observed in aged
393 ferrets (>4 years old) during, and in recovery from, illness [37]. With weight loss an
394 indicator of possible disease, accounting for the age of the animal is important for
395 contextual assessment of health status, and so further research is required to quantify
396 weight changes during ageing and precautionary close observation of weight loss in older
397 animals would be justified.

398 While the seasonal and water-regulation induced changes in weight were highly
399 stereotyped within an animal, and had common features across animals, we were unable
400 to generate a simple statistical model that could accurately predict the expected weight
401 changes. Pulling together our findings we would expect that animals should gain weight
402 in winter, with an initial increase of around roughly 1.4%/week, declining to 0.7%/week
403 after 10 weeks and 0.1%/week after 20 weeks. In contrast, expected weight loss in the
404 summer was roughly linear, with animals losing roughly 0.7%/week, except in weeks
405 which were preceded by access to free water in which weight loss was around 1.5%.
406 Superimposed upon these weekly changes are daily fluctuations in body weight that result
407 from fluid regulation which are typically of the order of 3-4% from a Monday morning to
408 Friday morning. These data therefore establish some normative benchmarks for
409 seasonal weight variation in female ferrets that can be incorporated along with other
410 indicators of well-being into the assessment of an animal's overall condition.

411

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