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3 **Seasonal variation in energy expenditure is not related to activity level**  
4 **or water temperature in a large diving bird.**

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## Abstract

32 There is considerable interest in understanding how the energy budget of an endotherm is  
33 modulated from a physiological and ecological point of view. In this paper, we used daily  
34 (24 h) heart rate (DHR), as a proxy of DEE across seasons, to test the effect of  
35 locomotion activity and water temperature on the energy budget of a large diving bird.

36 DHR was monitored continuously in common eiders (*Somateria mollissima*) during  
37 seven months together with measures of time spent flying and time spent feeding. DHR  
38 varied substantially during the recording period with numerous increases and decreases  
39 that occurred across seasons, although we could not find any relationship between DHR  
40 and the time spent active (feeding and flying). However, inactive heart rate (IHR)  
41 decreased as locomotion activity increases suggesting that common eiders were using  
42 some form of compensation when under a high work load. We were also unable to detect  
43 a negative relationship between water temperature and resting heart rate, a proxy of  
44 resting metabolic rate. This was unexpected based on the assumption that high  
45 thermoregulation costs would be associated with cold waters. We showed instead that  
46 high level of energy expenditure coincided with feather moult and warm waters, which  
47 suggest that the observed variable pattern of seasonal DEE was driven by these two  
48 factors. Nevertheless, our results indicate that compensation and possibly the timing of  
49 moult may be used as mechanisms to reduce seasonal variation in energy expenditure.

50

## 51 INTRODUCTION

52 How animals deal with their intrinsic needs and environmental variability is a fundamental  
53 question in physiology and ecology. Energy expenditure is thought to vary considerably in  
54 birds during the annual cycle as they need to reproduce, thermoregulate, grow feathers or  
55 even migrate. At the same time, food resources may vary seasonally in quantity and quality,  
56 while predation pressure may force animals to move and seek safer habitats. For these  
57 reasons, we expect the energy cost of life of birds to vary considerably in the course of the

58 annual cycle. This reasoning has been labelled the **increased demand hypothesis (ID)**  
59 (Weathers and Sullivan 1993). Recent studies on energetics of diving birds during the annual  
60 cycle support the ID hypothesis where peaks of EE are associated with productive costs just  
61 like during pre-breeding and feather moult (Guillemette et al. 2007, Green et al. 2009, White  
62 et al. 2011).

63

64 However, metabolic ceilings may impose a limit to the level of energy expenditure (  
65 Kirkwood 1983, Daan et al. 1990; Peterson et al. 1990; Weiner 1992). In such a case, we  
66 might expect daily energy expenditure (DEE) to stay relatively constant despite variation in  
67 activity level, thermoregulation and productive costs (Guillemette et al. 2012). Despite large  
68 and sophisticated efforts devoted recently to the study of metabolic ceilings in endotherms  
69 (reviewed by Bacigalupe and Bozinovic. 2002, Speakman and Król. 2011), it appears that the  
70 identification of such limits to DEE are at best difficult. Perhaps, such a difficulty is related to  
71 the possibility that an animal uses an array of behavioural or physiological strategies in order  
72 to maintain itself below a metabolic ceiling or to minimise energy expenditure, thus making  
73 the identification of such a ceiling elusive (Guillemette 2012). This is the **energy budget**  
74 **limitation hypothesis (EBL)**, which predicts that when facing such limits to DEE, the time-  
75 energy budget will be re-organised whether it is by the means of behavioural or physiological  
76 compensation (Pelletier et al. 2008). These authors found that a high level of flight, a costly  
77 activity for most wing-propelled diving birds like the common eider *Somateria mollissima* ,  
78 was associated with a reduction of the rest of the energy budget. However, Pelletier et al.  
79 (2008) could not discriminate if this was the effect of EBL from the lack of any requirement  
80 to move rapidly by flight. In a recent paper, Guillemette et al. (2012) selected a period of high  
81 energy turnover and compared DEE before and after moult migration and found that DEE was  
82 similar despite a large increase in foraging costs occurring before migration. Although, this  
83 latter study supports the EBL hypothesis, we do not know if such an hypothesis would be  
84 upheld during a longer time scale like the annual cycle.

85

86 The heart rate method using data loggers (DLs) implanted in the body cavity of birds  
87 allows the investigator to estimate the rate of energy expenditure continuously over a  
88 relatively long period of time, often in excess of a year (reviewed by Butler et al. 2004;

89 Green 2011). The heart rate (HR) method has been largely used for wild and farm  
90 animals, in addition to human beings (reviewed respectively by Green 2011, Brosh 2007;  
91 Atchen and Jeukendrup 2003). With this method, HR is calibrated against  $\dot{V}_{O_2}$  in the  
92 laboratory and HR measured in the field. Studies using this method have shown that  
93 variation in HR of wild birds is the major circulatory adjustment observed in relation to  
94 changes in oxygen demand and thus, any sustainable response to that demand should be  
95 reflected by variation of HR.

96

97 In the present paper, we test the two hypotheses stated above by quantifying seasonal  
98 variation of energy expenditure in a large sea duck, the common eider. Daily heart rate  
99 (DHR, in beats  $\text{min}^{-1}$ ), the total number of heart beats occurring in one day divided by  
100 1440 (the total number of minutes in 24 h) was converted into energy expenditure  
101 (Hawkins et al. 2000) and used as an index of DEE in this study. We determine if average  
102 DHR varies on a seasonal basis to test the ID hypothesis. Seasonal DHR was  
103 characterised by various oscillations during the recording period (seven months) and was  
104 related to seasonal water temperature in an effort to interpret these variations. We also  
105 examine the influence of locomotor activity (LA = time spent flying and feeding) to test  
106 the EBL hypothesis by partitioning DHR into feeding heart rate (FeHR), flight heart rate  
107 (FHR) and "inactive" heart rate (IHR). The analysis was performed first within (intra-)  
108 individuals and then conducted on the data from all the birds to examine how the pattern  
109 of seasonal variation in DHR is correlated with LA.

110

## 111 **METHODS**

112 The study was performed on Christiansø Island (55°19'N, 15°12'E), an old Danish  
113 fortress located in the southern Baltic Sea, 18 km from the Danish island of Bornholm.  
114 The general approach of our work involved the monitoring and deployment of data  
115 loggers on breeding females, partitioning of heart rate data, and using heart rate to  
116 estimate the daily energy expenditure (DEE).

117 Deployment of data loggers

118 We studied the breeding biology of common eiders by monitoring about 100 nests on the  
119 study plot every year (1999–2005). Nests of banded females were identified by numbered  
120 wooden sticks. In spring 2003, 20 females were surgically implanted with heart rate and  
121 pressure data loggers (DLs, as manufactured by Anthony J. Woakes from U.K.). We  
122 obtained a licence from Dyreforsøgtilsynet (Royal Veterinarian Corporation) in Denmark  
123 and birds were cared for in accordance with the principles and guidelines of the Canadian  
124 Council on Animal Care. All surgical procedures were conducted indoors 100 m from the  
125 experimental plot. The 20 DLs were 36 mm long ( $\pm$  SD = 0.5) x 28 mm (0.2) wide x 11  
126 mm thick (0.3) and weighed 21 g (0.3), that is 1.2% of body mass at implantation  
127 (Guillemette et al. 2002). Hydrostatic pressure and heart rate were sampled every 2 s.

128 Eighteen (90%) of the experimental females returned to the study area one year later,  
129 which is similar to the previously reported survival rate in this species (Coulson 1984).  
130 This is most likely related to the fact that implanted DLs do not alter aerodynamic or  
131 hydrodynamic properties of the instrumented individuals (Guillemette et al. 2002).  
132 However, the number of days per bird for which we had available information in the  
133 present study was variable (ranging from 45 to 220 days), most likely due of battery  
134 failure of the DLs. We analysed data from thirteen individuals, as these birds had loggers  
135 that recorded continually for about 7 months ( $n = 186$ -220 days), which covered the  
136 summer and the beginning of winter (mid-December).

### 137 Time activity budget and partitioning of heart rate data

138 The time budget data involved calculating the daily time spent: (1) flying, (2) feeding  
139 and (3) being inactive. The partitioning of heart rate data involved calculating the number  
140 of heart beats associated with each of these categories of behavior and subsequently  
141 counting the number of heart beats occurring in one day (daily number of heart beats).  
142 Flight schedules (number and duration of flights) were compiled for each bird following  
143 the method described by Pelletier et al. (2007). This method is based on the dramatic  
144 increases and decreases of heart rate upon take-offs and landings respectively, and a  
145 plateau phase during flight where heart rate is typically 3–4 times the resting level. For

146 every female, the daily time spent flying (TSF) was obtained by summing the duration of  
147 all flights that occurred during one day.

148 In birds, dives are usually performed in a series, where time spent submerged  
149 alternates with time breathing at the surface, which constitute a dive cycle. A feeding  
150 bout (> 1 dive) is defined as the succession of dive cycles and the daily time spent  
151 feeding (TSFe) was obtained by summing all feeding bouts occurring in one day. Finally,  
152 the daily time spent “inactive” (TSI) was obtained for each day and each female sampled  
153 by subtracting the time spent active (feeding + flying) from 1440 min. From visual  
154 observations, we know that “inactive” behavior is composed of swimming, preening and  
155 resting (Guillemette 2001).

156 The heart rate data were partitioned into useful quantities like feeding heart rate  
157 (FeHR), flight heart rate (FHR) and inactive heart rate (IHR). Thus, for every category of  
158 behavior and for every female, we summed the total number of heart beats associated  
159 with that behavior and divided this by the number of minutes the bird spent engaged in  
160 that activity each day. Thus, we obtained averages of FHR, FeHR and IHR for the  
161 thirteen females. A similar procedure was followed for the total (daily) heart rate by  
162 dividing the total number of heart beats obtained in one day by 1440 minutes.

163 A customized computer program (written by J.M. Grandbois) was run to calculate  
164 all these quantities from the raw data. Finally, we estimated the minimum heart rate for  
165 each bird during each day of sampling and took this to be the resting heart rate (RHR). To  
166 do so, we wrote a computer program in order to find the minimum average value within  
167 an interval of 5 min which was then compared with similar 5 min intervals obtained  
168 through the day. This time interval was a compromise between smaller intervals,  
169 incompatible with the observed decrease in heart rate during diving, and larger intervals  
170 that compose a larger portion of the day. Since each new 5 min interval was searched 20 s  
171 later than the one before, we obtained 4,306 such intervals for each day of sampling. The  
172 end result of that procedure was the selection of the 5 min interval with the lowest mean  
173 heart rate. We used that quantity as an estimate of the resting heart rate (RHR) for that  
174 day and for a specific bird.

175 Conversion into rate of energy expenditure

176 We used the calibration study of Hawkins *et al.* (2000) to convert HR data into mass-  
177 specific metabolic rate (sMR). Hawkins *et al.* (2000, Table 4) related HR (beats min<sup>-1</sup>)  
178 and mass-specific rate of oxygen consumption,  $s\dot{V}_{O_2}$  (ml O<sub>2</sub> kg<sup>-1</sup> min<sup>-1</sup>) for six common  
179 eiders that were monitored continuously for two days on a water flume, exercised at  
180 various speeds for up to 6 h per day and fed with waterfowl diet pellets. The functional  
181 (reduced major axis) relationship was:  $s\dot{V}_{O_2} = 0.146HR + 9.677$  ( $r^2 = 0.753$ ,  $p = 0.023$ ).  
182 One liter of oxygen consumed was multiplied by 20.083 kJ (Schmidt-Nielsen 1997) to  
183 obtain sMR of birds.

184

185 A different procedure was required to estimate flight costs as it has been shown  
186 that there is a different relationship between HR and  $\dot{V}_{O_2}$  when birds are  
187 swimming/running compared with when they are flying (Ward *et al.*, 2002). Pelletier *et al.*  
188 (2008) used the two physiological models of Bishop (Bishop 1997) based on heart  
189 mass and heart rate, and found that estimates of energy expenditure during flight were  
190 similar to estimates generated by allometric and theoretical models. Using the second  
191 model of Bishop (Bishop 1997), Pelletier *et al.* (2008) estimated mass-specific flight  
192 costs of flight to be 79.5 W kg<sup>-1</sup> and this estimate was used in the present study. Finally,  
193 mass-specific daily energy expenditure (DEE) was obtained by summing the MR of birds  
194 while flying, feeding and being inactive.

195

196 One potential difficulty when converting HR into estimates of DEE for migratory  
197 birds is that they show labile body and organ mass during the annual cycle (Biebach  
198 1998, Piersma 1998, McWilliams and Karasov 2001). This is because changes in organ  
199 mass and body composition may alter the relationship between HR and  $\dot{V}_{O_2}$  and thus  
200 affect estimates of energy expenditure. This may well be the case in our study, as female  
201 common eiders recover from incubation fast and prepare themselves for migration, wing  
202 and body moult and the onset of winter. Thus, an implicit assumption of the heart rate  
203 method is that heart mass is an isometric function of body mass. Variation in heart mass

204 is a determinant of the cardiac stroke volume: for a given rate of oxygen uptake and  
205 oxygen extraction, HR varies inversely with stroke volume (see Butler et al. 2004).  
206 Violation of that assumption would potentially confound our results regarding DEE. At  
207 the inter-specific level, Bishop (1997) computed a reduced major-axis regression from  
208 228 species of birds and found that heart mass scales with body mass with a slope of 0.93  
209 ( $\pm 95\%$  CI = 0.02), which is only slightly shallower than the theoretical (isometric) slope  
210 of 1. Guillemette et al. (2007) collected female common eiders during winter and post-  
211 hatching phases and found that they varied greatly in terms of body mass (1191–2002 g)  
212 and heart mass (12.1–19.8 g). A reduced major-axis regression on these log-transformed  
213 data resulted in a slope of 1.03 (intercept = -2.12,  $n = 17$ ,  $r^2 = 0.61$ ). These results indicate  
214 that variation in body mass is tracked by a similar variation in heart mass, suggesting that  
215 although there is some small variability in the relationship between heart mass and body  
216 mass, it is reasonable to assume that the relative stroke volume remains constant during  
217 the present study period. However, only measurements at that intra-individual level  
218 would properly test the assumption that heart mass variation tracks body mass variation.  
219

220         Perhaps, the best calibration study performed so far between HR and  $\dot{V}_{O_2}$  on a  
221 migratory bird is that by Portugal et al. (2009). These authors found that five out of the  
222 six relationship between HR- $\dot{V}_{O_2}$ , derived from various sampling periods throughout the  
223 year, did not differ significantly despite significant changes in body mass and body  
224 composition (Portugal et al. 2007). This indicates that the heart rate method is robust in  
225 relation to most of the seasonal variation of body condition. It suggests also that the  
226 single calibration study (Hawkins et al. 2000) used in the present paper to covert HR into  
227  $\dot{V}_{O_2}$  and then into EE of common eiders may be valid when comparing seasons within the  
228 whole study period (208 days on average). However, Portugal et al. (2009) found that the  
229 calibration curve derived from captive barnacle geese was different from that for wild  
230 geese, indicating that for a similar  $\dot{V}_{O_2}$ , HR is lower for wild individuals. This suggests  
231 that our study may underestimate the actual value of EE. Therefore, our results are  
232 presented as a two-step process. A first one where a minimum of assumptions are made



233 to test the effect of locomotion activity and water temperature on HR and a second one  
234 where estimates of DEE are made for the minima and maxima HRs observed during the  
235 period of recording (seven months) giving an umbrella of values that can be compared to  
236 other studies.

237

### 238 Water temperature

239 The females nesting on Christiansø Island have been ringed for several years (Lyngs  
240 2000) and from this information we know that most females move from east to west  
241 during moult migration, mostly toward the eastern part of the Baltic and the southern part  
242 of the Kategat and some individuals into the Wadden sea. Using this information together  
243 with the time spent flying and flight speed, we were able to position every female in this  
244 study into a spatial polygon. For each female in the Baltic, the surface water temperature  
245 was obtained from a monitoring program (Finnish Institute of Marine Research) along the  
246 route of the ferry Finnpartner (Travemünde - Helsinki)  
247 (<http://www3.fimr.fi/en/itamerikanta/pfsearch/>). In the Wadden sea, similar information  
248 was obtained from the marine station of Oldenburg University ([http://las.physik.uni-  
249 oldenburg.de/landstation/](http://las.physik.uni-oldenburg.de/landstation/)). Although this approach is coarse, it has the advantage of  
250 being robust when the increases and decreases in water temperature of these three regions  
251 are compared. Thus, for each day of recording, the water temperature was averaged  
252 across females.

253

### 254 **Data analysis**

255 We performed an average and individual analysis of the data collected.

256

#### 257 Individual level

258 One salient feature of the heart rate data presented here is that they were recorded  
259 continuously, every 2 s. for a long period of time (208 days). We thus compute the  
260 Pearson correlation coefficient between two quantities for each individual separately  
261 (Table 1). We then calculated the average intra-individual correlation coefficients for  
262 which we calculate confidence intervals using the bootstrap method using ten thousand  
263 re-samplings (Lunneborg 2000). Inspection of the sampling distribution resulting from

264 that procedure demonstrated that the distribution was symmetric. Thus, we were able to  
265 use the Student-t version of the bootstrap (Lunneborg 2000). When this interval excludes  
266 zero, we declared the average correlation coefficient to be significantly different from  
267 zero at the 95 % level.

268

#### 269 Average level

270 When averaged over the 13 experimental females, DHR varies greatly on a seasonal basis  
271 with steady increases and decreases. Thus, the data points are obviously not independent  
272 from each other (Fig. 1a). This is an important point, as any positive autocorrelation will  
273 inflate the p value of any statistical tests. By implication, positive autocorrelation reduces  
274 the effective sample size (Guillemette et al. 2004). We argue that this characteristic of the  
275 data does not interfere with the analysis proposed here. Clifford et al. (1989) and  
276 Dutilleul (1993) suggest independently a method to adjust the sample size in relation to  
277 the level of autocorrelation observed in serial data. When performing a statistical test (  
278 for example for a correlation) we correct  $n_{\text{sample}}$  into  $n'$  by adjusting the observed p value  
279 to its real (unbiased) value. Numerical simulations using artificial data with known  
280 autoregressive structures confirm that this method is valid (Dale and Fortin 2002).

281

282 One important point in the application of this method however is that all  
283 autocorrelation lags must be included in the calculation of the effective sample sizes,  
284 even if the individual values do not seem to be significant. If this is not done, an error in  
285 the correction factor may arise because negative autocorrelations require deflation rather  
286 than inflation (Dutilleul 1993, Dale and Fortin 2002). Given that the level of  
287 autocorrelation of the data presented here is alternatively positive and negative (Fig. 1)  
288 with a preponderance of negative values (data not shown), this suggests that our sample  
289 size should be increased, not decreased. Nevertheless, we used the original number of  
290 days recorded in our analysis ( $n = 208$ ) as a conservative estimate of the p-value derived  
291 from our correlation analysis.

292 The EBL hypothesis predicts that DHR and LA are not related and is similar to  
293 the statistical null hypothesis of no relationship. On the other hand, when we remove all  
294 the heart beats associated with LA from DHR (= IHR), the EBL hypothesis predicts that

295 IHR and LA should be inversely related. We first computed the correlation coefficient for  
296 the original data and then chose permutation re-samples from the data without  
297 replacement in a way that is consistent with the null hypothesis of no relationship. We  
298 then constructed the permutation distribution of the statistic from its values in ten  
299 thousands of re-samples (the permutation approach is distribution free). Finally, we found  
300 the *P*-value by locating the original statistic on the permutation (Lunneborg 2000).

301

302 When averaged across individuals, DHR varies greatly on a seasonal basis with  
303 various oscillations (Fig. 1a). We used that characteristic of the data to perform our  
304 analysis and test if the various decreases or increases of DHR occurring during the annual  
305 cycle were different from zero. For each individual, we averaged DHR for a five days  
306 period (lows and highs of Fig. 1a) that we subtracted from the average obtained during  
307 the preceding five days period to obtain a difference ( $\Delta$ ) that estimates the rate of change  
308 of DHR. By repeating this over all 13 individuals sampled, we obtained the average rate  
309 of change for which we calculated 95 % confidence intervals using the bootstrap method  
310 and ten thousand re-samplings, as described above. The statistical significance ( $\alpha$ )  
311 was set at  $p = 0.05$ . RESAMPLING STATS v. 2 ([www.resample.com](http://www.resample.com)) was used for  
312 statistical analyses. Values are means  $\pm$  standard deviation (s. d.) unless otherwise stated.

313

## 314 RESULTS

315

### 316 HR and DEE

317 One obvious feature of the curve depicted in Fig. 1A is the numerous oscillations in HR  
318 that occur during the 7 months of recordings. For five deltas, the confidence intervals  
319 excluded the zero line which indicates that these increases or decreases in DHR were  
320 significantly different from zero (Fig. 1B). Thus, this analysis indicates that DHR of the  
321 instrumented females varied significantly during the seven months of recording.

322

323 One frequent concern when converting HR to energy expenditure is how accurate the  
324 estimate can be. Given the uncertainties associated with the calibration obtained from  
325 captive birds and applied to wild birds (see Methods), we did not want to misrepresent

326 the accuracy of our estimates by interpreting small variations in EE as being significant.  
327 Green (2011) performed simulations for four species of birds showing that a variation of  
328 9-31 % in HR would most likely result in a real difference in EE (depending on species,  
329 calibration available and sample size). In our study, seasonal and significant increases  
330 and decreases in DHR are associated with 8-39 % variation when compared to mean  
331 DHR (Fig.1). Therefore, the seasonal DHR minima and maxima of Fig. 1 most probably  
332 translate into real variation in EE (Table 2) although we cannot associate estimates of  
333 DEE with error bars. Average specific DEE was  $955 \text{ kJ.kg}^{-1}$  and varied between 885 and  
334  $1028 \text{ kJ.kg}^{-1}$  during the period of recording (Table 2).

335

### 336 HR and locomotion

337 These variations in HR were not positively related to level of activity (LA)  
338 although it varied between 70 and 300 min per day (Fig 2A). On the contrary, we found a  
339 small, negative correlation (Fig 2A,  $r = -0.132$ ,  $p = 0.023$ ,  $n = 208$ ) between LA (flying +  
340 feeding) and DHR. By removing all the activity heart beats from DHR (see Methods),  
341 we obtained the inactive heart rate (IHR), which is highly correlated with DHR ( $r =$   
342  $0.880$ ,  $n = 208$ , Fig. 1A et 1C) indicating that the pattern of variation was similar for  
343 these two variables. Moreover, when LA is related to IHR ( $r = -0.377$ ,  $p < 0.0001$ ,  $n =$   
344  $208$ ) we found a stronger negative correlation (Fig. 2B) indicating that a high level of  
345 LA is associated with a decrease of IHR.

346

347 At the individual level, the correlation between DHR and LA varied greatly and  
348 when the coefficient of correlation is averaged across individuals ( $r = 0.132$ ), the  
349 relationship is positive and marginally significant (Table 1). In contrast, a negative and  
350 significant relationship emerges when relating IHR and LA ( $r = -0.150$ ).

351

### 352 HR and water temperature

353 RHR is the minimum HR occurring over a five min interval during each day for each  
354 female (see Methods) and is used here as a proxy for resting metabolic rate. RHR was  
355 positively correlated to DHR within each of the 13 experimental females (Table 1, range  
356 of coefficients  $r = 0.342-0.875$ ). Thus the amount of intra-individual variability in DHR

357 explained by RHR ranged from 12 % to 75 % (Table 1) with an average of 47 %. At the  
358 average level, the curve depicting RHR (Fig. 3B) and DHR (Fig.1A) were also very  
359 similar ( $r = 0.783$ ,  $n = 208$ ). This is an interesting result given that only 5 min of  
360 minimum heart rate per day can predict DHR.

361

362 Water temperature ranged from 6 ° C to 22 ° C during the study period with the  
363 coldest period occurring upon the return of the females on the water in spring, and at the  
364 onset of winter (Fig. 3A). In order to determine whether the return on the water incurred  
365 thermoregulation costs for breeding females, we compared RHR when on the nest for the  
366 last five days of incubation with RHR once on the water for a similar time interval. RHR  
367 increased significantly from  $74.2 \pm 14.7$  beats  $\text{min}^{-1}$  when on nest to  $90.8 \pm 17.2$  beats  
368  $\text{min}^{-1}$  when on water five days after departure, giving an average difference of 16.6 beats  
369  $\text{min}^{-1}$  (bootstrap 95 % confidence intervals, 7.6 and 25.7 beats  $\text{min}^{-1}$ ). Because the  
370 confidence intervals of the average difference exclude zero, we conclude that RHR  
371 increased significantly when females left the colony for the water.

372

373 Contrary to our expectation, neither RHR nor DEE varied negatively with water  
374 temperature on a seasonal basis (Fig. 3A et 3B). In fact, there was no significant  
375 relationship between these variables and water temperature (Fig. 3C,  $r = 0.276$ ,  $p > 0.05$ ).  
376 This suggests that, at the proximal level, we cannot detect any effect of variation in water  
377 temperature on energy expenditure (Table 2).

378

## 379 **DISCUSSION**

380

381 We used the heart rate method to test two hypotheses about the seasonal variation of  
382 energy expenditure of a large diving bird, the increased demand (ID) hypothesis  
383 (Weathers and Sullivan 1993) and the energy budget limitation (EBL) hypothesis  
384 (Pelletier et al. 2008). The average level of DHR and DEE of female common eiders  
385 varied widely during the annual cycle and we discuss to what extent this variability is  
386 related to locomotor activity, water temperature and productive costs.

387

388 Energy management strategies

389 Given the observed increases and decreases in the level of seasonal DHR and DEE, our  
390 study supports the increased demand hypothesis. Two annual cycle curves published so  
391 far for diving birds (Green et al. 2009, White et al. 2011) support the increased demand  
392 hypothesis as the reproductive season is characterised by the highest level of energy  
393 expenditure when compared to the rest of the annual cycle. This is most probably related  
394 to the increased demands of producing tissues or by the extra energy required to feed the  
395 chicks, or both. But even outside the reproductive season, these two studies show large  
396 seasonal variation in DEE, a situation similar to our study (Fig. 1). Some obvious factors  
397 that may explain these variations in seasonal DEE are water temperature, production  
398 costs (e. g. moult) and LA (see below).

399

400 However, increases in LA of common eiders did not increase DHR. This is  
401 despite the fact that feeding heart rate (FeHR) and flying heart rate (FHR) are 50 % and  
402 121 %, respectively, higher than IHR, (Guillemette et al. 2012). There are two reasons  
403 that may explain such a result. The first one is related to the fact that the active period  
404 (flying + feeding) represents a small proportion of the day (5-22 %), ranging about from  
405 70 to 320 min, thus limiting any influence of the activity level on the 24 h energy budget.  
406 Another reason is related to compensation as predicted by the EBL hypothesis. There are  
407 four lines of evidence supporting this hypothesis in our study. First, average DHR and  
408 average LA were not positively correlated giving a very similar pattern of DHR and IHR  
409 in relation to time (Fig. 1A et 1C). Second, average IHR was inversely and significantly  
410 related to average LA, suggesting that a high level of activity is compensated by a lower  
411 heart rate when inactive (Fig. 2B). Third, an analysis performed at the individual level  
412 gives similar results (where IHR is inversely related to LA, Table 1). Fourth, in a  
413 companion paper, Guillemette et al. (2012) identified the high level of activity observed  
414 in the present study (Fig. 2) as migration hyperphagia.

415 In their paper, Guillemette et al. used a before-after and inter-individual approach  
416 showing that when fueling for migration (hyperphagia) these birds compensate for their  
417 high level of activity by decreasing time spent flying and IHR. Similarly, studies with  
418 captive birds (Bautista et al. 1998, Deerenberg et al. 1998, Wiersma and Verhulst 2005)

419 and wild birds (Moreno et al. 1999, Welcker et al. 2010) testing the effect of increasing  
420 LA, found no evidence of associated increases of DEE. One study (Deerenberg et al.  
421 1998) found that increasing LA elicits physiological compensation (a decrease in resting  
422 MR during the night, whereas another study (Moreno et al. 1999) found that increasing  
423 LA had a negative effect on reproductive output.

424

425         How do we reconcile our interpretation that both the ID and the EBL hypotheses  
426 are supported by our data? We suggest here that the two hypotheses are not mutually  
427 exclusive and propose that the energy management strategies of eider ducks is a step  
428 process. Because behavioural adjustments are energetically cheaper and faster than  
429 physiological adjustments, we might expect a change in behaviour to be the first response  
430 to an increase in metabolic demands (Vézina and Salvante 2010). Visual observations of  
431 focal eiders during spring hyperphagia support this view, as the time spent feeding was  
432 inversely related to comfort, swimming and resting activities (Guillemette 2001), which  
433 are grouped in the present study under “inactive” behaviour. Therefore, the various  
434 components of the time and energy budget would be inherently related and the reduction  
435 of inactive heart rate would be driven by a shift from high cost (comfort) to low cost  
436 behaviour (resting, see Supplementary Information, Pelletier et al. 2007). Then,  
437 physiological compensation, such as reductions in body temperature (Butler and Woakes  
438 2001, Wojciechowski and Pinshow 2009), body mass and body organs (Biebach 1998,  
439 Piersma 1998, McWilliams and Karasov 2001), would arise to decrease further energy  
440 expenditure. However, such compensation to reduce energy expenditure might not be  
441 sufficient to buffer completely the seasonal variation in DEE.

442

443 What are the determinants of seasonal HR?

444 RHR should represent a proxy of maintenance cost in nature (Guillemette et al. 2007).  
445 We used this quantity to determine if water temperature could explain the observed  
446 seasonal variation of HR. Although the passage from a terrestrial to an aquatic habitat  
447 indicates that cold water caused a significant increase in RHR by 22 %, we did not find  
448 any evidence at the proximate level that water temperature could have been a determinant  
449 of RHR during the rest of year (Fig 3C), when common eiders spend 95-100% of their

450 time on the water (Pelletier et al. 2008). In contrast, at the ultimate level, variations in  
451 water temperature may have shaped the timing of moult in this population.

452

453 A fascinating aspect of our results is how well synchronised the wing moult was  
454 in relation to the warmest waters of the year (Fig. 3). Although this apparent  
455 synchronicity between water temperature and wing moult might be coincidental, it cannot  
456 be concluded that, on an evolutionary scale, that water temperature had no effect on  
457 RHR. Indeed, it suggests that water temperature may have shaped this important phase of  
458 the annual cycle of female eiders by determining what would be the best timing to start  
459 wing and body moult in this population. Photoperiod is known to be the main trigger of  
460 moult in birds (Gwinner 2003) and water temperature and photoperiod are highly  
461 correlated in the study population (Guillemette M. unpublished). Therefore, we suggest  
462 that the timing of moult is such to minimise energy expenditure during feather growth  
463 and we hypothesize that individuals that initiate moult later when water temperature is  
464 decreasing will have to pay an extra cost in terms of thermoregulation (Jensen et al.  
465 1989).

466

467 Given that neither water temperature nor LA explain the seasonal variation in  
468 DHR in the present study, the question is: Which factors are causing that variation? We  
469 suggest that productive processes like feather growth may explain such variation. Firstly,  
470 the highest level of DHR and RHR coincided with wing moult (Fig. 3). Moult is a costly  
471 process as it requires energy to produce the building material of feathers and tissues  
472 responsible for their biosynthesis (Dietz et al. 1992, Brown and Bryant 1996). The  
473 intensity of wing moult is high in synchronous species like common eiders meaning that  
474 individuals grow many feathers at any one time. Guillemette et al. (2007) estimated the  
475 cost of wing moult in this species to be 12 % of resting metabolic rate and 9 % of DEE.  
476 We suggest that the RHR peak arising after wing moult (143 days from hatching, Fig.  
477 3B) is also caused by feather growth since available evidence indicates that body moult  
478 occurs after wing moult in eider ducks (Guillemette et al. 2007). Secondly, DHR  
479 decreases steadily from peak # 4 (Fig. 1a) and we hypothesize that such a decrease is



480 caused by a cessation of body moult and the occurrence of a better plumage insulation at  
481 the onset of winter.

482 Evidence in support of this is provided by consideration of the lower critical  
483 temperature in air for this species, which is 7 °C for breeding females compared to 0 °C  
484 for winter acclimatized birds (Gabrielsen et al, 1991, Jenssen et al. 1989) Thirdly, we  
485 speculate that the increase of RHR (Fig. 3B) that occurred 10 days after hatching is also  
486 the result of feather growth. Indeed, incubating females are characterized by a paucity of  
487 down feathers on their abdomen when nesting as they use down feathers plucked from  
488 their abdomen to line their nest, which also improves the transfer of heat from the body to  
489 the eggs when they incubate. However, the lack of down feathers on the abdomen of  
490 females when they go back to the water may tend to cause an increase in the cost of  
491 thermoregulation and it is expected that females replace these down feathers shortly after  
492 the end of nesting. Finally, other changes in production costs may occur during the study  
493 period. For instance, those related to the decrease in HR that occurred 35 days after  
494 hatching (Fig. 1). Although this feature occurred in all 13 females, there is not any logical  
495 explanation for it. Altogether, these data suggest that complex metabolic adjustments  
496 occur during post-hatching and it is unclear which process would generate the high  
497 variation of DHR observed during this period.

498

499 In conclusion, the energetic cost of life varied during the annual cycle of a diving bird,  
500 which supports the increased demand hypothesis. The present study suggests that the  
501 energetic costs of locomotion (flying and diving) are buffered by behavioural  
502 compensation, which supports the energy budget limitation hypothesis. We are thus  
503 unable to discriminate between these two hypotheses in the present study, most probably  
504 because they are not mutually exclusive. Nevertheless, our results indicate that  
505 behavioural compensation and possibly the timing of moult may be used as mechanisms  
506 to reduce seasonal variation in energy expenditure.

507

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518

519

## REFERENCES

520

- 521 Achten, J., and Jeukendrup, A. E. (2003). Heart rate monitoring — applications and  
522 limitations. *Sports Med.* **33**, 517–538.
- 523 Bacigalupe, L. D., and Bozinovic, F. (2002). Design, limitations and sustained metabolic  
524 rate: lessons from small mammals. *J. Exp. Biol.* **205**, 2963-2970.
- 525 Bautista, L. M., Tinbergen, J., Wiersma, P. and Kacelnik, A. (1998). Optimal foraging  
526 and beyond: How starlings cope with changes in food availability. *Am. Nat.* **152**, 543-  
527 561.
- 528 Biebach, H. (1998). Phenotypic organ flexibility in Garden Warblers *Sylvia borin* during  
529 long-distance migration. *J. Avian Biol.* **29**, 529-535.
- 530 Bishop, C. M. (1997). Heart mass and the maximum cardiac output of birds and  
531 mammals: implications for estimating maximum aerobic power input of flying  
532 animals. *Phil. Trans. R. Soc. London: B* **352**, 447-456.
- 533 Brosh, A. (2007). Heart rate measurements as an index of energy expenditure and energy  
534 balance in ruminants: a review. *J. of Anim. Sc.* **85**, 1213–1227.
- 535 Brown, C. R., and Bryant, D. M. (1996). Energy expenditure during molt in Dippers  
536 (*Cinclus cinclus*): no evidence of elevated costs. *Physiol. Zool.* **69**:1036–1056.
- 537 Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). Measuring metabolic  
538 rate in the field: the pros and cons of the doubly labelled water and heart rate  
539 methods. *Func. Ecol.* **18**,168-183.

- 540 Clifford, P., Richardson S. and Hémond, D. (1989). Assessing the significance of a  
541 correlation between two spatial processes. *Biometrics* **45**, 123-134.
- 542 Coulson, J. C. (1984). The population dynamics of the Eider duck *Somateria mollissima*  
543 and evidence of extensive non-breeding by adult ducks. *Ibis* **126**, 525-543.
- 544 Daan, S., Masman, D. and Groenewold, A. (1990). Avian basal metabolic rates: their  
545 association with body composition and energy expenditure in nature. *Am. J. Physiol. -*  
546 *Reg. Integr. Comp. Physiol.* **259**, R333-R340.
- 547 Dale, M. R. T. and Fortin, M. J. (2002). Spatial autocorrelation and statistical tests in  
548 ecology. *Écoscience* **9**, 162-167.
- 549 Deerenberg, C., Overkamp, G. J. F., Visser, G. H. and Daan, S. (1998). Compensation in  
550 resting metabolism for experimentally increased activity. *J. Comp. Physiol. B:*  
551 *Biochem. Syst. Environ. Physiol.* **168**, 507-512.
- 552 Dietz, M. W., Daan, S., and Masman, D. (1992). Energy requirements for molt in the  
553 kestrel *Falco tinnunculus*. *Physiol. Zool.* **65**, 1217–1235.
- 554 Dutilleul, P. (1993). Spatial heterogeneity and the design of ecological field experiments.  
555 *Ecology* **74**, 1646-1658.
- 556 Gabrielsen, G.W., Mehlum, F., Karlsen, H.E., Andersen, O., Parker, H. (1991). Energy  
557 cost during incubation and thermoregulation in the female common eider *Somateria*  
558 *mollissima*. *Norsk Polar. Skr.* **195**, 51–62.
- 559 Green, J. A., Boyd, I. L., Woakes, A. J., Warren, N. L. and Butler, P. J. (2009).  
560 Evaluating the prudence of parents: daily energy expenditure throughout the annual  
561 cycle of a free-ranging bird. *J. Avian Biol.* **40**, 529-538.
- 562 Green, J. A. (2011). The heart rate method for estimating metabolic rate: review and  
563 recommendations. *Comp. Biochem. Physiol. - Part A: Mol. Integr. Physiol.* **158**, 287-  
564 304.
- 565 Guillemette, M. (2001). Foraging before spring migration and before breeding in  
566 Common Eiders: does hyperphagia occur? *Condor* **103**, 633-638.
- 567 Guillemette, M., D. Pelletier, J.-M. Grandbois, and P. J. Butler. (2007). Flightlessness  
568 and the energetic cost of wing molt in a large sea duck. *Ecology* **88**, 2936-2945.
- 569 Guillemette, M., Woakes, A. J., Flagstad, A. and Butler, P. J. (2002). Effects of data-  
570 loggers implanted for a full year in female Common Eiders. *Condor* **104**, 448-452.

- 571 Guillemette, M., Woakes, A. J., Henaux, V., Grandbois, J.-M. and Butler, P. J. (2004).  
572 The effect of depth on the diving behaviour of Common Eiders. *Can. J. Zool.* **82**,  
573 1818-1826.
- 574 Guillemette, M., Richman, S. E., Portugal, S. J. and Butler, P. J. (2012). Behavioural  
575 compensation reduces energy expenditure during migration hyperphagia in a large  
576 bird. *Func. Ecol.* (In press).
- 577 Gwinner, E. (2003). Circannual rhythms in birds. *Current opinion in Neurobiology* **13**,  
578 770-778.
- 579 Hawkins, P. A. J., Butler, P. J., Woakes, A. J. and Speakman, J. R. (2000). Estimation of  
580 the rate of oxygen consumption of the Common Eider duck (*Somateria mollissima*),  
581 with some measurements of heart rate during voluntary dives. *J. Exp. Biol.* **203**, 2819-  
582 2832.
- 583 Jenssen, B. M., Ekker, M. & Bech, C. (1989). Thermoregulation in winter-acclimatized  
584 common eiders (*Somateria mollissima*) in air and water. *Can. J. of Zool.* **67**, 669 –  
585 673.
- 586 Kirkwood, J. K. (1983). A limit to metabolisable energy intake in mammals and birds.  
587 *Comp. Biochem. Physiol. Part A: Physiology* **75**, 1-3.
- 588 Lunneborg, C. E. (2000). Data analysis by resampling: concepts and applications.  
589 Duxbury Press, Pacific Grove, California.
- 590 Lyngs, P. (2000). Status of the Danish breeding population of eiders *Somateria*  
591 *mollissima*. *Dan. Ornithol. Foren. Tidsskr.* **94**, 12-18.
- 592 McWilliams, S. R., & Karasov, W. H. (2001). Phenotypic flexibility in digestive system  
593 structure and function in migratory birds and its ecological significance. *Comparative*  
594 *Biochemistry Physiology - Part A: Molecular Integrative Physiology* **128**, 577-591.
- 595 Moreno, J., S. Merino, J. Potti, A. de León, and R. Rodríguez. (1999). Maternal energy  
596 expenditure does not change with flight costs or food availability in the Pied  
597 Flycatcher (*Ficedula hypoleuca*): costs and benefits for nestlings. *Behav. Ecol.*  
598 *Sociobiol.* **46**, 244-251.
- 599 Pelletier, D., Guillemette, M., Grandbois, J.-M. and Butler, P. J. (2007). It is time to  
600 move: linking flight and foraging behaviour in a diving bird. *Biol. Lett.* **3**, 357-359.

- 601 Pelletier, D., Guillemette, M., Grandbois, J.-M. and Butler, P. J. (2008). To fly or not to  
602 fly: high flight costs in a large sea duck do not imply an expensive lifestyle. *Proc. R.*  
603 *Soc. London Ser B Biol. Sci.* **275**, 2117-2124.
- 604 Peterson, C. C., Nagy, K. A. & Diamond J. M. (1990). Sustained metabolic scope. *PNAS*  
605 **87**, 2324 – 2328.
- 606 Piersma, T. (1998). Phenotypic flexibility during migration: optimization of organ size  
607 contingent on the risks and rewards of fuelling and flight? *J. Avian Biol* **29**, 511-520.
- 608 Portugal, S. J., Green J. A. and Butler, P. J. (2007). Annual changes in body mass and  
609 resting metabolism in captive barnacle geese (*Branta leucopsis*): the importance of  
610 wing moult. *J. Exp. Biol.* **210**, 1391-1397.
- 611 Portugal, S. J., Green J. A., Cassey, P., Frappel, P. B. and Butler, P. J. (2009). Predicting  
612 the rate of oxygen consumption from heart rate in barnacle geese *Branta leucopsis*:  
613 effects of captivity and annual changes in body condition. *J. Exp. Biol.* **212**, 2941-  
614 2948.
- 615 Speakman, J. R., and Król, E. (2011). Limits to sustained energy intake. XIII. Recent  
616 progress and future perspectives. *J. Exp. Biol.* **214**, 230-241.
- 617 Vézina, F., and Salvante, K. (2010). Behavioral and physiological flexibility are used by  
618 birds to manage energy and support investment in the early stages of reproduction.  
619 *Current Zool.* **56**, 767-792.
- 620 Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J. (2002). Heart rate and the rate of  
621 oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and  
622 bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.
- 623 Weathers, W.W. and Sullivan, K.A. (1993). Seasonal allocation of time and energy in  
624 juncos. *Physiol. Zool.* **66**, 511-536.
- 625 Welcker, J., Moe, B., Bech, C., Fyhn, M., Schultner, J., Speakman, J. R. and Gabrielsen  
626 G. W. (2010). Evidence for an intrinsic energetic ceiling in free-ranging kittiwakes  
627 *Rissa tridactyla*. *J. Anim. Ecol.* **79**, 205-213.
- 628 Weiner, J. (1992). Physiological limits to sustainable energy budgets in birds and  
629 mammals: ecological implications. *Trends Ecol. Evol.* **7**, 384-388.

630 White, C. R., Grémillet, D., Green, J. A., Martin, G. R. and Butler, P.J. (2011). Metabolic  
631 rate throughout the annual cycle reveals the demands of an Arctic existence in Great  
632 Cormorants. *Ecology*, **92**, 475-486

633 Wiersma, P., and Verhulst, S. (2005). Effects of intake rate on energy expenditure,  
634 somatic repair and reproduction of zebra finches. *J. Exp. Biol.* **208**, 4091-4098.

635

### 636 **Figure Legends**

637

638 Figure 1. (a) Mean values of daily heart rate (DHR) during 208 days of recording for  
639 female common eider ducks (N = 13) synchronised in relation to hatching of eggs. Ups  
640 and downs are identified by a bold trait covering five days of recording. (b) Average  
641 deltas representing the difference between contiguous DHR ups and downs and their  
642 respective confidence intervals. When the confidence intervals exclude the zero line, the  
643 delta is declared significant at 5 % level (see Methods). (c) Mean values of inactive heart  
644 rate (IHR) during 208 days of recording.

645

646 Figure 2. (a) Mean daily heart rate in relation to mean time spent active for 13 females  
647 eiders. (b) Mean inactive heart rate in relation to mean time spent active for 13 females  
648 eiders. The time budget is obtained from continuous recordings of flight and feeding (see  
649 Methods) whereas inactive behaviour was obtained by subtracting active time (flying +  
650 feeding) from 1440 min (24 h). The significance level of both Pearson correlation  
651 coefficients were calculated with a permutation test (see Methods).

652

653 Figure 3. (a) Mean water temperature for female common eiders (N = 13) positioned at  
654 various locations in the Baltic and Wadden sea during the recording period. (b) Mean  
655 values of resting heart rate (RHR) during 208 days of recording for female common  
656 eiders (N = 13) synchronised in relation to hatching of eggs. Mean date (arrows) of moult  
657 and fall migrations are positioned along the season (days from hatching) together with the  
658 occurrence of wing moult (open symbols). (c) Relationship between mean resting heart  
659 rate (n = 13) and mean water temperature shown in Table 2.

660

661

662

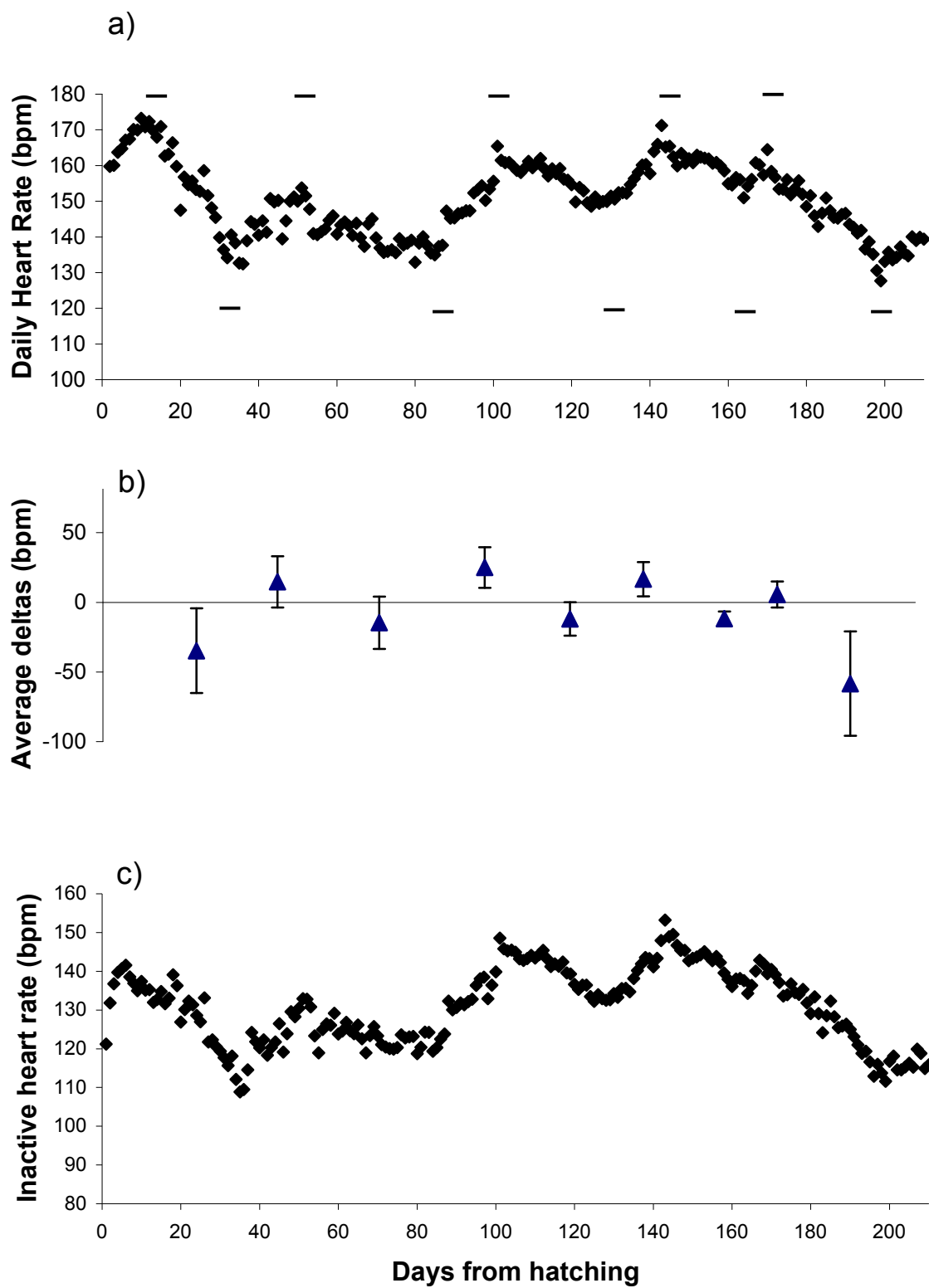


Fig.1



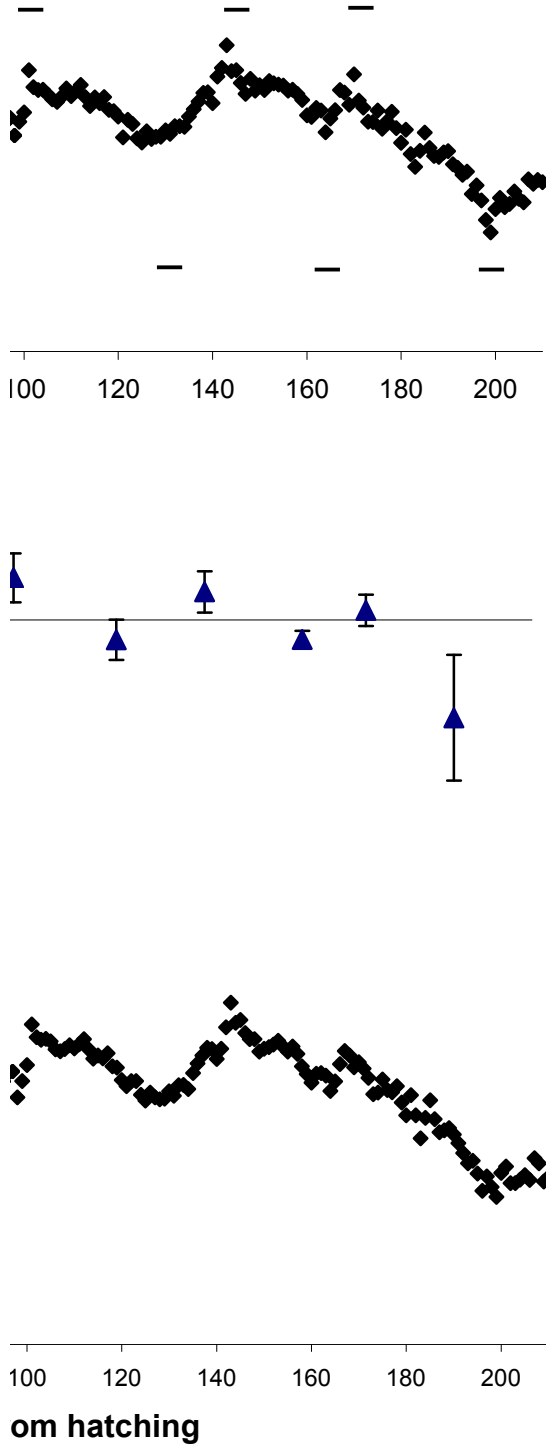


Fig.1

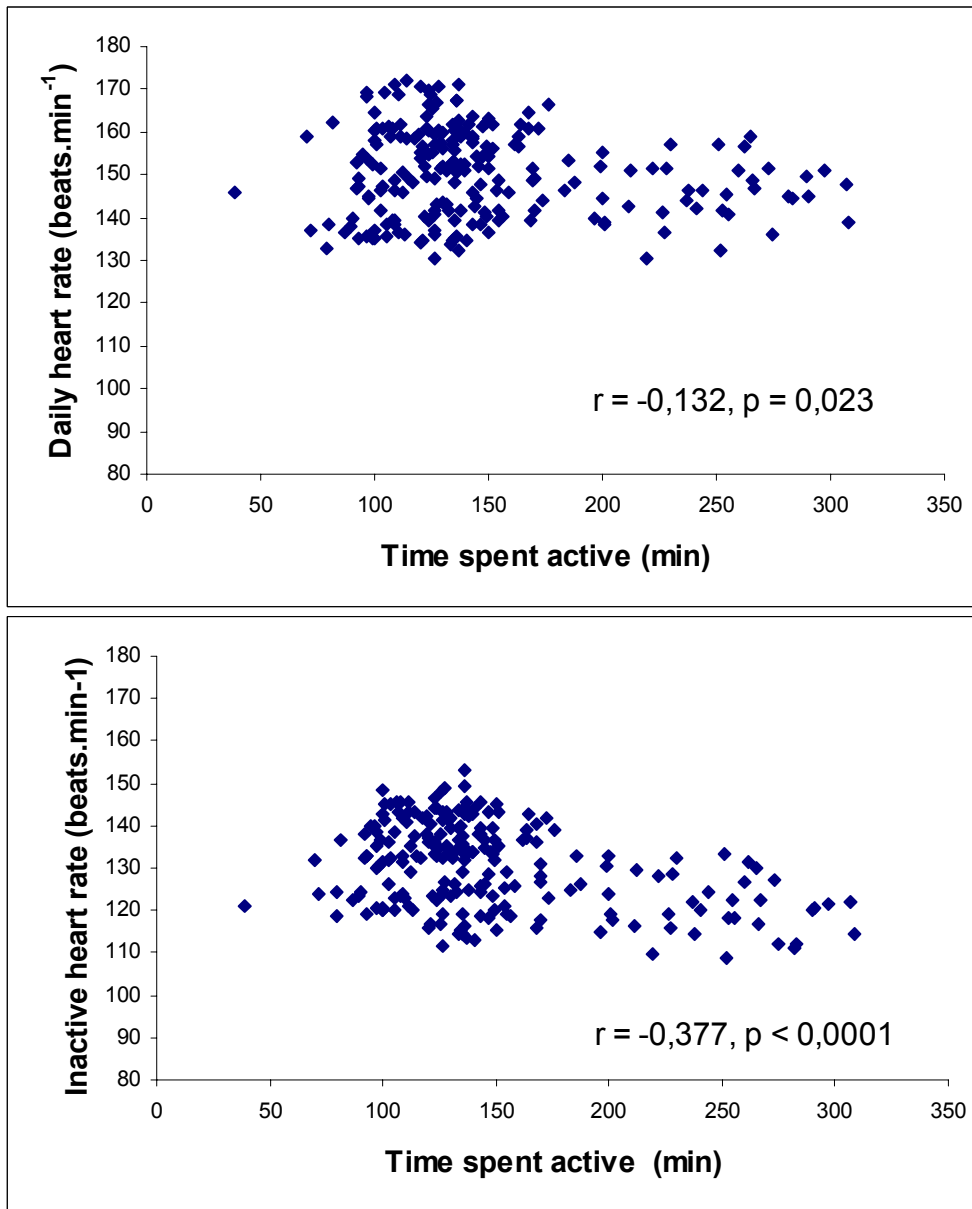


Fig.2

Fig.3.xls

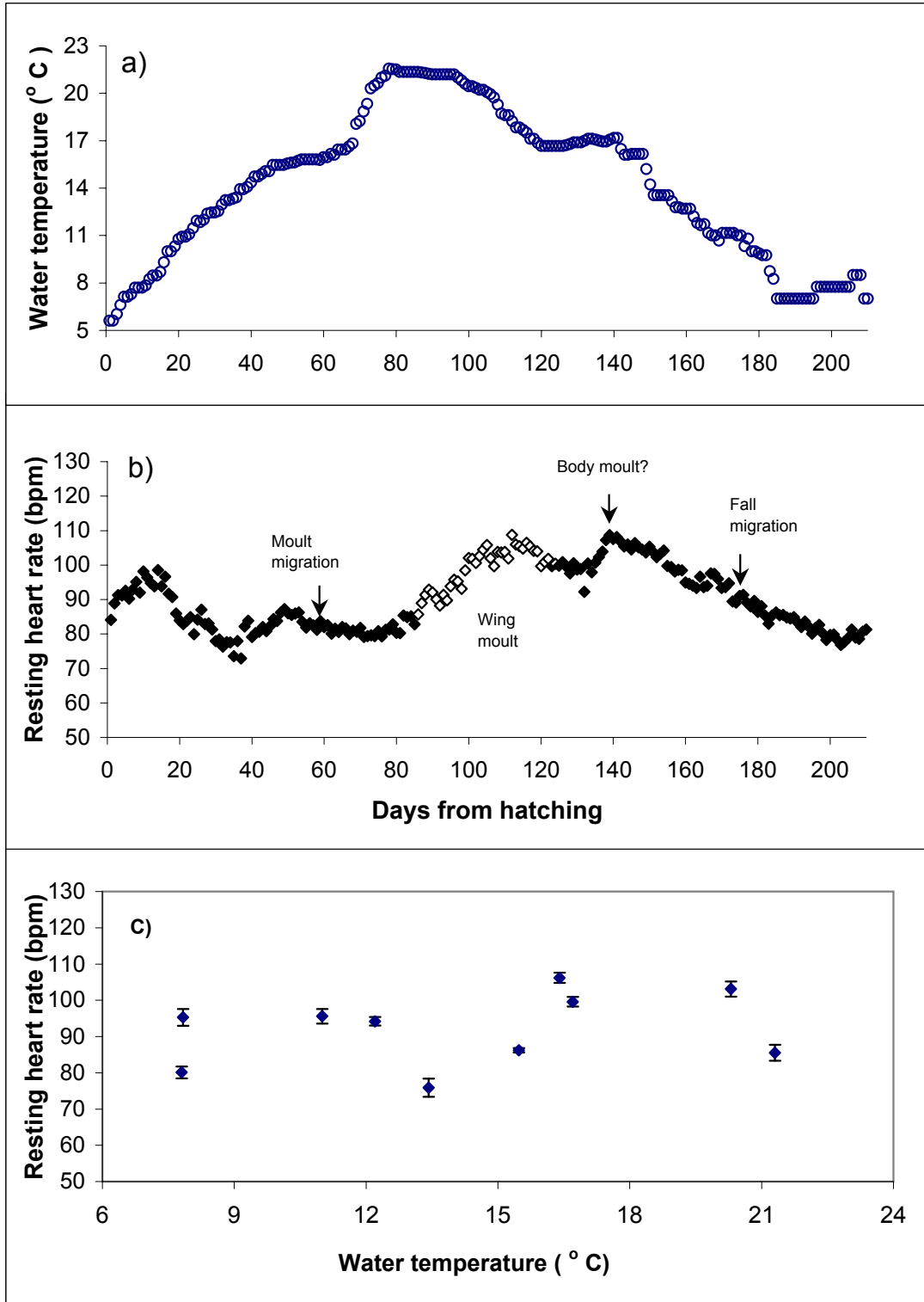


Fig. 3

Table 1. Pearson correlation coefficients ( $r$ ) and coefficients of determination ( $r^2$ ) between various combinations of daily heart rate (DHR), inactive heart rate (IHR), resting heart rate and locomotion activity (LA) at the intra-individual level for 13 females common eiders. Intra-individual correlation coefficients were averaged for which, we calculated confidence intervals using the bootstrap method. When this interval excludes zero, we declared the average correlation coefficient to be significantly different than zero at the 95 % level.

Individual	Cor. DHR.RHR		Cor. DHR.IHR		Cor. DHR.LA		Cor. IHR.LA	
	$r$	$R^2$	$r$	$R^2$	$r$	$R^2$	$r$	$R^2$
WB	0,856	0,733	0,917	0,841	-0,293	0,086	-0,614	0,377
BR	0,799	0,638	0,985	0,970	0,096	0,009	0,282	0,080
OO	0,694	0,482	0,990	0,979	0,229	0,052	-0,050	0,003
OR	0,808	0,653	0,942	0,888	-0,068	0,005	-0,278	0,077
OY	0,500	0,249	0,951	0,905	0,092	0,008	-0,177	0,031
YR	0,649	0,421	0,831	0,691	0,253	0,064	-0,206	0,042
YW	0,875	0,765	0,960	0,921	-0,014	0,000	-0,391	0,153
YY	0,574	0,329	0,770	0,592	0,591	0,349	-0,007	0,000
RW	0,868	0,754	0,936	0,875	0,156	0,024	-0,156	0,024
WO	0,403	0,162	0,935	0,874	0,462	0,213	0,166	0,028
YB	0,342	0,117	0,960	0,921	-0,004	0,000	-0,230	0,053
RR	0,733	0,537	0,943	0,889	0,047	0,002	-0,155	0,024
RB	0,466	0,217	0,937	0,879	0,175	0,031	-0,128	0,016
Mean	0,659	0,466	0,927	0,863	0,132	0,065	-0,150	0,070
sd	0,186		0,061		0,227		0,227	
CI	0,108		0,036		0,132		0,133	
CI+	0,767		0,963		0,264		-0,017	
CI-	0,551		0,891		0,000		-0,282	

Table1

Table 2

Table 2. Estimates of daily energy expenditure of female common eiders at various times (lows and highs of Fig. 1a) after hatching their eggs estimated from daily heart rate. Also shown is the time spent active and water temperature.

Days from hatching	DEE (kJ.kg <sup>-1</sup> )	Time active <sup>1</sup> (min)	Water temperature <sup>2</sup> (°C)
10	971	117	7,8
35	922	262	13,4
50	1003	204	15,5
85	885	86	21,3
103	970	104	20,3
126	923	123	16,7
143	1028	135	16,4
162	1000	129	12,2
169	1022	146	11
199	903	131	7,8

1 Pearson correlation coefficient between DEE and time spent active is 0,091

2 Pearson correlation coefficient between DEE and water temperature is 0,145