Title:

"A circadian clock drives behavioral activity in Antarctic krill (*Euphausia superba*) and provides a potential mechanism for seasonal timing"

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Abstract

Antarctic krill is a species with fundamental importance for the Southern Ocean ecosystem. Their large biomass and synchronized movements, like diel vertical migration (DVM), significantly impact ecosystem structure and the biological carbon pump. Despite decades of research, the mechanistic basis of DVM remains unclear. Circadian clocks help organisms anticipate daily environmental changes, optimizing adaptation. In this study, we used a recently developed activity monitor to record swimming activity of individual, wild-caught krill under various light conditions and across different seasons. Our data demonstrate how the krill circadian clock, in combination with light, drives a distinct bimodal pattern of swimming activity, which could facilitate ecologically important behavioral patterns, such as DVM. Rapid damping and flexible synchronization of krill activity indicate that the krill clock is adapted to a life at high latitudes and seasonal activity recordings suggest a clock-based mechanism for the timing of seasonal processes. Our findings advance our understanding of biological timing and high-latitude adaptation in this key species.

Introduction

Antarctic krill (*Euphausia superba,* hereafter referred to as krill) is a pelagic, up to 6 cm long crustacean, which is endemic to the Southern Ocean. With an estimated biomass of 300 to 500 million tons (*1*), krill is amongst the most abundant wild species worldwide (*2*) and an important prey for predators such as whales, seals and penguins (*3*). Its huge abundance and central position in the food web makes krill a key species of fundamental importance for the functioning of the Southern Ocean ecosystem (*4, 5*).

Krill is mostly found in swarms, the largest of which can be hundreds of meters long and tens of meters in height, likely reflecting a behavioral adaptation to minimize the risk of predation (*6, 7*). Along with their pronounced swarming behavior, krill display diel vertical migration (DVM), a common behavior among many pelagic zooplankton organisms across different taxa found in all oceans (*8*). DVM is commonly characterized by an ascent to the surface layers in the dark of the night to feed and a descent to darker parts of the water column during the day to avoid visually hunting predators (*8, 9*). The regular, synchronized movement of this large biomass significantly shapes the structure of ecosystems (*10*) and has large impacts on biogeochemical cycles (*11, 12*). This includes the recycling of nutrients, stimulation of primary production as well as transport and storage of carbon in the deep ocean (*4*). Although such a "regular" DVM pattern has been commonly observed for krill swarms (*13, 14*), there is considerable behavioral variation, including reverse DVM, higher frequency migrations or no synchronized migrations at all (*13, 15*). While the adaptive advantage of balancing increased predation pressure during the day with the need to feed seems obvious, the underlying mechanisms of DVM and its modulations are not understood. Light is thought to be the most reliable and thus important environmental cue for DVM (*16*). However, a study in the marine copepod *Calanus finmarchicus* indicates that DVM behavior is underpinned by a circadian

57 clock, which helps the animals to adjust behavior, physiology and gene expression to the day-
58 night cycle (*17*). Similarly, a study on Norwegian krill, *Meganyctiphanes norvegica*, suggested
59 swimming activity in krill is under clock control, with increased activity during the dark phase
60 (*18*). In Antarctic krill, similar studies have been conducted to test the hypothesis of a clock
61 involvement in DVM behavior (*19, 20*). While these studies gave first hints that daily
62 behavioral activity in krill is partly under endogenous control, a large variability hindered a
63 clear characterization of the circadian behavioral activity of krill.
64 Previous studies on endogenous rhythms in krill identified the molecular components of a krill
65 circadian clock (*21, 22*) . It has further been shown that the krill circadian clock drives daily
66 rhythms in gene expression, metabolism and behavior (*20, 23, 24*). Part of krill's success lies
67 in its strong adaptation to the extreme seasonality of the Southern Ocean environment (*25*),
68 which is reflected in a fundamental, seasonal regulation in body composition, metabolic
69 activity, feeding, growth (*26, 27*), sexual maturity (*28, 29*) and gene expression (*30, 31*). Results
70 from laboratory studies showed that photoperiod is a major driver for seasonal
71 ecophysiological changes in krill (*32-34*), and it has been suggested that an endogenous
72 timekeeping system (i.e. biological clock) is involved, which uses photoperiod as a
73 synchronizing cue (*33, 35*).
74 Circadian clocks are widespread and well-studied endogenous timing mechanisms which allow
75 an organism to fine-tune biological functions to specific times of the day-night cycle. The
76 endogenous oscillation is produced by transcriptional-translational feedback loops (TTFL) of a
77 set of clock genes and their translation products (*36*), which, under the absence of external
78 cues, produce a temporal signal with a period (τ) of about 24 h. Under natural conditions, the
79 rhythm of the central oscillator is synchronized to exactly 24 h by predictable environmental
80 cycles, called *Zeitgeber*, of which light is usually the most important (*37*). The rhythmic signal

81 of the central oscillator is superimposed on downstream processes, which results in the
82 temporal regulation of several biological functions, such as gene expression, physiology and
83 behavior. Thus, the circadian clock allows organisms to anticipate daily environmental cycles
84 and adapt their biology, ultimately increasing survival and fitness (*38*). Since the circadian
85 clock implicitly measures day length, it is also capable of providing seasonal information.
86 Consequently, the circadian clock has also been shown to be involved in the timing of seasonal
87 functions, such as the induction of reproduction or diapause (*39, 40*).
88 While most knowledge about circadian clocks and their involvement in organism's daily and
89 seasonal processes stems from terrestrial model organisms, little is known about clocks in the
90 marine environment (*41*). This is especially true for marine habitats in polar regions,
91 characterized by extreme photic conditions, including polar night and midnight sun phases.
92 An understanding of the mechanistic underpinnings of biological timing is essential to
93 comprehend how organisms adapt to their specific environment. This is particularly important
94 in times of rapid environmental change, which can alter the temporal synchronization of
95 trophic interactions, including DVM, feeding and swimming behaviour, and, thus, the
96 functioning of whole ecosystems (*42*).
97 In this study, we use the novel Activity Monitor for Aquatic Zooplankter (AMAZE) (*43*) to
98 investigate the mechanistic basis of swimming activity of wild-caught Antarctic krill under
99 controlled environmental conditions onboard a commercial krill fishing vessel. We provide
100 novel evidence that the circadian clock underlies ecologically important behaviors, such as
101 DVM, and discuss how the krill circadian clock could control the timing of seasonal life cycle
102 functions in krill.

103

Results

Two sets of experiments were conducted for this study. The first set consists of two experiments, recording krill swimming activity under simulated short days (experiment 1) or long days (experiment 2), followed by constant darkness conditions (DD). The second set consists of four experiments (experiment S1-S4), recording the swimming activity under DD conditions of krill sampled from the field in summer, late summer, autumn, and winter.

A circadian clock is involved in the daily regulation of swimming activity

To investigate whether a circadian clock is involved in regulating daily rhythms of swimming activity, we sampled krill from the field under short-day conditions in winter (experiment 1, sampling date: May 31st 2021, local photoperiod: 5.5 h) and under long-day conditions in late summer (experiment 2, sampling date: February 16th 2022, local photoperiod: 15.3 h; see Tab. 1 for details). We exposed 11 and 9 sampled individuals to 3 days of light-dark conditions in the activity monitor simulating short-day conditions, and to 5 days of LD simulating long-day conditions, respectively. The photoperiod in the activity monitor approximated the natural photoperiod in the field during the time of sampling. The period of LD was followed by 5 days of constant darkness. The data under LD conditions show that krill swimming activity at both individual and group level increased during the dark phase and showed a strong synchronization with the light-dark cycle provided (Fig. S2b).

As synchronization between individuals and robustness of activity patterns under DD within individuals was strongest in krill from experiment 1 (short-day), we used these data for initial assessments of circadian activity rhythms. Under constant darkness conditions in the short-day experiment, krill swimming activity continued in several individuals (n = 6; 54%) with significant circadian rhythmicity over the 5 days of DD conditions (Fig. 1a and b, Fig. S3),

127 indicating the involvement of a circadian clock in the daily regulation of swimming activity.
128 Nevertheless, in most individuals the amplitude of the rhythm decreased over time (Fig. 1a,
129 Fig. 3a, Fig. S3). The patterns of swimming activity observed at the individual level were
130 reflected at the group level, showing a strong synchronization of increased swimming activity
131 with the dark phase under light-dark conditions (Fig 2a, Fig. S2), and persistent circadian
132 swimming activity under constant conditions with a continuously decreasing amplitude
133 throughout the experiment (Fig. 1c; Fig. 3a: Paired t-test, p-value = 0.005, Fig. 3c: Paired t-
134 test, p-value = 0.012).

135

136 **Light affects the amplitude and phase of swimming activity**

137 Comparing the mean swimming activity of rhythmic individuals over an average day from
138 experiments 1 and 2 under LD conditions reveals a clear synchronization of swimming activity
139 with the dark phase (Fig. 2), visible in the sharp increase in activity around the times of lights-
140 off and lights-on. Furthermore, the time of increased activity during the dark phase exhibits a
141 discernible pattern comprising three distinct activity bouts (Fig. 2). First, an increase in activity
142 is observable in the evening (i.e., end of illumination), followed by a decrease in activity
143 towards midnight. A second increase in swimming activity is observable in the latter half of
144 the dark phase, followed by a decline in activity just before morning. A third short activity bout
145 is observed in the morning (i.e., onset of illumination). This distinctive activity pattern during
146 the dark phase is observed regardless of the photoperiod, resulting in a compression of the
147 three activity bouts in short nights, while they spread out in long nights (Fig. 2), demonstrating
148 that krill can entrain to the long and short days provided. Furthermore, comparing the activity
149 pattern during short-day LD conditions with the respective activity pattern under DD

150 conditions reveals the persistence of evening and late-night activity increases under constant
151 darkness conditions. In contrast, the morning activity bout is not visible (Fig. S5). This suggests
152 that the circadian clock drives the evening and late-night activity bouts, while the morning
153 activity bout is triggered by the onset of illumination in the experimental set-up. Furthermore,
154 the amplitude of swimming activity is higher under LD than DD conditions (Fig. 3 B, Mann-
155 Whitney-U test: *p-value* < 0.001; Fig. 3 D, *p-value* < 0.001).
156

157 **Circadian swimming activity persists with a stable phase after entrainment to a wide range**
158 **of natural photoperiods**

159 To gain insights into potential seasonal differences in the regulation of krill swimming activity,
160 we sampled krill from the field in summer (experiment S1), late summer (experiment S2),
161 autumn (experiment S3), and winter (experiment S4) and recorded their swimming activity for
162 4 to 8 days under constant darkness conditions, without previous entrainment in the activity
163 monitor (see Tab. 1 for details).
164 Similar to our previous analysis of individual krill swimming activity under constant darkness
165 conditions, a large proportion of the recorded individuals in each experiment (44% to 100%)
166 exhibited significant rhythmicity in the circadian range (Fig. S7-10), which is also reflected in
167 rhythmic swimming activity at the group level (Fig. 4a-d, Fig. S6). Interestingly, the pattern of
168 circadian swimming activity, with two peaks during the dark phase, appears to become clearer
169 and higher in amplitude with shortening photoperiods towards winter (Fig. 4a-d). This is
170 supported by rhythm analysis of the group behavior, which revealed a higher power and a
171 period estimation closer to 24 h with shortening photoperiod (Fig. S11). While the morning
172 peak is mainly visible in autumn and winter, the late-night activity peak is visible throughout
173 the year, at the same time of the subjective 24-h-day (Fig. 4e-h). This pattern appears

irrespective of the natural photoperiod the animals were entrained to in their natural environment before they were sampled for the respective experiment.

Synchronized diel vertical migration in the field is present across a wide range of natural photoperiods

To investigate whether krill swarms exhibited daily behavioral patterns in swimming behavior in the field before they were sampled for seasonal experiments, hydroacoustic data were recorded from the fishing vessel in the days preceding sampling for seasonal experiments described above (for vessel positions during hydroacoustic recordings see Fig. S1). The data provide information about the vertical distribution of krill swarms in the upper water column (<220 m) below the vessel over time (Fig. 5a-d). The hydroacoustic data in summer, late summer and autumn show that krill swarms performed diel vertical migration, highly synchronized with the local light regime (Fig. 5a-c). The swarms regularly ascended to the surface layers (<50 m) around sunset and returned to deeper layers (>100 m) around sunrise. In winter, little signal is visible in the upper water layer, indicating very low density or the absence of krill swarms (Fig. 5d) and consequently, krill for experiment S4 was sampled from deeper waters (185 m). This agrees with observations that krill shift their distribution to deeper waters in winter, where krill have been observed to perform DVM also during this time of year(*15*). The available data only covers the upper 220 m of the water column, meaning that the dynamics of swarms below this depth are missed.

194 ## Discussion:

195 Our study revealed continued rhythmic swimming activity under constant darkness, showing
196 the involvement of a circadian clock in swimming behavior of krill, as has been previously
197 shown for other marine pelagic crustaceans (*17, 18, 44*). To date, only three studies have
198 investigated the involvement of a circadian clock in the regulation of swimming behavior in
199 krill species, including *E. superba* (*19, 20*) and *Meganyctiphanes norvegica* (*18*). Data from
200 Piccolin et al. (*20*) showed a strong damping of the amplitude and indication of a remarkably
201 short (~12 h) free running period (FRP) of vertical swimming behavior of a group of krill under
202 constant darkness (*20*). The short period complements the pattern of swimming activity under
203 DD conditions on the individual level found in the present study, suggesting that the ~12 h
204 rhythm in group swimming behavior in Piccolin et al. (*20*) could have resulted from a bimodal
205 activity pattern at the individual level. Bimodal locomotor activity rhythms are well known
206 across different species, possibly most prominently in the fruit fly (*Drosophila melanogaster*),
207 were two coupled endogenous oscillators control morning and evening activity bouts (*45*). In
208 our experiments, we observed a strong weakening of the evening activity under constant
209 conditions, especially after entrainment to long days, while the late-night activity peak
210 appeared more robust. The differential variation in the morning and late-night activity
211 suggests that the circadian activity bouts could also be controlled separately at the neuronal
212 level in krill.

213 Comparing the daily pattern of swimming activity under LD conditions with those under DD,
214 it is clear that the amplitude of the rhythm was significantly higher under LD than under DD.
215 In addition, the activity under LD was organized into three activity phases, one in the evening,
216 one in the late night and a smaller one in the morning, whereas under DD there were a
217 maximum of two activity phases during the subjective night. Light is known to play a dual role

218 in regulating daily behavioral activity. On the one hand, light functions as a *Zeitgeber*, which
219 synchronizes the circadian clock with the day-night cycle, a process called *entrainment* (*46*).
220 On the other hand, it can directly affect clock output functions such as physiology and
221 behavior, known as *masking* (*46, 47*). The masking response depends on the activity type of
222 the organism, and light was shown to promote activity in diurnal animals while inhibiting
223 activity in nocturnal ones (*48, 49*). Krill are regularly observed performing nocturnal DVM (*13,*
224 *14*), including in the present study, with higher activity during the night, including the times of
225 twilight, related to foraging and feeding activity and vertical ascend and descend movements
226 (*50, 51*). The observed effect of light on reducing krill swimming activity under LD conditions
227 in our study is thus in line with previous concepts of the exogenous effects of light on nocturnal
228 organisms (*46, 47*). However, under LD conditions, the observed swimming activity pattern in
229 krill is not solely driven by light. The increase in activity in the evening and the second half of
230 the dark phase under LD conditions flexibly adjust to the length of the night and seem to
231 persist under constant darkness, suggesting these bouts to be under clock control, as
232 discussed above.
233 The reduced amplitude in swimming activity after the switch to DD conditions and further
234 damping of amplitude over time under constant darkness has been found in other studies in
235 krill (*20*) and other marine zooplankton organisms (*17, 44*). A rapidly damping output signal
236 under constant conditions could indicate a weak, low amplitude circadian oscillator, where
237 the damping amplitude of the clock output signal is caused by a rapid desynchronization of
238 clock neurons, when Zeitgeber signals are absent (*52*). Interestingly, weak oscillators have
239 been found in different fruit fly species (*53, 54*), the Svalbard reindeer (*55*), and Svalbard
240 ptarmigan (*56*), native to high latitudes and species that exhibit pronounced seasonal
241 rhythmicity, such as aphids (*57, 58*). It is known that weaker clocks are more flexible and can

242 be entrained more efficiently in comparison to those that are more rigid and self-sustained
243 (*52, 59*). This could confer a significant adaptive advantage to species inhabiting environments
244 characterized by extreme photic conditions (*53, 54, 60*) or species that rely on precise
245 photoperiodic time measurement for accurate seasonal adaptation. In the Southern Ocean,
246 photoperiod becomes extreme around the solstices, and its daily change is rapid around the
247 spring and autumn equinoxes. A weak oscillator could, therefore, ensure that krill remains
248 synchronized with the extreme and rapidly changing photic conditions and, thus, its
249 environment.
250 Indeed, our data from activity recordings under DD conditions of krill sampled in different
251 seasons indicate persistent circadian swimming activity, irrespective of the season. Our
252 findings suggest that the krill circadian clock remains functional and drives rhythmic
253 behavioral output in natural conditions throughout the year. This finding is complemented by
254 the hydroacoustic recordings in the days preceding krill sampling for activity experiments. In
255 summer, late summer and autumn, krill swarms exhibited apparent diel vertical migration
256 behavior, synchronized to the natural photoperiod. While we did not observe DVM in the
257 upper water column during sampling in winter, acoustic recordings from other studies in
258 winter indicate that krill may perform DVM also in winter (*61*), potentially in deeper layers.
259 Our data show that light acts in concert with, and is likely modulated by, the circadian clock
260 to achieve the observed daily regulation in krill swimming activity. Nevertheless, further
261 studies at the behavioral, molecular, and neuronal levels are required to elucidate the
262 endogenous and exogenous effects of light and the circadian clock on the different behavioral
263 aspects observed and to gain further insights into the characteristics of the molecular
264 mechanism of the krill clock.
265

266 In a high-latitude region, such as the Southern Ocean, the timing of crucial life history
267 functions to seasonal fluctuations of light, food availability, and sea ice extent is key to
268 organisms' success. In contrast to early studies, which suggested that only food availability
269 was the main driver of krill's seasonal physiological functions (*62*), later studies have revealed
270 that photoperiod, likely in concert with circannual timing, drives the seasonal regulation of
271 key lifecycle functions (*26, 32-35*).
272 Although the exact molecular mechanism of seasonal timing is still unknown, there is clear
273 evidence that the circadian clock measures day length and provides information about the
274 seasonal progression (*39, 40*). Several studies in insects (*63*) and plants (*64*) support the
275 external coincidence model (*65*). This model explains how the circadian clock regulates the
276 expression of a light-sensitive substance during the late dark phase. Under long days, this
277 substance is degraded by morning light, suppressing a winter response, while the substance
278 accumulates under short days (and long nights), inducing a winter-like response. In the case
279 of krill, this response could include sexual regression, increased lipid accumulation (*28, 33*),
280 and a reduction in overall metabolism (*26, 35*). Evidence from photoperiodic studies in insects
281 indicates that the clock protein TIMELESS (TIM) may be a suitable candidate to fulfil the role
282 of the light-sensitive substance (*40*). In krill, phylogenetic and molecular characterizations
283 have indicated a light-dependent degradation of TIM via the clock photoreceptor
284 CRYPTOCHROME 1 (*21*). As the basic molecular architecture of circadian clocks is highly
285 conserved across arthropods, these findings could indicate that TIM may be involved in
286 seasonal timing in krill as well.
287 Our data on krill circadian swimming activity of animals sampled from the field in different
288 seasons show a remarkably stable peak in late-night activity (Fig. 4e-h). Assuming that the
289 behavioral activity under constant darkness reflects the direct influence of the circadian clock,

290 our findings suggest that the krill circadian clock can measure the length of the day (or night)
291 via external coincidence. The increased rhythmicity and synchronization of animals under
292 shortening photoperiods indicate the potential of precise daylength measurement especially
293 during seasonal physiological changes (*26*). The day length information could subsequently be
294 used directly to synchronize physiological functions with the environment, and/or indirectly
295 to entrain a circannual clock (*66*), which subsequently drives rhythmic annual output. While
296 the individual activity recordings provide first insights into potential mechanisms for the
297 control of daily and seasonal processes in krill, it must be noted that behavioral output does
298 not always reflect the detailed characteristics of the circadian oscillator (*67*). Further
299 investigations into the molecular clock mechanism and its downstream functions across
300 seasons are needed to clarify the involvement of the circadian clock in photoperiodism and
301 circannual rhythms in krill.
302
303 Circadian clocks have the potential to anticipate the daily light-dark cycle and thereby enable
304 a fine-tuned synchronization of behavioral and physiological functions with environmental
305 cycles, which increases an organism's fitness (*38*). Consequently, synchronizing krill behavioral
306 output with the day-night cycle provides potential adaptive advantages on various levels.
307 DVM of zooplankton seems to be a trade-off between predation risk and feeding (*16*). As it is
308 complex to directly sense predation risk, light is a robust proxy (*68*) and a circadian clock
309 provides an additional mechanism to anticipate and synchronize the times of vertical
310 migration, active feeding and foraging with nighttime. Our data show that light, in addition to
311 the clock, ensures robust, synchronized activity patterns. Differences between entrained and
312 free-running conditions further indicate that evening and late-night activity are under clock
313 control. At the same time, the activity peak in the morning is a response to the onset of light.

314 A differential regulation of certain phases of DVM has previously been suggested for marine
315 zooplankton species (*17, 44*). A clock driven evening ascent would allow for the anticipation
316 of sunset and thus the timely initiation of migration when krill swarms are in deeper water
317 layers, resulting in optimized surface feeding time. The clock-controlled late-night activity
318 could support a second feeding bout and preparation for a timely morning descent before
319 sunrise increases visual predation risk.
320 In-situ hydroacoustic observations have demonstrated increased swimming speeds during
321 nighttime for both *M. norvegica* and *E. superba* (*50, 51*). This increase is likely related to active
322 foraging and feeding behavior, as higher swimming speeds increase the chance of
323 encountering food particles and thus increase food uptake. Nevertheless, swimming in krill is
324 energetically costly and can make up more than 70% of the total energy expenditure (*69*).
325 Reducing swimming activity to the baseline level during the day thus represents a significant
326 opportunity for energy conservation.
327 At high latitudes, biological clocks are exposed to extreme changes in photoperiod throughout
328 the year, including phases of midnight sun and polar night. Many organisms inhabiting polar
329 regions show arrhythmicity, at least during parts of the year, and potential mechanisms
330 include direct impacts on the central oscillator and uncoupling between output functions and
331 the circadian clock (*60*). Previous investigations of krill clock gene expression in the laboratory
332 indicated a weaker oscillation of the krill clock and metabolic output functions under
333 simulated extreme photic conditions (i.e. LL and LD 3:21), compared to more balanced day-
334 night cycles (*70*). Data on the transcriptomic and organismic level further indicate that krill can
335 flexibly adapt to the photic conditions at different latitudes (*31, 33*). This aligns with our
336 findings, where krill, sampled across a wide range of photoperiods, remain behaviorally
337 synchronized with the local photoperiod under entrained conditions, but show reduced

338 endogenous rhythmicity under DD after entrainment to long day conditions. Krill inhabits a
339 broad range of latitudes, including regions with clear day-night cycles and regions with polar
340 night and midnight sun during the seasonal extremes (*71*). At the same time krill is transported
341 rapidly between regions via ocean currents (*72*), potentially exposing the same individuals to
342 various photic conditions during their life span. A weak circadian clock in close interaction with
343 the ambient light regime could thus allow krill to adopt the best strategy for the environmental
344 conditions prevailing in the current location. For example, at high latitudes, the adaptive
345 advantage of predator evasion with DVM diminishes in summer when the upper water column
346 is illuminated constantly. In this case, arrhythmic behavior would allow flexibly exploiting food
347 patches at any time of day, when the need for food is high to fuel reproductive processes (*73*).
348 Further, a study on the marine annelid *Platynereis dumerilii* recently highlighted a differential
349 regulation of clock-controlled processes, where behaviorally arrhythmic individuals show
350 increased rhythmicity in physiological output (*67*), indicating the possibility of flexibly adapting
351 timed processes to environmental conditions.
352 Adapting to a fluctuating environment involves significant seasonal physiological changes in
353 krill to survive the harsh, food-scarce winter (*26, 74*). Crucial processes, such as accumulating
354 enough lipids, must begin long before winter, requiring an anticipatory mechanism. Recent
355 evidence suggests that photoperiod and circannual timing drive these seasonal changes in krill
356 physiology, using photoperiod as a proxy for seasonal progression (*26, 32-35*). Rapid warming
357 in the Southern Ocean is observed to cause shifts in phytoplankton bloom timing (*75*), which
358 may lead to temporal mismatches between photoperiod synchronized krill physiology and
359 their primary food source, with the potential to reduce reproductive success (*42*) and
360 negatively impact the krill-dependent ecosystem. Understanding the mechanistic basis of

species adaptation and their flexibility is therefore crucial in times of rapid environmental change (*42*).

We used a new krill activity monitor to identify the endogenous underpinnings of swimming activity of wild-caught Antarctic krill. The results of our experiments provide clear evidence that a circadian clock underlies krill behavior, driving a distinct bimodal pattern of clock-controlled swimming activity. The damping of the activity amplitude under constant darkness suggests that krill possesses a "weak", highly flexible circadian clock adapted to a life under extreme and variable conditions. These findings are further supported by experiments conducted under simulated short-day and long-day conditions, demonstrating a flexible adaptation of the krill swimming activity pattern to a wide range of photoperiods. The results provide novel insights into the combined impacts of the circadian clock and light on the daily regulation of swimming activity. Furthermore, hydroacoustic recordings demonstrate that most krill swarms sampled exhibited synchronized DVM in the field, indicating that in this region, krill remain behaviorally synchronized across a wide range of photoperiods. Seasonal recordings of field-entrained circadian swimming activity suggest that the krill circadian clock may be involved in measuring day length and, consequently, the timing of seasonal events. The findings provide novel insights into the mechanistic underpinnings of daily and seasonal timing in Antarctic krill, a marine pelagic key species, endemic to a high-latitude region. Mechanistic studies are a prerequisite for understanding the processes by which krill adapt to their specific environment and their flexibility in responding to environmental changes.

Methods:

Animal collection

Sampling of Antarctic krill (*Euphausia superba*) for experimental purposes was conducted at various points throughout the seasonal cycle from the Bransfield Strait and South Orkney Island regions (for detailed locations, please refer to Tab. 1 and Fig. S1) by use of the continuous fishing system onboard the krill fishing vessel Antarctic Endurance. During fishing, the vessel trawled at a speed of 1.5–2 knots using a commercial trawl. The krill was pumped on board by creating a vacuum in a hose connected to the cod-end of the trawl. On board, the krill was separated from the water on a metal grate, from which the krill was sampled. Sampled krill were kept in surface seawater at densities of ~1 Ind. per L, at 1°C under constant darkness for an acclimation period between 4 - 10 h, to reduce the impact of sampling stress and to check for the condition of krill individuals before transfer to the experimental set-up. Prior to the start of each experiment, the experimental columns were filled with seawater sampled from the surface. For experiments conducted during summer, late summer and autumn, when high food concentrations were to be expected, seawater was filtered through a 0.25 µm filter. During winter, field measurements showed a very low surface chlorophyll a level (below 0.5 µg/L; data not shown) and any potential larger particles in the water were excluded by sedimentation. The columns were distributed to the set-up and the temperature was adjusted to 0.8°C. Only krill that were unharmed and lively were selected for behavioral experiments in the set-up. At the end of each experiment, the krill were removed from the set-up, evaluated for overall condition and total length (measured from the front of the eye to the tip of the telson, excluding setae), and their sex was determined under a stereo microscope.

407 **Krill swimming activity recording and experimental design**

408 Krill swimming activity was recorded using the AMAZE setup, described in detail in Hüppe et
409 al. (*43*). In short, the recording principle is based on krill swimming in vertical acrylic
410 experimental columns (height: 80 cm, diameter: 9 cm). Each column contains five infrared (IR)
411 detector modules, equally spaced over the height of the column. Vertical movements of krill
412 are detected by IR light beam breaks. A computer on each column controls data acquisition
413 and stores beam break data. The experimental columns are placed in a compressor cooled
414 incubator, which allows for a precise temperature control. Programmable LED light bars in the
415 top of the incubator, simulate underwater light spectra and daily light intensity cycles.

416 To investigate the behavioral activity of individual krill under light-dark conditions, 12 krill
417 were exposed to three days of simulated short-day conditions (experiment 1), and 10 krill to
418 5 days of simulated long-day conditions (experiment 2). Due to technical problems, only data
419 from 11 krill were analyzed from experiment 1 and data from 9 krill from experiment 2. Light-
420 dark cycles were adjusted to a photoperiod of 5.5 h simulating short days and 15 h simulating
421 long days. Day length was arranged symmetrically around local solar noon, to approximate
422 the natural photoperiodic light regime at the time of sampling. The intensity and spectrum in
423 the activity monitor were adjusted to field measurements, as described in Hüppe et al.(*43*). In
424 short, light regime increased and decreased in a linear fashion from a maximum light intensity
425 of 8.8 $mW*m^{-2}$ during midday. To further investigate the involvement of an endogenous clock
426 in krill activity, the initial phase of day-night simulations was followed by 5 days of constant
427 darkness conditions in both experiments.

428 To investigate seasonal changes in krill circadian activity, four additional experiments (S1-S4)
429 were conducted in summer (sampling date: January 22nd 2022), late summer (sampling date:

430 March 1st 2022), autumn (sampling date: March 15th 2022) and winter (sampling date: June
431 10th 2022). For these experiments, krill were caught at the dates described above and after
432 acclimation, 10 animals per experiment were distributed to the activity monitor, where their
433 activity was recorded for 4 to 8 days under constant darkness conditions (see Tab. 1 for
434 details).

435 **Behavioral data analysis**

436 *Experiments 1 and 2 (LD-DD)*

437 Swimming activity of krill individuals was calculated from raw beam breaks as described in
438 Hüppe et al.(*43*). In short, we only considered upward swimming movements of individuals to
439 separate baseline activity from increased activity, as krill are negatively buoyant. Beam breaks
440 caused by upward swimming were summed over 10-minute intervals and normalized between
441 0 and 1 for each individual. Data were smoothed by a centered moving average, with a
442 smoothing window of 6 data points under light-dark simulations and 24 data points under
443 constant darkness conditions.

444 To calculate the group activity of rhythmic krill under DD conditions, we first detrended the
445 normalized swimming activity data of each individual to account for individual variability in
446 baseline shifts of activity over time. This was done by subtracting the daily mean activity from
447 each activity value of the same day. As the individual free running period (FRP) varies between
448 individuals, in the next step we corrected the data for each individual's FRP and assigned them
449 to a 24 h day. To achieve this, we tested the detrended and smoothed activity data of each
450 krill for rhythmic activity and determined the period of the rhythm using the Lomb-Scargle-
451 Periodogram analysis (LSP). We considered individuals with a period τ between 20 and 28
452 hours, a power of >= 50, and a p-value of less than 0.01 to be significantly rhythmic. The
453 activity data of each rhythmic krill were subsequently assigned a new modified timestamp to

454 cover the individual FRP within a 24-hour experimental day. This maintains the original
455 resolution of the time series data while allowing for comparative analysis of individuals with
456 differing FRPs. Group behavior was determined by calculating the mean and standard error of
457 the mean (s.e.m.) per 10-minute time interval and subsequent smoothing, as described above.
458 Average day analysis for a group of krill under LD cycles was done by determining the mean
459 and s.e.m. per 10-minute time interval of a 24 h day over all experimental days and all
460 individuals in one experiment and subsequent smoothing. Average day analysis for a group of
461 krill under DD conditions were done in the same way, but based on the detrended and FRP-
462 corrected data. The amplitude of swimming activity under LD and DD conditions was based
463 on individual, normalized, detrended, and smoothed activity data, and was calculated for
464 every experimental day as the difference between the daily maximal and minimal swimming
465 activity. Differences in the activity amplitude between light conditions (i.e. LD vs. DD) were
466 tested with the Mann-Whitney-U-Test and amplitude differences between the first and last
467 day under DD conditions were tested with a paired t-test (R *stats* package version 4.1.2). All
468 statistical analysis assumed a significance level of $p > 0.05$.
469
470 *Seasonal experiments S1-S4 (DD)*
471 Group activity and average day activity for seasonal experiments (experiments S1-S4) was
472 done as for DD conditions described above, but only the first 4 days of activity of each
473 experiment were considered. This was to allow for a better comparison and to account for the
474 effect of damping rhythms after several days under DD.
475 All data handling, analysis and visualization was done with the *R programming language*
476 (version 4.1.2 (*76*)) in *RStudio* (version 2023.12.1.402), using the *tidyverse* package (version

2.0.0 (*77*)). Rhythm analysis and period estimation was done with the R package *lomb* (*78*), local sun data (i.e. sunset and sunrise) were retrieved from the *suncalc* package (version 0.5.1).

Hydroacoustic data recording and visualization

Hydroacoustic data were collected using a hull-mounted SIMRAD ES80 echosounder (Kongsberg Maritime AS) aboard the Antarctic Endurance in the days preceding the sampling for each of the seasonal behavioral experiments (S1-S4, i.e. summer, late summer, autumn, and winter). The signal received from the 200 kHz band was used to visualize the vertical distribution of krill swarms beneath the ship. The raw acoustic data from the summer, late summer, and autumn periods were converted to mean volume backscattering strength and binned to a time resolution of 1 s and depth bins of 0.5 m using Echopype (*79*). For the period of winter, raw acoustic data were not available. An alternative approach was employed, utilizing a novel method proposed by Bahlburg et al. (*80*), to reconstruct the backscattering signal from a dataset of screenshots displaying the visualized hydroacoustic signal of the echosounder.

As data is only used during fishing periods and the vessel is specifically targeting *E. superba*, which occurs in large monospecific aggregations, it is highly probable that the recorded signal represents krill swarms. Data handling and visualization were done with R in RStudio, using the packages *tidyverse* and *scico* (version 1.3.1).

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References

1. A. Atkinson, V. Siegel, E. A. Pakhomov, M. J. Jessopp, V. Loeb, A re-appraisal of the total biomass and annual production of Antarctic krill. *Deep Sea Research Part I: Oceanographic Research Papers* **56**, 727-740 (2009).
2. Y. M. Bar-On, R. Phillips, R. Milo, The biomass distribution on Earth. *Proc Natl Acad Sci U S A* **115**, 6506-6511 (2018).
3. P. N. Trathan, S. L. Hill, in *Biology and Ecology of Antarctic Krill,* V. Siegel, Ed. (Springer International Publishing, Cham, 2016), chap. Chapter 9, pp. 321-350.
4. E. L. Cavan *et al.*, The importance of Antarctic krill in biogeochemical cycles. *Nature Communications* **10**, 1-13 (2019).
5. R. Trinh, H. W. Ducklow, D. K. Steinberg, W. R. Fraser, Krill body size drives particulate organic carbon export in West Antarctica. *Nature* **618**, 526-530 (2023).
6. G. A. Tarling *et al.*, Variability and predictability of Antarctic krill swarm structure. *Deep Sea Research Part I: Oceanographic Research Papers* **56**, 1994-2012 (2009).
7. W. M. Hamner, P. P. Hamner, Behavior of Antarctic krill (Euphausia superba): schooling, foraging, and antipredatory behavior. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 192-202 (2000).
8. K. Bandara, Ø. Varpe, L. Wijewardene, V. Tverberg, K. Eiane, Two hundred years of zooplankton vertical migration research. *Biological Reviews*, (2021).
9. G. C. Hays, A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. *Hydrobiologia* **503**, 163-170 (2003).
10. R. C. Nichols *et al.*, Intra-seasonal variation in feeding rates and diel foraging behaviour in a seasonally fasting mammal, the humpback whale. *R Soc Open Sci* **9**, 211674 (2022).
11. O. Aumont, O. Maury, S. Lefort, L. Bopp, Evaluating the Potential Impacts of the Diurnal Vertical Migration by Marine Organisms on Marine Biogeochemistry. *Global Biogeochemical Cycles*, (2018).
12. K. M. Archibald, D. A. Siegel, S. C. Doney, Modeling the Impact of Zooplankton Diel Vertical Migration on the Carbon Export Flux of the Biological Pump. *Global Biogeochemical Cycles* **33**, 181-199 (2019).
13. M. Godlewska, Vertical migrations of krill. *Euphausia superba*, 9-63 (1996).

14. I. Everson, Variations in vertical distribution and density of krill swarms in the vicinity of South Georgia. (1983).
15. D. Balburg *et al.*, Plasticity and seasonality of the vertical migration behaviour of Antarctic krill using acoustic data from fishing vessels. *R Soc Open Sci* **10**, 230520 (2023).
16. J. Ringelberg, E. van Gool, On the combined analysis of proximate and ultimate aspects in diel vertical migration (DVM) research. *Hydrobiologia* **491**, 85-90 (2003).
17. N. S. Häfker *et al.*, Circadian Clock Involvement in Zooplankton Diel Vertical Migration. *Current Biology* **27**, 2194-2201.e2193 (2017).
18. J.-P. Velsch, G. Champalbert, Swimming activity rhythms in Meganyctiphanes norvegica. *COMPTES RENDUS DE L ACADEMIE DES SCIENCES SERIE III-SCIENCES DE LA VIE-LIFE SCIENCES* **317**, 857-862 (1994).
19. E. Gaten, G. Tarling, H. Dowse, C. Kyriacou, E. Rosato, Is vertical migration in Antarctic krill (Euphausia superba) influenced by an underlying circadian rhythm? *Journal of Genetics* **87**, 473-483 (2008).
20. F. Piccolin, L. Pitzschler, A. Biscontin, S. Kawaguchi, B. Meyer, Circadian regulation of diel vertical migration (DVM) and metabolism in Antarctic krill Euphausia superba. *Scientific Reports* **10**, 16796 (2020).
21. A. Biscontin *et al.*, Functional characterization of the circadian clock in the Antarctic krill, Euphausia superba. *Scientific Reports* **7**, 1-13 (2017).
22. C. Shao *et al.*, The enormous repetitive Antarctic krill genome reveals environmental adaptations and population insights. *Cell*, (2023).
23. M. Teschke, S. Wendt, S. Kawaguchi, A. Kramer, B. Meyer, A Circadian Clock in Antarctic Krill: An Endogenous Timing System Governs Metabolic Output Rhythms in the Euphausid Species Euphausia superba. *PLoS ONE* **6**, e26090 (2011).
24. A. Biscontin *et al.*, Analysis of the circadian transcriptome of the Antarctic krill Euphausia superba. *Scientific Reports* **9**, 1-11 (2019).
25. H. W. Ducklow *et al.*, Marine pelagic ecosystems: the west Antarctic Peninsula. *Philos Trans R Soc Lond B Biol Sci* **362**, 67-94 (2007).
26. B. Meyer *et al.*, Seasonal variation in body composition, metabolic activity, feeding, and growth of adult krill Euphausia superba in the Lazarev Sea. *Marine Ecology Progress Series* **398**, 1-18 (2010).
27. L. B. Quetin, R. M. Ross, Behavioral and Physiological Characteristics of the Antarctic Krill, Euphausia superba. *American Zoologist* **31**, 49-63 (1991).
28. S. Kawaguchi, T. Yoshida, L. Finley, P. Cramp, S. Nicol, The krill maturity cycle: a conceptual model of the seasonal cycle in Antarctic krill. *Polar Biology* **30**, 689-698 (2007).
29. V. Siegel, Krill stocks in high latitudes of the Antarctic Lazarev Sea: seasonal and interannual variation in distribution, abundance and demography. *Polar Biology* **35**, 1151-1177 (2012).
30. P. J. Seear, W. P. Goodall-Copestake, A. H. Fleming, E. Rosato, G. A. Tarling, Seasonal and spatial influences on gene expression in Antarctic krill Euphausia superba. *Marine Ecology Progress Series* **467**, 61-75 (2012).
31. F. Höring *et al.*, Seasonal gene expression profiling of Antarctic krill in three different latitudinal regions. *Marine Genomics*, 100806 (2020).
32. M. Brown, S. Kawaguchi, R. King, P. Virtue, S. Nicol, Flexible adaptation of the seasonal krill maturity cycle in the laboratory. *Journal of Plankton Research* **33**, 821-826 (2011).
33. F. Höring, M. Teschke, L. Suberg, S. Kawaguchi, B. Meyer, Light regime affects the seasonal cycle of Antarctic krill (*Euphausia superba*): impacts on growth, feeding, lipid metabolism, and maturity. *Canadian Journal of Zoology* **96**, 1203-1213 (2018).
34. M. Teschke, S. Kawaguchi, B. Meyer, Simulated light regimes affect feeding and metabolism of Antarctic krill, Euphausia superba. *Limnology and Oceanography* **52**, 1046-1054 (2007).
35. F. Piccolin *et al.*, The Seasonal Metabolic Activity Cycle of Antarctic Krill (Euphausia superba): Evidence for a Role of Photoperiod in the Regulation of Endogenous Rhythmicity. *Frontiers in Physiology* **9**, (2018).

36. K. Tomioka, A. Matsumoto, A comparative view of insect circadian clock systems. *Cellular and Molecular Life Sciences* **67**, 1397-1406 (2010).
37. T. Roenneberg, S. Daan, M. Merrow, The Art of Entrainment. *Journal of Biological Rhythms* **18**, 183-194 (2003).
38. S. Krittika, P. Yadav, Circadian clocks: an overview on its adaptive significance. *Biological Rhythm Research* **51**, 1109-1132 (2019).
39. D. S. Saunders, Dormancy, Diapause, and the Role of the Circadian System in Insect Photoperiodism. *Annu Rev Entomol* **65**, 373-389 (2020).
40. C. Helfrich-Förster, Neuropeptidergic regulation of insect diapause by the circadian clock. *Curr Opin Insect Sci*, 101198 (2024).
41. K. Tessmar-Raible, F. Raible, E. Arboleda, Another place, another timer: Marine species and the rhythms of life. *BioEssays* **33**, 165-172 (2011).
42. B. Helm *et al.*, Annual rhythms that underlie phenology: biological time-keeping meets environmental change. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20130016-20130016 (2013).
43. L. Hüppe *et al.*, A new Activity Monitor for Aquatic Zooplankter (AMAZE) allows the recording of swimming activity in wild-caught Antarctic krill (Euphausia superba). *Sci Rep* **14**, 16963 (2024).
44. J. H. Cohen, R. B. Forward, Diel vertical migration of the marine copepod Calanopia americana. II. Proximate role of exogenous light cues and endogenous rhythms. *Marine Biology* **147**, 399-410 (2005).
45. C. Helfrich-Förster, The locomotor activity rhythm of Drosophila melanogaster is controlled by a dual oscillator system. *Journal of Insect Physiology* **47**, 877-887 (2001).
46. J. Aschoff, Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol* **25**, 11-28 (1960).
47. N. Mrosovsky, Masking: history, definitions, and measurement. *Chronobiol Int* **16**, 415-429 (1999).
48. J. Aschoff, C. von Goetz, Masking of Circadian Activity Rhythms in Canaries by Light and Dark. *Journal of Biological Rhythms* **4**, 29-38 (1989).
49. J. Aschoff, C. von Goetz, Masking of circadian activity rhythms in hamsters by darkness. *Journal of Comparative Physiology A* **162**, 559-562 (1988).
50. M. Zhou, R. D. Dorland, Aggregation and vertical migration behavior of Euphausia superba. *Deep Sea Research Part II: Topical Studies in Oceanography* **51**, 2119-2137 (2004).
51. T. A. Klevjer, S. Kaartvedt, Krill (Meganyctiphanes norvegica) swim faster at night. *Limnology and Oceanography* **56**, 765-774 (2011).
52. A. B. Webb, S. R. Taylor, K. A. Thoroughman, F. J. Doyle, 3rd, E. D. Herzog, Weakly circadian cells improve resynchrony. *PLoS Comput Biol* **8**, e1002787 (2012).
53. E. Bertolini *et al.*, Life at High Latitudes Does Not Require Circadian Behavioral Rhythmicity under Constant Darkness. *Current Biology*, (2019).
54. M. Beauchamp *et al.*, Closely Related Fruit Fly Species Living at Different Latitudes Diverge in Their Circadian Clock Anatomy and Rhythmic Behavior. *Journal of Biological Rhythms* **33**, 602-613 (2018).
55. W. Arnold *et al.*, Circadian rhythmicity persists through the Polar night and midnight sun in Svalbard reindeer. *Scientific Reports* **8**, 1-12 (2018).
56. D. Appenroth, G. C. Wagner, D. G. Hazlerigg, A. C. West, Evidence for circadian-based photoperiodic timekeeping in Svalbard ptarmigan, the northernmost resident bird. *Curr Biol* **31**, 2720-2727 e2725 (2021).
57. K. Beer, J. Joschinski, A. Arrazola Sastre, J. Krauss, C. Helfrich-Förster, A damping circadian clock drives weak oscillations in metabolism and locomotor activity of aphids (Acyrthosiphon pisum). *Sci Rep* **7**, 14906 (2017).

58. M. Barbera, J. M. Collantes-Alegre, D. Martinez-Torres, Characterisation, analysis of expression and localisation of circadian clock genes from the perspective of photoperiodism in the aphid Acyrthosiphon pisum. *Insect Biochem Mol Biol* **83**, 54-67 (2017).
59. U. Abraham *et al.*, Coupling governs entrainment range of circadian clocks. *Mol Syst Biol* **6**, 438 (2010).
60. G. Bloch, B. M. Barnes, M. P. Gerkema, B. Helm, Animal activity around the clock with no overt circadian rhythms: patterns, mechanisms and adaptive value. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20130019 (2013).
61. B. Cisewski, V. H. Strass, M. Rhein, S. Krägefsky, Seasonal variation of diel vertical migration of zooplankton from ADCP backscatter time series data in the Lazarev Sea, Antarctica. *Deep Sea Research Part I: Oceanographic Research Papers* **57**, 78-94 (2010).
62. T. Ikeda, P. Dixon, Body shrinkage as a possible over-wintering mechanism of the Antarctic krill, Euphausia superba Dana. *Journal of Experimental Marine Biology and Ecology* **62**, 143-151 (1982).
63. D. S. Saunders, Time measurement in insect photoperiodism: external and internal coincidence. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **210**, 513-525 (2024).
64. G. Vicentini *et al.*, Environmental control of rice flowering time. *Plant Commun* **4**, 100610 (2023).
65. C. S. Pittendrigh, D. H. Minis, The Entrainment of Circadian Oscillations by Light and Their Role as Photoperiodic Clocks. *The American Naturalist* **98**, 261-294 (1964).
66. Y. Miyazaki, in *Insect Chronobiology,* H. Numata, K. Tomioka, Eds. (Springer Nature Singapore, Singapore, 2023), pp. 229-249.
67. N. S. Häfker *et al.*, Molecular circadian rhythms are robust in marine annelids lacking rhythmic behavior. *PLoS Biol* **22**, e3002572 (2024).
68. K. J. Benoit-Bird, M. A. Moline, Vertical migration timing illuminates the importance of visual and nonvisual predation pressure in the mesopelagic zone. *Limnology and Oceanography*, (2021).
69. U. Kils, Swimming behaviour, swimming performance and energy balance of Antarctic krill Euphausia superba. *BIOMASS Sci. Ser.* **3**, 1-122 (1981).
70. F. Piccolin *et al.*, Photoperiodic modulation of circadian functions in Antarctic krill Euphausia superba Dana, 1850 (Euphausiacea). *Journal of Crustacean Biology* **38**, 707-715 (2018).
71. V. Siegel, J. L. Watkins, in *Biology and Ecology of Antarctic Krill,* V. Siegel, Ed. (Springer International Publishing, Cham, 2016), pp. 21-100.
72. B. A. Fach, E. E. Hofmann, E. J. Murphy, Transport of Antarctic krill (Euphausia superba) across the Scotia Sea. Part II: Krill growth and survival. *Deep Sea Research Part I: Oceanographic Research Papers* **53**, 1011-1043 (2006).
73. L. B. Quetin, R. M. Ross, Environmental Variability and Its Impact on the Reproductive Cycle of Antarctic Krill. *American Zoologist* **41**, 74-89 (2001).
74. B. Meyer, The overwintering of Antarctic krill, Euphausia superba, from an ecophysiological perspective. *Polar Biology* **35**, 15-37 (2012).
75. S. J. Thomalla, S.-A. Nicholson, T. J. Ryan-Keogh, M. E. Smith, Widespread changes in Southern Ocean phytoplankton blooms linked to climate drivers. *Nature Climate Change* **13**, 975-984 (2023).
76. R Core Team. (R Foundation for Statistical Computing, Vienna, Austria, 2021).
77. H. Wickham *et al.*, Welcome to the Tidyverse. *Journal of Open Source Software* **4**, (2019).
78. T. Ruf, The Lomb-Scargle Periodogram in Biological Rhythm Research: Analysis of Incomplete and Unequally Spaced Time-Series. *Biological Rhythm Research* **30**, 178-201 (1999).
79. W.-J. Lee *et al.*, Echopype: A Python library for interoperable and scalable processing of water column sonar data for biological information. *arXiv preprint arXiv:2111.00187*, (2021).
80. D. Bahlburg, T. Böhrer, L. Hüppe, An open and lightweight method to analyze the vertical distribution of pelagic organisms using echogram screenshots. *bioRxiv*, 2023.2004.2016.537064 (2023).

Data availability

The datasets and analysis scripts used for the analysis of krill swimming activity, as well as of hydroacoustic recordings will be made freely available on the Zenodo repository upon publication. Supplementary figures are available in the Supplementary Material file.

Author Contributions

LH, BM and CHF designed the study. LH, RD and DB conducted the behavioral experiments. DB processed and analyzed the hydroacoustic data. LH analyzed the behavioral data and wrote the initial draft of the manuscript. All authors revised the initial draft and approved the submitted version.

Competing Interests statement

The authors declare no competing interests

Figures

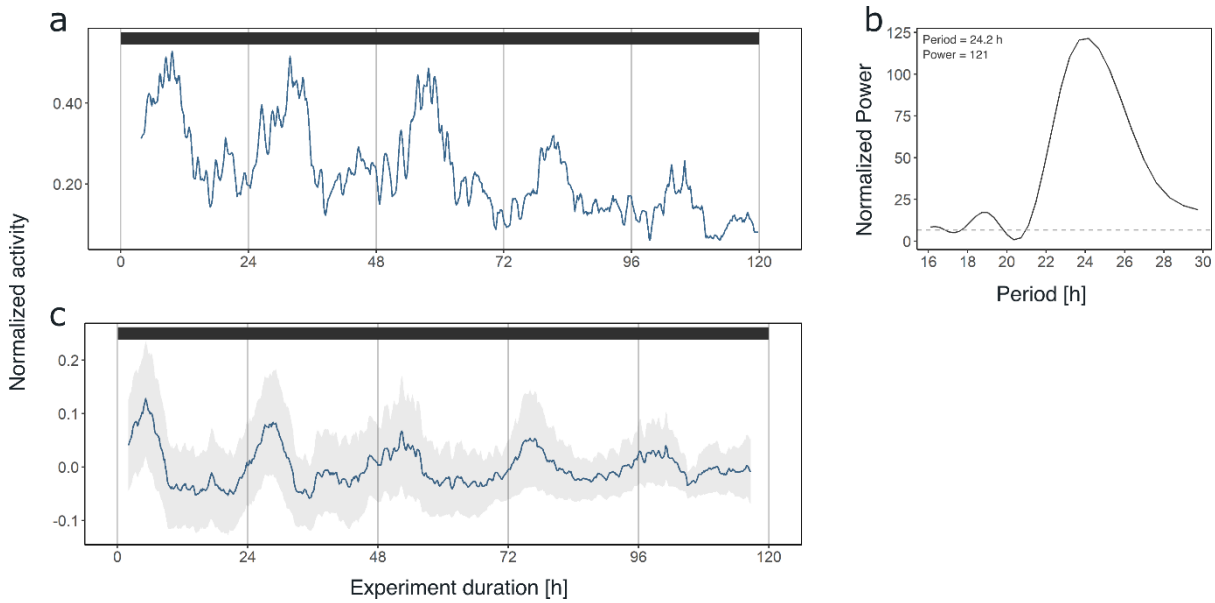
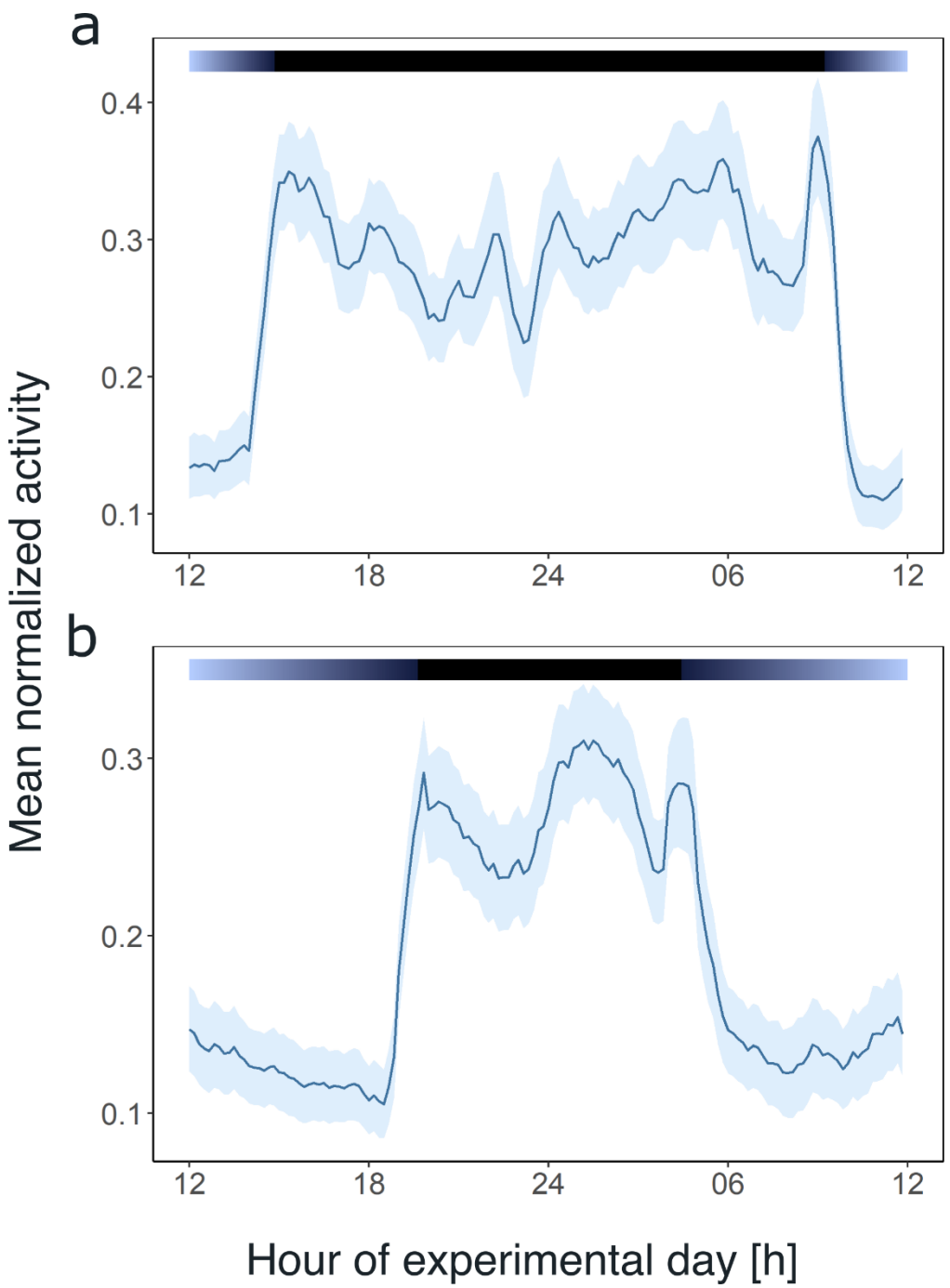
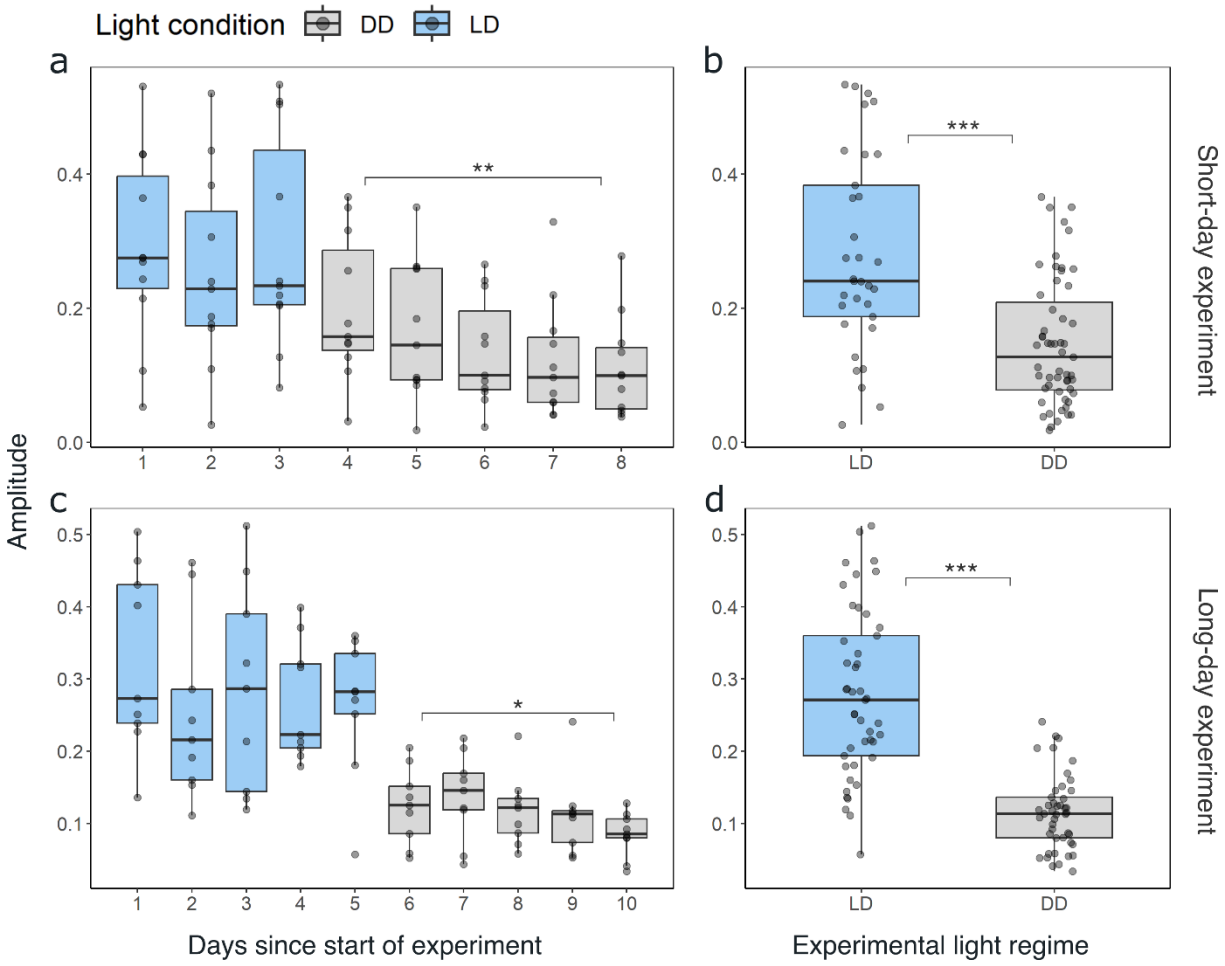


Figure 1: Krill swimming activity persists under constant conditions. Swimming activity under 5 days of DD conditions in experiment 1 of individual #9 after entrainment to simulated short-day conditions (a) and group mean swimming activity of rhythmic individuals under 5 days of DD conditions from the same experiment (n = 6, c). The result from LSP analysis shows significant circadian rhythmicity of swimming activity of individual #9 during DD conditions of experiment 1 (period: 24.2 h, b). Grey shading represents standard error of the mean (s.e.m.). Color bars at the top indicate light conditions (constant darkness).



717

718 **Figure 2: Light and the circadian clock shape the daily activity profile of krill.**
719 Average day analysis of the group mean swimming activity over 3 days of short-day
720 simulations (experiment 1, n = 11, a) and 5 days of long-day simulations (experiment 2, n = 9,
721 b). Color bars at the top indicate light regime under day-night simulations. Shading around the
722 line represents the s.e.m.. Data in (b) from Hüppe et al. (*43*).



723

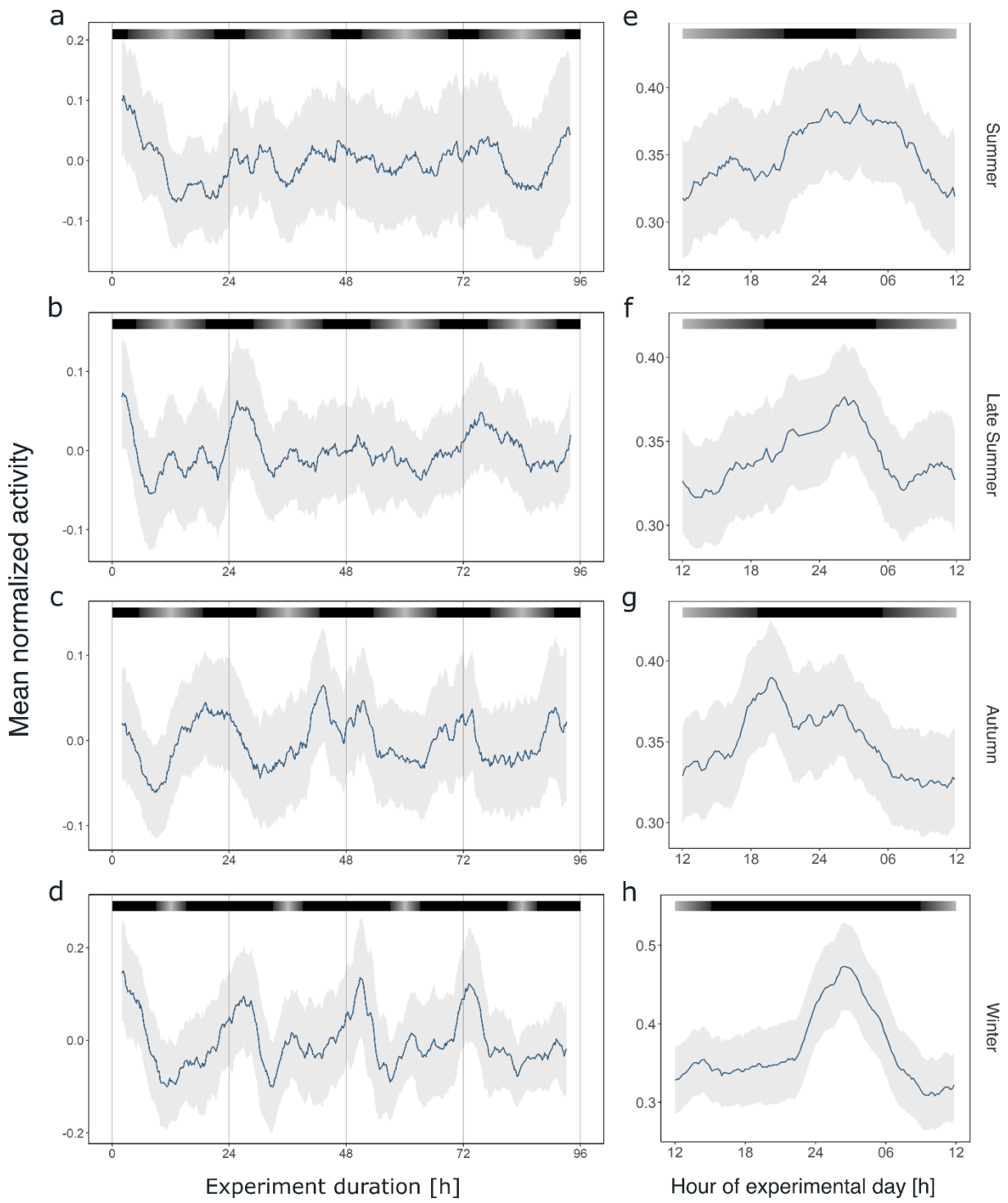
724 **Figure 3: The amplitude of swimming activity decreases in DD conditions.**

725 Distribution of the daily amplitudes of swimming activity visualized with boxplots for every
726 day of the LD-DD experiments for short-day (experiment 1, a), and long-day simulations
727 (experiment 2, b). Differences in the daily amplitude of swimming activity between LD and DD
728 conditions for short-day (b) and long-day (d) experiments. Lower and upper hinges of the
729 boxes correspond to the 25th and 75th percentiles, the upper and lower whiskers extend to
730 the largest and smallest value no further than 1.5 of the interquartile range, respectively, the
731 horizontal line shows the median. Points represent daily amplitudes of individuals for each
732 day and light condition. Differences in amplitude between first and last day under DD tested
733 with paired t-test for experiment 1 (a; n = 11, t = 3.197, df = 10, p-value = 0.005) and
734 experiment 2 (c, n = 9, t = 2.731, df = 8, p-value = 0.012), and between LD and DD conditions

735 with Mann-Whitney-U-Test for experiment 1 (b; n = 88, p-value = < 0.001) and experiment 2

736 (d; n = 90, p-value < 0.001). Significance levels: p < 0.05: *, p < 0.01: **, p < 0.001: ***.

737



738 **Figure 4: Circadian swimming activity persist across seasons.**

Group mean swimming activity of rhythmic individuals during the first four days under DD conditions for krill sampled in summer (experiment S1, n individuals = 5, a), late summer (experiment S2, n individuals = 8, b), autumn (experiment S3, n individuals = 10, c), and winter (experiment S4, n individuals = 4, d), as well as corresponding average day analysis of the first four days in summer (e), late summer (f), autumn (g), and winter (h). Grey shading represents the s.e.m.. Color bars at the top indicate the natural photoperiod at the day of sampling.

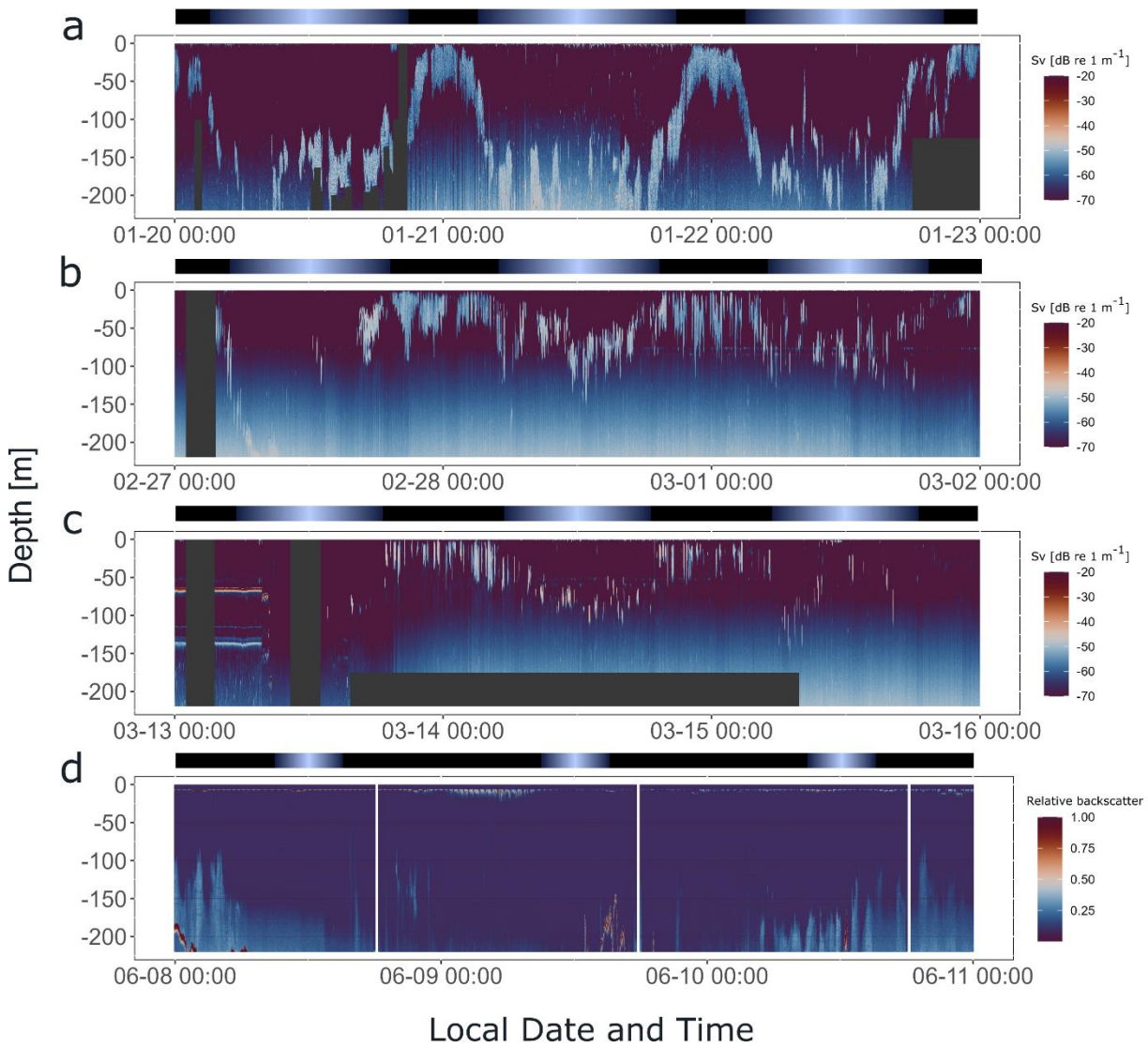


Figure 5: Krill display synchronized DVM in the field across a wide range of photoperiods. Hydroacoustic recordings showing the vertical distribution of krill swarms in the upper water column (<220 m) below the vessel, visualized by the mean volume backscattering signal (200

kHz), on the days preceding krill sampling for experiments S1-S4, in summer (a), late summer (b), autumn (c), and winter (d). Diffuse blue shading between ~100-220 m represents instrument noise. Grey areas depict missing data. Color bars at the top indicate the natural light regime.

Tables

Table 1: Meta data for behavioral experiments.
For each experiment (Experiment ID), the meta data show the light regime during the experiment in the activity monitor (LD: light-dark cycle, DD: constant darkness), the duration of the experiments under the respective light condition, the local date and time, Region (BS: Bransfield Strait, SOI: South Orkney Islands), exact location, and depth for krill sampling, the local natural photoperiod at the time of krill sampling, the number of krill used for each experiment, and their mean length and standard deviation.

Experiment ID	Experimental light regime	Experiment duration [days]	Sampling Date [UTC-2]	Sampling Time [UTC-2]	Sampling Region	Sampling location [Latitude °S/Longitude °W]	Sampling Depth [m]	Natural photoperiod [h]	n Krill [total/male/female]	Mean ±SD length [mm]
Experiment 1	LD-DD	3 (LD), 5 (DD)	2021-05-31	13:02	BS	62.95/57.77	170	5.5	11/5/6	45 ±2.6
Experiment 2	LD-DD	5 (LD), 5 (DD)	2022-02-16	15:50	SOI	60.04/46.45	94	15.3	9/4/5	44.4 ±3.7
Experiment S1	DD	8	2022-01-22	17:03	SOI	60.08/46.46	157	17.5	9/8/1	42.4 ±1.5
Experiment S2	DD	6	2022-03-01	11:00	SOI	60.0/44.73	86.5	14.0	9/4/5	48.9 ±4.4
Experiment S3	DD	7	2022-03-15	08:40	SOI	60.28/46.43	119.2	12.6	10*/4/5	51.3 ±1.6*
Experiment S4	DD	4	2021-06-10	12:56	SOI	60.5/46.11	185	5.9	9/2/7	49.2 ±3.9

* Length and sex of one individual undetermined.

Supplementary Figures

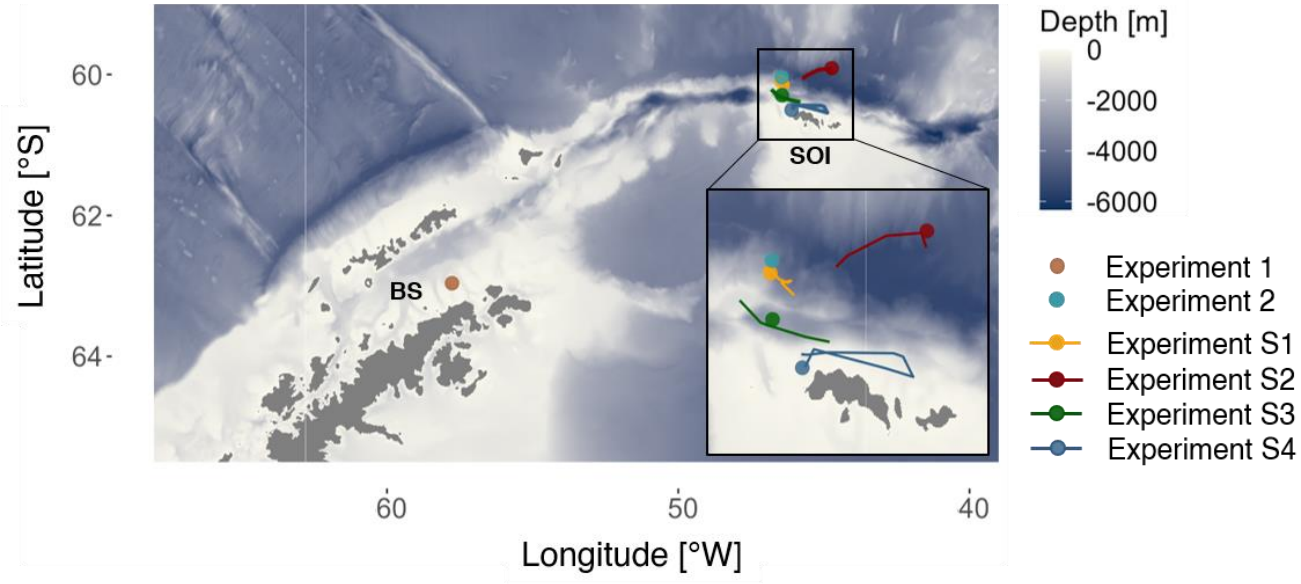


Figure S1: Locations of krill sampling and hydroacoustic recordings for behavioral experiments. Colored dots indicate sampling locations for the respective behavioral experiments. Colored lines indicate the cruise track of the fishing vessel during recording of hydroacoustic data prior to sampling for behavioral experiments S1-S4. BS: Bransfield Strait. SOI: South Orkney Islands. Bathymetric data were retrieved from the International Bathymetric Chart of the Southern Ocean (500m× 500 m resolution, https://ibcso.org/).

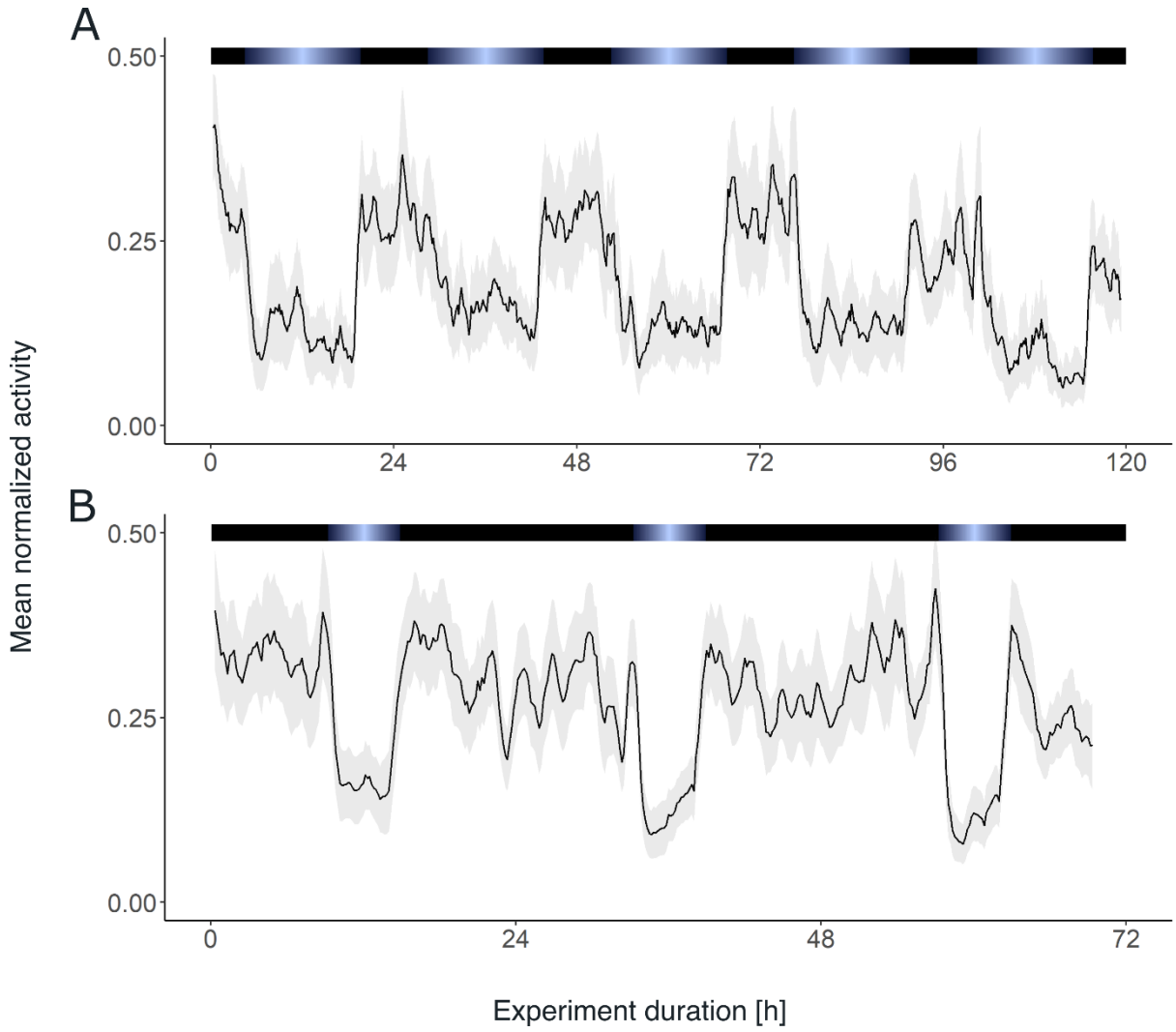


Figure S2: Krill group activity under LD conditions.
Group mean swimming activity of krill under long-day (experiment 2, n=9, A) and short-day (experiment 1, n=11, B) simulations. Shading around the line depicts the standard error of the mean (s.e.m.). Color bars at the top indicate the light regime provided. Data for (A) from Hüppe et al..

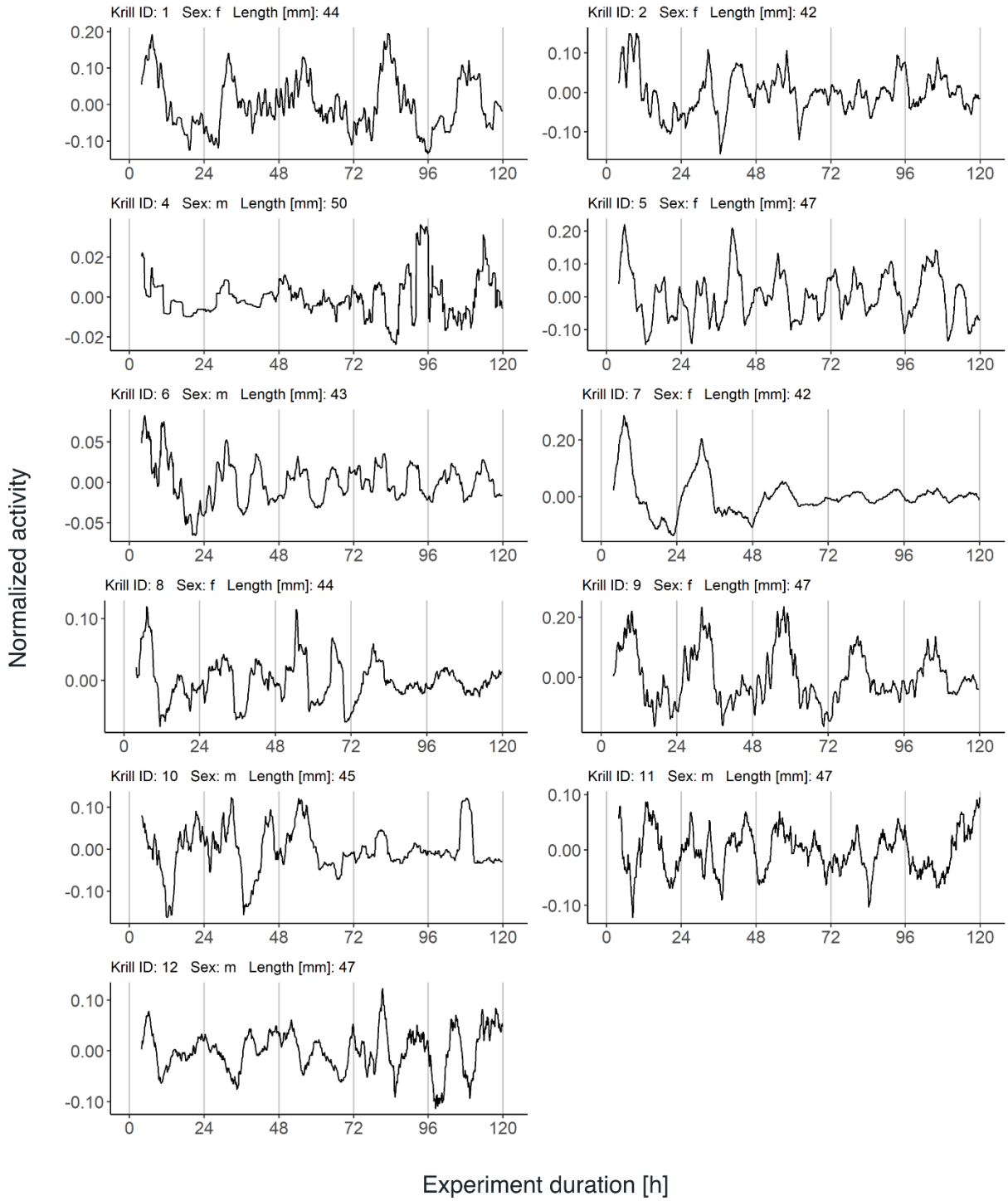


Figure S3: Swimming activity of individual krill under DD conditions.
Normalized and detrended swimming activity under 5 days of constant darkness (DD) after entrainment to simulated short-day conditions (experiment 1) for each individual. Color bars at the top indicate subjective day and night.

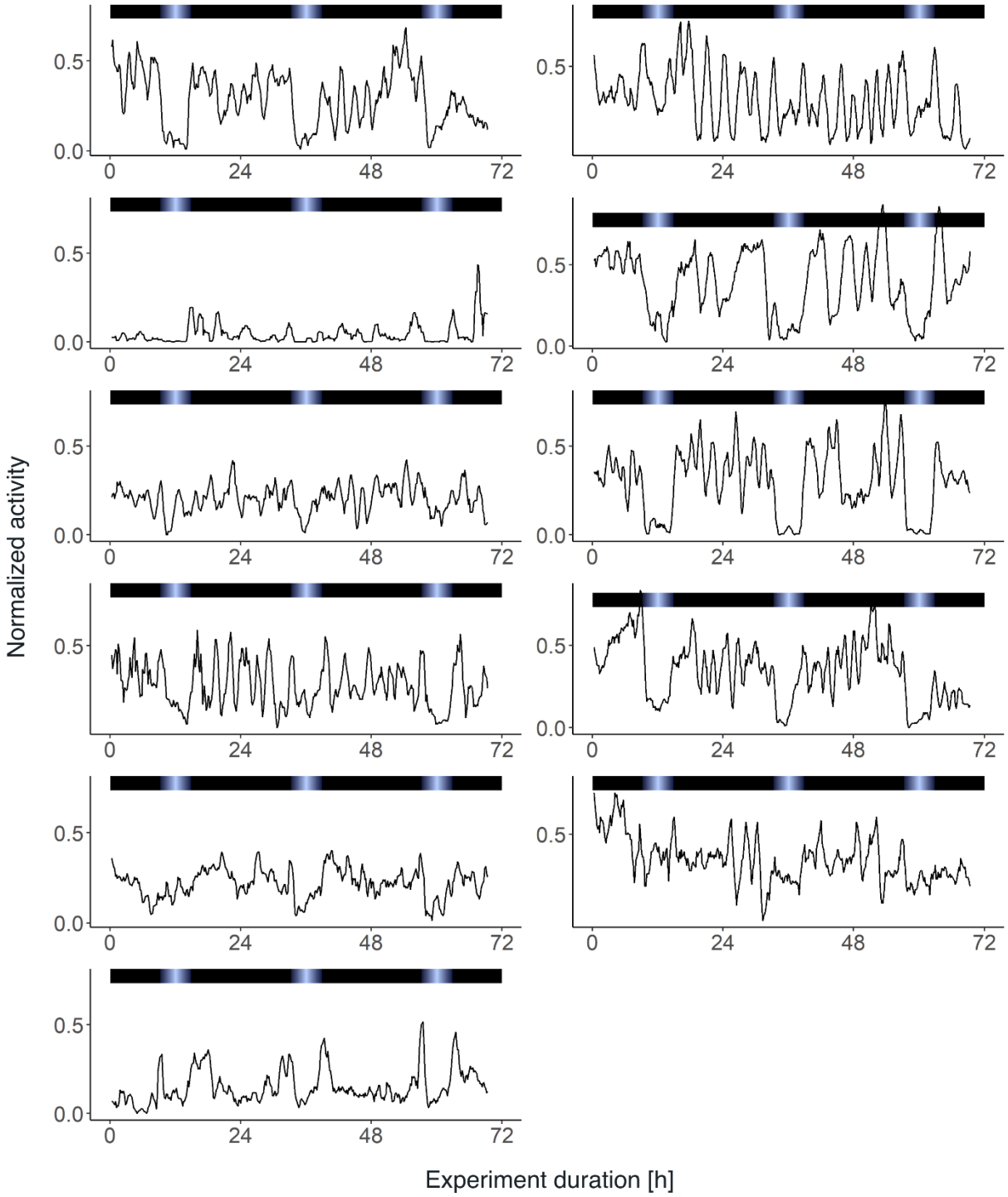


Figure S4: Swimming activity of individual krill under LD conditions.
Normalized swimming activity under 5 days of simulated short-day (experiment 1) conditions for each individual. Color bars at the top indicate the light regime provided.

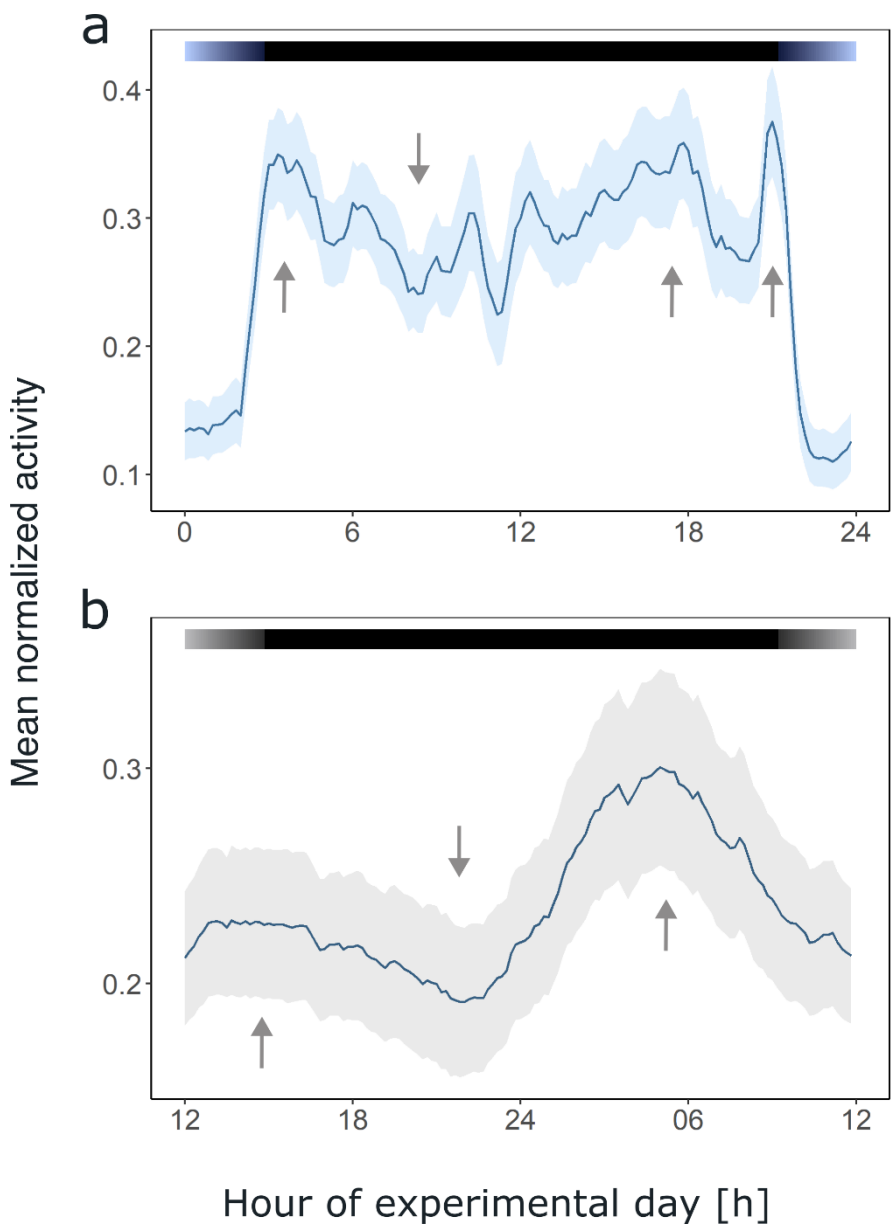


Figure S5: Comparison of daily swimming activity between LD and DD conditions. Group mean swimming activity of rhythmic individuals over an average day under 3 days of simulated short-day conditions (A) and 5 days of constant darkness (B) during experiment 1. Color bars at the top indicate the light regime provided, as well as subjective day and night. Arrows indicate times of increased or decreased activity which lead to the characteristic activity pattern during the dark phase. Shading indicates the s.e.m..

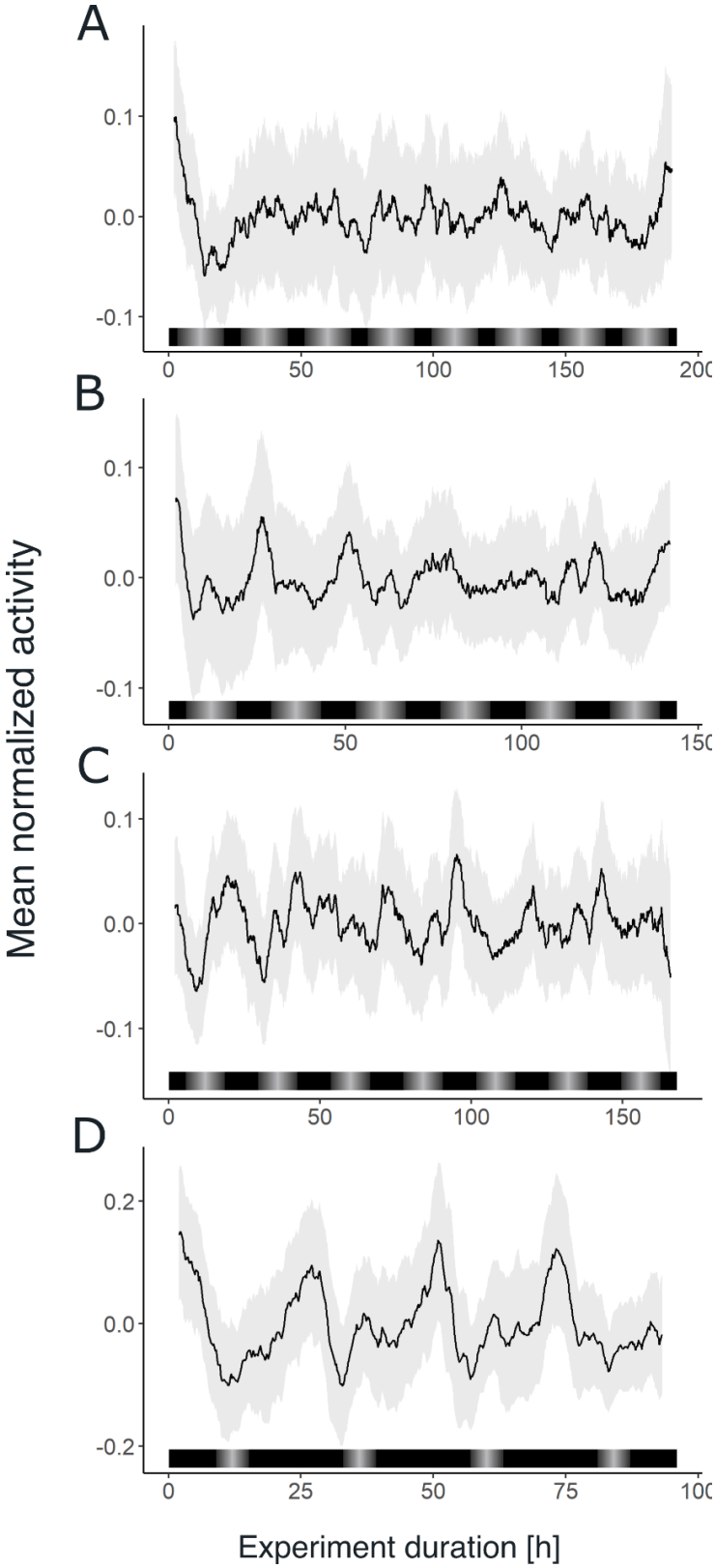


Figure S6: Group swimming activity under DD conditions across seasons.
Group mean swimming activity of rhythmic individuals under DD conditions after individuals have been sampled from their natural environment during summer (experiment S1, A), late summer (experiment S2, B), autumn (experiment S3, C), and winter (experiment S4, D). Grey shading indicates the s.e.m..

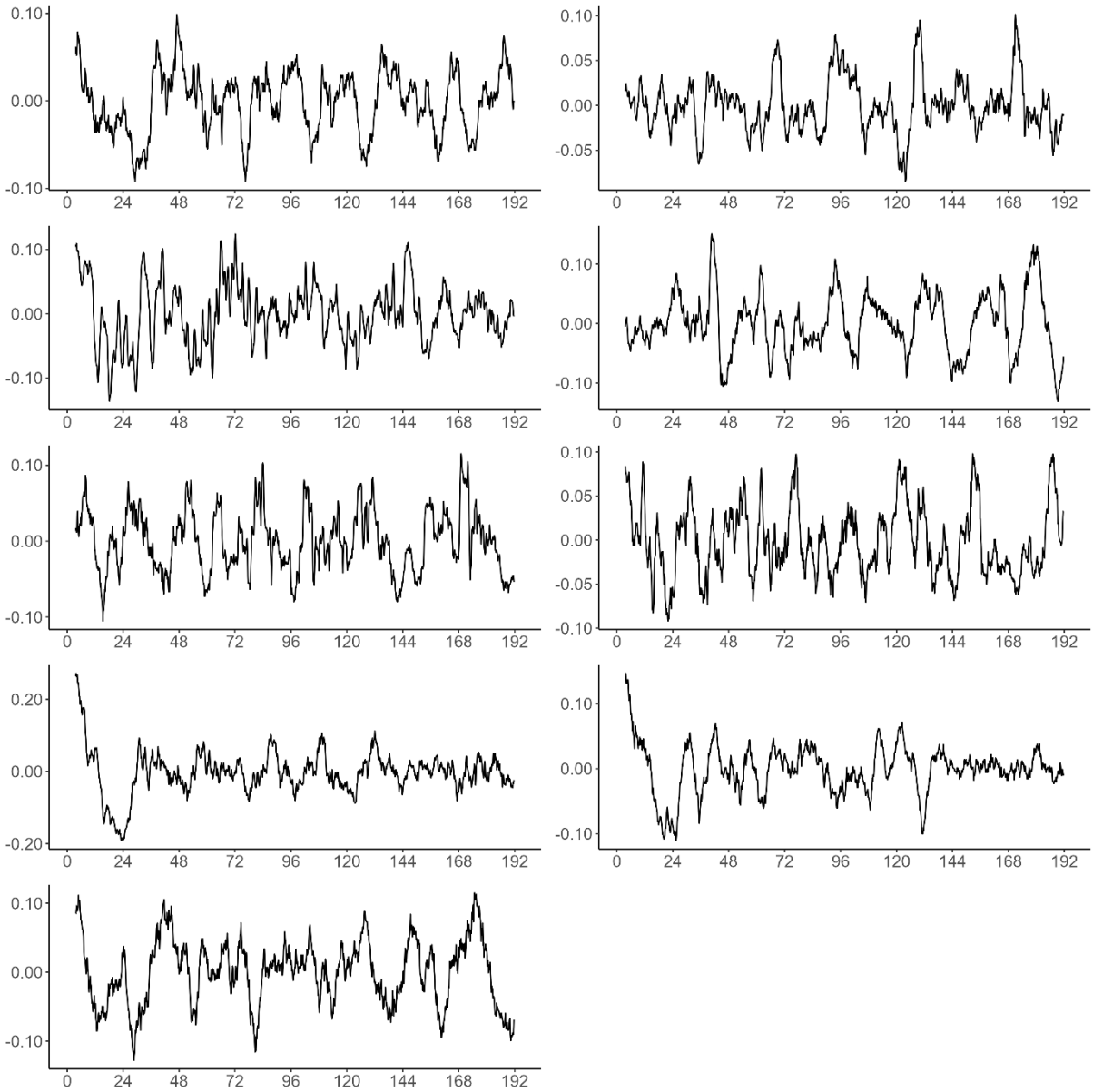


Figure S7: Individual swimming activity under DD conditions of krill sampled in Summer.

Normalized and detrended swimming activity under 8 days of constant darkness (DD) after individuals have been sampled from their natural environment during summer (experiment S1, photoperiod: 17.5 h).

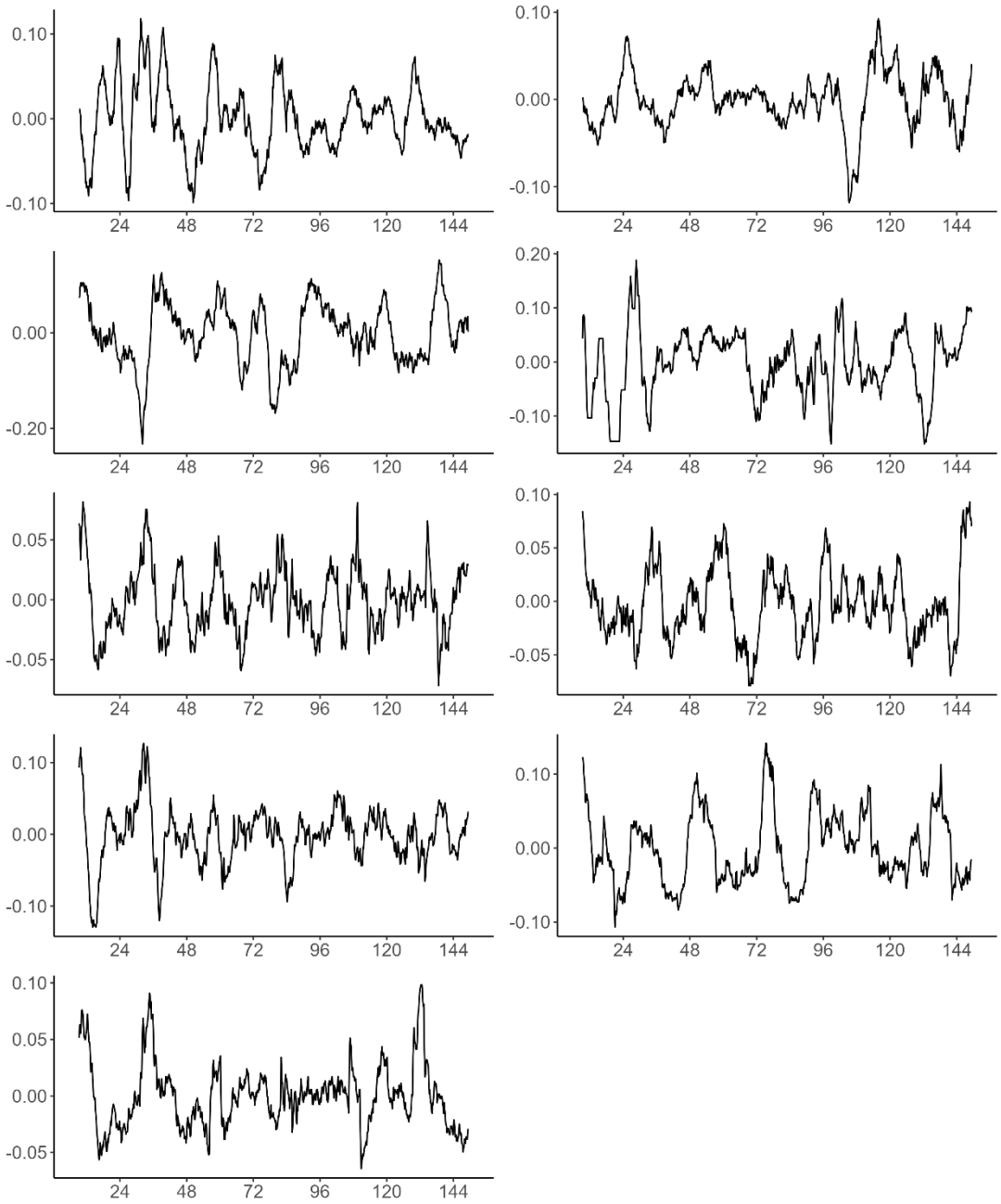


Figure S8: Individual swimming activity under DD conditions of krill sampled in Late Summer.

Normalized and detrended swimming activity under 6 days of constant darkness (DD) after individuals have been sampled from their natural environment during late summer (experiment S2, photoperiod: 14 h).

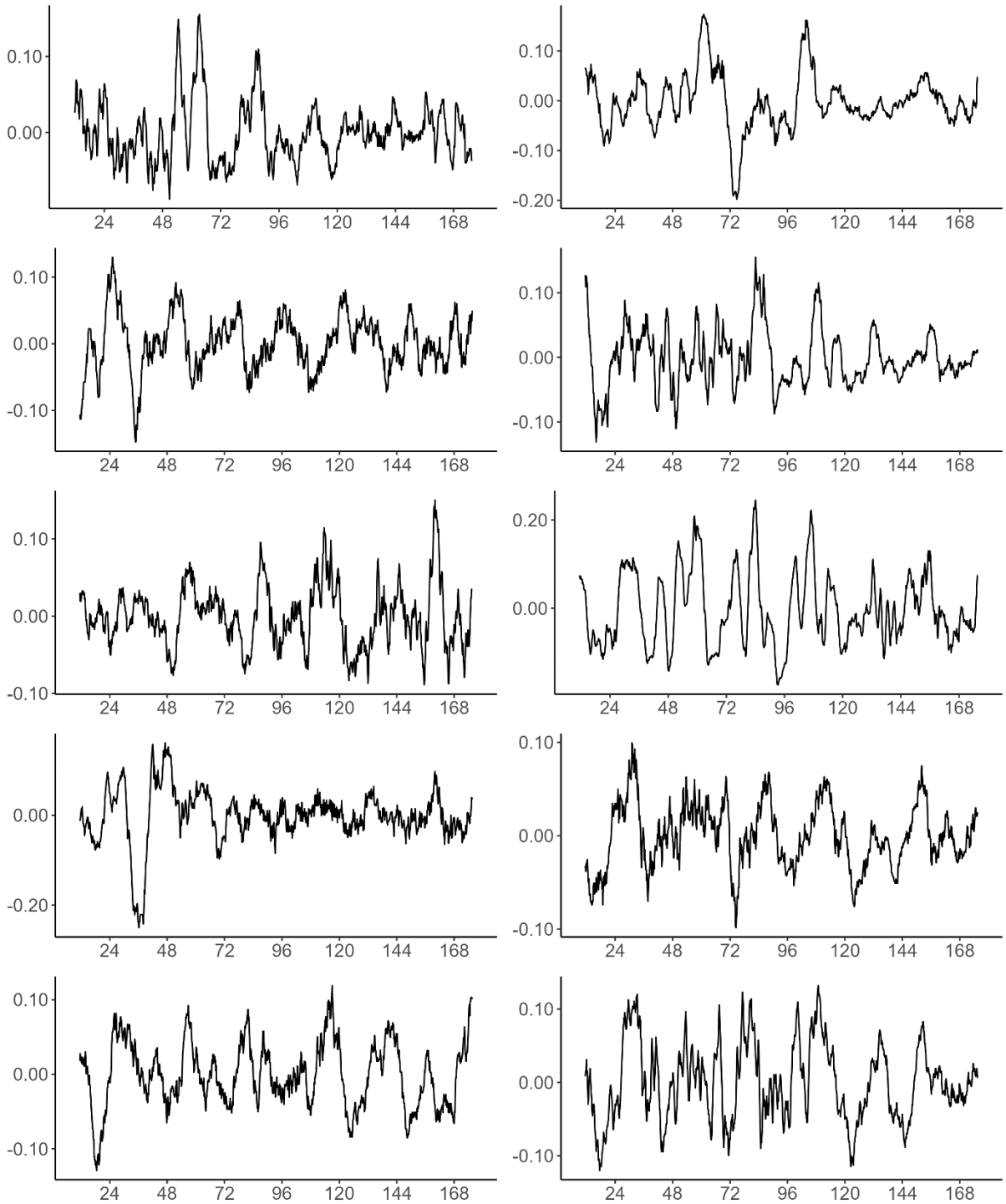


Figure S9: Individual swimming activity under DD conditions of krill sampled in Autumn.

Normalized and detrended swimming activity under 7 days of constant darkness (DD) after individuals have been sampled from their natural environment during autumn (experiment S3, photoperiod: 12.6 h).

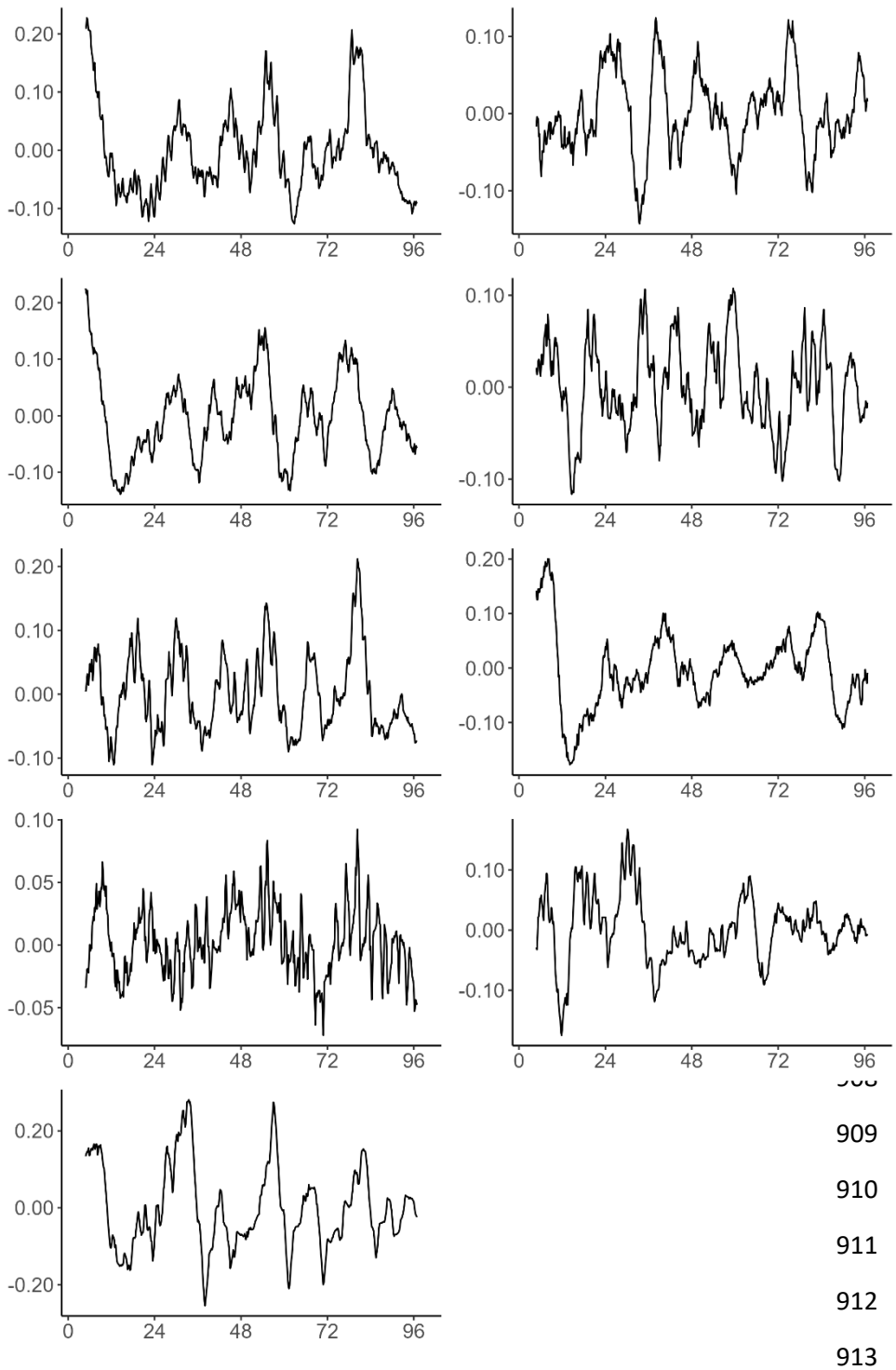


Figure S10: Individual swimming activity under DD conditions of krill sampled in Winter.

Normalized and detrended swimming activity under 4 days of constant darkness (DD) after individuals have been sampled from their natural environment during winter (experiment S4, photoperiod: 5.9 h).

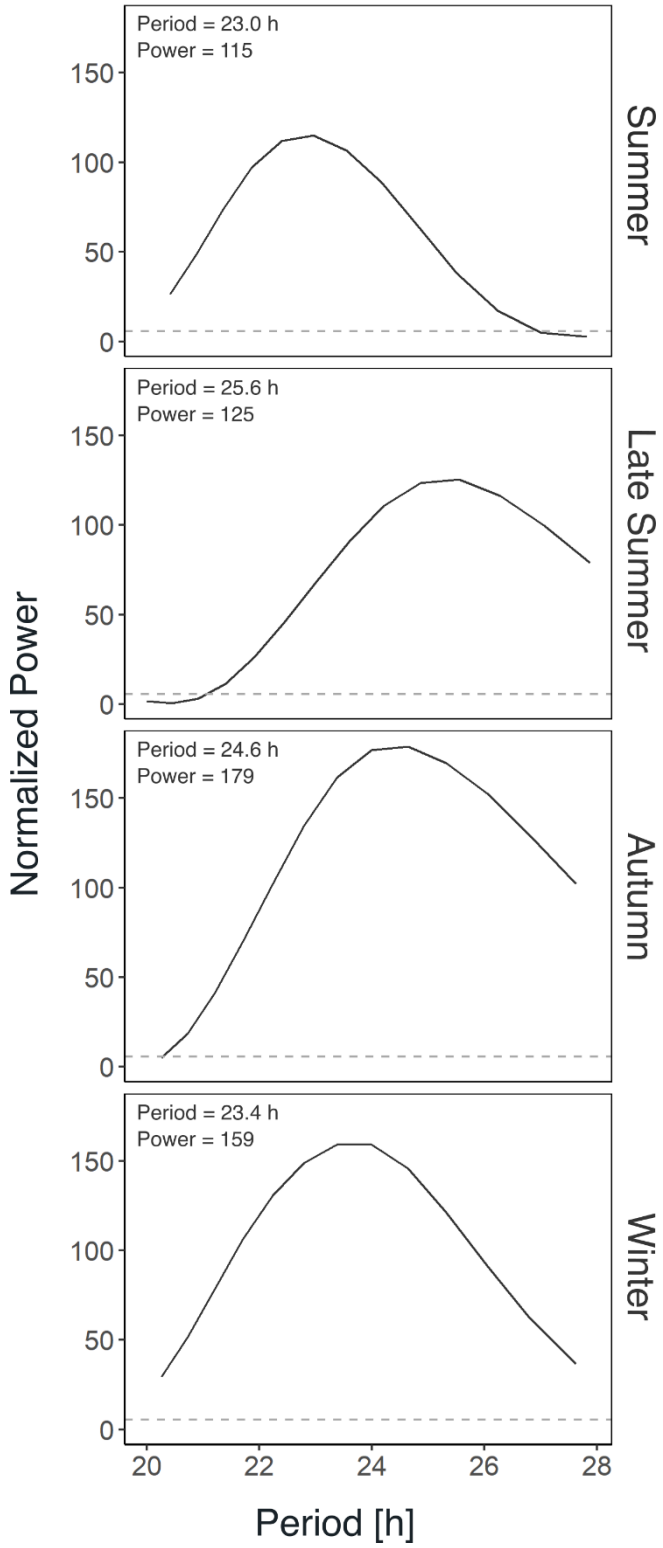


Figure S11: Rhythm analysis of group swimming activity under DD conditions across seasons. Periodogram derived from Lomb-Scargle Periodogram analysis (LSP) of group mean swimming activity under DD conditions after individuals have been sampled from their natural environment in different seasons (experiments S1-S4, see suppl. fig. 6).