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1 Circadian clock involvement in zooplankton diel vertical migration

2

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15 Summary

16 Biological clocks are a ubiquitous ancient and adaptive mechanism enabling organisms to
17 anticipate environmental cycles and to regulate behavioral and physiological processes
18 accordingly [1]. Whilst terrestrial circadian clocks are well understood, knowledge of clocks
19 in marine organisms is still very limited [2–5]. This is particularly true for abundant species
20 displaying large-scale rhythms like diel vertical migration (DVM) that contribute significantly
21 to shaping their respective ecosystems [6]. Here, we describe exogenous cycles and
22 endogenous rhythms associated with DVM of the ecologically important and highly abundant
23 planktic copepod *Calanus finmarchicus*. In the laboratory, *C. finmarchicus* shows circadian
24 rhythms of DVM, metabolism, and most core circadian clock genes (*clock*, *period1*, *period2*,
25 *timeless*, *cryptochrome2*, *clockwork orange*). Most of these genes also cycle in animals
26 assessed in the wild though expression is less rhythmic at depth (50-140 m) relative to
27 shallow caught animals (0-50 m). Further, peak expressions of clock genes generally occurred
28 at either sunset or sunrise, coinciding with peak migration times. Including one of the first
29 field investigations of clock genes in a marine species [5,7] this study couples clock gene
30 measurements with laboratory and field data on DVM. While the mechanistic connection
31 remains elusive, our results imply a high degree of causality between clock gene expression
32 and one of the planet's largest daily migration of biomass. We thus suggest that the circadian
33 clocks increase zooplankton fitness by optimizing the temporal trade-off between feeding and
34 predator avoidance especially when environmental drivers are weak or absent [8].

35

36 Key words: *Calanus finmarchicus*, circadian clock, clock genes, diel vertical migration,
37 respiration, zooplankton

38 Results & Discussion

39 Diel vertical migration (DVM) in one of the most abundant and ecologically important marine
40 copepods, *Calanus finmarchicus*, is paralleled by endogenous circadian rhythmicity at
41 behavioral, physiological and molecular levels. In the laboratory, copepods collected from an
42 actively migrating field population showed endogenous rhythms of swimming, respiration
43 and core circadian clock gene oscillations under constant darkness. In the field, most clock
44 gene oscillations mimicked laboratory findings with some genes becoming less rhythmic in
45 animals collected from depth. Peaks of gene expression follow sunset/sunrise, the periods of
46 greatest vertical migrations over the solar day. Our data indicates that circadian timekeeping
47 is an important component of DVM and particularly adaptive at maintaining migratory
48 rhythmicity in habitats where the principle exogenous driver of DVM, light, is limiting.

49 DVM of marine zooplankton is one of the most profound coordinated movements of
50 organisms on the planet. It contributes fundamentally to ecological interactions in both marine
51 and freshwater habitats [9], and to global biogeochemical cycles [10]. DVM also structures
52 predator prey-interactions, since increased predation risk from visually hunting predators
53 drives zooplankton to depths during the day, whilst at night they return to the surface to feed
54 [8]. Current mechanistic knowledge of DVM suggests that diel light changes are the main
55 environmental cue of migration behavior [11]. However, paradoxically DVM still occurs in
56 deepwater habitats or at high latitudes during the winter where light is limiting suggesting
57 alternative control mechanisms [12–15].

58 In terrestrial organisms, endogenous temporal synchronization is achieved by a circadian
59 clock cellular machinery involving an intricate network of gene/protein feedback loops that
60 create a cycle of ~24 h length [16]. The clock is primarily entrained by light to ensure
61 synchronization with the environment and is a potent tool of rhythm regulation controlling
62 diel activity patterns [17]. However, studies addressing the role of molecular clock
63 mechanisms in marine organisms are scarce [2–4,6], primarily due to the non-model nature of

64 most marine species and a lack of genetic resources. Furthermore, marine organisms are often
65 difficult to maintain in the laboratory and sampling them in the field is often expensive and
66 labor intensive. However, understanding marine clock mechanisms, especially in key
67 ecological species, is crucial to predicting how the rhythmic life of these organisms may be
68 affected by changes in environmental conditions [18].

69 Copepods occupy a central position in marine pelagic food webs, providing an important
70 energy source for their predators [19]. *C. finmarchicus* accumulates large lipid reserves [20]
71 and is the main link between phytoplankton and higher trophic levels in the North Atlantic
72 thereby sustaining one of the world's most productive fisheries [21]. It is well recognized that
73 *C. finmarchicus* undergoes DVM [22] and recently published transcriptomic resources [23,24]
74 make it an ideal model to examine the molecular clock machinery.

75

76 Vertical migration in the field

77 To determine DVM of copepods in their natural environment an acoustic mooring was
78 deployed in Loch Etive in the Bonawe deep (~145 m), UK (56°45'N, 5°18'W, Fig. S1).
79 Acoustic Doppler Current Profilers (ADCPs) generated backscatter profiles as sound
80 scattering layers representing the vertical distribution of zooplankton biomass. The ADCP
81 generated data indicated clear DVM behavior of zooplankton with near 24 h periodicity
82 during the field campaign (May 2015) (Fig. 1). The main scattering layer was located in the
83 upper 40 m depth at night, whereas during daytime this was typically between 40 and 80 m
84 depth. The timing of the upward and downward migrations coincided closely with the time of
85 local sunset (20:12) and sunrise (4:24).

86 *C. finmarchicus* is the dominant zooplankton species in Loch Etive [25]. As such, the
87 recorded DVM signals were assumed to primarily reflect the vertical migration of *C.*
88 *finmarchicus*. This assumption was supported by net catches (data not shown) that established
89 high abundance of these animals in the water column during ADCP recordings.

90

91 Phenotypic rhythmicity

92 DVM behavior and respiration were determined in *C. finmarchicus* collected from Loch
93 Etive, to investigate if the cyclic migrations observed in the field also persist under entrained
94 and constant laboratory conditions. The animals were exposed to a simulated light-dark (LD)
95 photoperiod (LD 16 h: 8 h) mimicking field conditions, followed by constant darkness (DD).
96 The copepods exhibited 24 h cycling in DVM under LD and near 24 h rhythms under DD
97 conditions with clear downward movement in the subjective morning (Fig.2A, Tab.S1). These
98 data clearly suggest an endogenous circadian regulation of DVM behavior. The rapid evening
99 ascent and morning descent under LD, with light triggering a direct negative phototactic
100 response, contrasted with the more gradual depth change and lower amplitude rhythm under
101 DD, which dampened over time. Endogenous DVM rhythms have previously been described
102 for zooplankton species and several of these studies also reported lower amplitude DVM
103 rhythms under DD [26,27]. While some of these studies found more robust endogenous
104 rhythms of zooplankton DVM than detailed here, direct comparisons are not appropriate as
105 DVM differ between species and life stages [22]. Nevertheless, the persistence of DVM in
106 copepods under constant darkness strongly suggests circadian clock involvement.

107 Swimming during vertical migration requires energy and is therefore accompanied by
108 increased metabolic activity [28]. Respiration experiments revealed that oxygen consumption
109 under LD increased in *C. finmarchicus* during the late afternoon/early night, a pattern
110 repeated over the subsequent two days under DD (Fig.2B, Tab.S1). While the peak respiration
111 in the second night between the two DD days was phase delayed by ~8 hours toward the late
112 night, peak respiration was once again in phase by the last night of the experiment suggesting
113 that the endogenous rhythm was still running “on time”. The delay initially observed under
114 DD could be related to the transition from LD to constant darkness constituting “after-effects”

115 suggested to reflect an adaption of the endogenous rhythm to unnatural changes in light
116 regime [29].

117 The evening increase in respiration matches the time when the copepods undertake the
118 energy demanding migration towards the surface [28] whereas the decrease in respiration
119 towards sunrise may reflect passive copepod sinking or reduced energy costs for downward
120 migration facilitated through negative buoyancy [30]. Of relevance here is that respiration
121 increases before the time of upward migration, indicating an endogenously regulated
122 anticipatory process. Rudjakov [12] hypothesized that DVM may actually be a result of an
123 endogenous rhythm of metabolic activity that initiates upward migration around sunset
124 followed by passive sinking around sunrise. Overall, these data reveal that *C. finmarchicus*
125 possesses an endogenous rhythm of metabolic activity that matches to DVM swimming
126 behavior and is in line with previous finding [31].

127

128 Clock gene expression

129 To investigate the expression of clock genes under controlled conditions, copepods were
130 collected in Loch Etive and, as for DVM and respiration experiments, transferred to the
131 laboratory where they were exposed to LD and DD conditions. Only “core” clock genes that
132 interact via gene/protein feedback loops to create endogenous circadian rhythms were
133 investigated [16]. The results indicated strong 24 h rhythmicity in six of eight core clock
134 genes: *clock (clk)*; *period1 (per1)*; *period2 (per2)*; *timeless (tim)*; *cryptochrome2 (cry2)* and;
135 *clockwork orange (cwo)*. The two remaining core genes *cycle (cyc)* and *vriille (vri)* showed
136 weak rhythmicity (Fig.3A-H, Tab.S2). Times of peak gene expression were closely associated
137 with the time of sunset or sunrise and generally matched expression patterns of terrestrial
138 model species [32,33]. Rhythmic gene expression persisted under DD, confirming the
139 endogenous nature of the clock in *C. finmarchicus*.

140 The presence and rhythmic expression of a mammalian type *cry2* gene which peaks in the
141 evening, indicates a clock mechanism similar to the ancestral clock model known from the
142 monarch butterfly *Danaus plexippus*, where *cry2* acts as a transcriptional repressor [33].
143 Laboratory studies in this insect found rhythmic *cry2* expression to peak in the early day, as
144 with the Antarctic krill *Euphausia superba*, the water flea *Daphnia pulex* and the marine
145 annelid *Platynereis dumerilii* [3,6,33,34]. In contrast, *C. finmarchicus* *cry2* expression in the
146 laboratory peaked at sunset (Fig.3F).

147 In addition to the core clock genes, expression was also measured in a suite of genes
148 associated with the modification and localization of core clock proteins (*doubletime2*,
149 *widerborst1*, *twins*, *casein kinase II α* , *shaggy*) or light entrainment (*cryptochrome1*) [35]. In
150 accordance with previous findings, none of these clock-associated genes showed consistent
151 circadian expression (Tab.S2) [35].

152 To investigate the functioning of a circadian clock in the field we conducted a 28 h
153 sampling campaign at Bonawe deep. Clock gene expression of *C. finmarchicus* was measured
154 in two depth layers (5-50 m, 50-140 m). Generally, the expression patterns of the clock genes
155 resembled those recorded in the laboratory (Fig.3I-O). However, gene rhythms were less
156 overt in the field and the number of rhythmic genes was reduced, especially in copepods from
157 the deeper layer (Tab.S2). Temperature changes and food availability can entrain clock
158 activity [36,37] and it is possible that the vertical migration through layers of different
159 temperature and phytoplankton concentration (Fig.S2) may have affected clock gene
160 expression and resulted in more labile rhythms when compared with laboratory experiments.
161 Further the overall reduced rhythmicity at 50-140 m could reflect the physiological state of
162 the copepods. At the time of the sampling animals in the deep layer may already have started
163 transitioning to seasonal diapause, a phase of inactivity in deep waters characterized by
164 metabolic downregulation and without any known diel activity cycle [25,38]. Data collected
165 later in the year (not shown) suggests that cyclic clock gene expression ceases during

166 diapause. It is also noteworthy that the more labile gene rhythms at 50-140 m depth were
167 mirrored by the weaker DVM signal acoustically recorded in this layer in Loch Etive (Fig.1)
168 further suggesting a coupling between clock and DVM. Nevertheless, the existence of clock
169 gene cycles in animals in the deeper layer shows that circadian clocks can operate under very
170 low light intensities providing an explanation for the observations of diel migrations in meso-
171 /bathypelagic habitats [13] and at high latitudes during winter months [14,15].

172 In summary, circadian clock gene expression in *C. finmarchicus* demonstrates
173 pronounced rhythms well-suited for evoking the observed rhythms in DVM and respiration.
174 Expression patterns mostly persist in the field, strongly suggesting that the copepod possesses
175 an endogenous clock that is also functioning under natural conditions.

176

177 Ecological implications

178 The adaptive significance of a circadian clock underpinning DVM in *C. finmarchicus* and
179 other vertically migrating organisms is clear. Primarily the clock would provide a mechanism
180 for the copepods to anticipate the day/night cycle, thereby temporally adjusting behavioral
181 functions, physiology, and gene expression accordingly. However, circadian clocks have also
182 been implicated in the sensitivity to predator cues and avoidance behavior [39]. Copepods and
183 many other planktic organisms are prey to visual predators during the day [40]. The circadian
184 clock would provide a mechanism for anticipating sunrise to return to deep, dark waters
185 before sufficient light enables visual predation. For example, the sea urchin *Centrostephanus*
186 *coronatus* shows an endogenous cycle in nocturnal foraging which is closely tuned to the
187 resting times of its predator, a diurnally active fish [41], increasing the urchins chance of
188 survival and also maximizing the time it can spend foraging. Circadian clock involvement in
189 vertical swimming may also explain “midnight sinking” behavior which is characterized by a
190 descent to intermediate depth in the middle of the night followed by a second upward
191 migration closely before sunrise [12,27]. This behavior has been suggested to be an avoidance

192 response to larger vertically migrating predators, which ascend later and descend earlier [42].
193 While predation risk can usually not be sensed until the predator is present, circadian clocks
194 are highly suitable for controlling crepuscular activity patterns [12] and could thus explain the
195 two upward migrations at sunset and sunrise characteristic for “midnight sinking”.

196 Circadian clocks would also be adaptive for maintaining DVM rhythms in
197 photoperiodically extreme environments such as high latitudes during the polar night and the
198 meso-/bathypelagic zone. In both these habitats light as an entrainment cue is only
199 temporarily available and/or extremely weak and food levels are relatively constant over the
200 course of the day [43,44]. Indeed, DVM occurrence in polar night habitats and the
201 synchronized evening ascent of animals from the aphotic depths beyond 1000 m support the
202 hypothesis that DVM is underpinned by a circadian clock [13–15]. Interestingly, a recent
203 study found that vertical migration shifted from diel (24 h) to lunar-day (24.8 h) cycles under
204 the influence of the full moon during the darkest part of the Arctic polar night [15]. This may
205 indicate that during the polar night strong lunar light can either override endogenous
206 rhythmicity or can act as an entrainment cue lengthening the period of a circadian clock
207 underlying the vertical migration pattern.

208 Furthermore, *C. finmarchicus* digestive enzymes are probably produced before feeding to
209 speed up digestion thereby increasing the overall amount of food that can be consumed and
210 digested while being at the surface for a limited time [31]. A similar preparatory mechanism
211 could be involved in the endogenous and light-entrained feeding rhythms in the copepod
212 *Acartia tonsa* [45] as too the clock-controlled anticipatory enzyme production in the shrimp
213 *Palaemon squilla* [46].

214 Circadian clocks have the capacity to regulate seasonal rhythmicity by measuring
215 photoperiod [47]. This can be achieved by a light sensitive phase at the transition between day
216 and night, which is associated with clock gene peak activity (external coincidence model,
217 [48]). The presence or absence of light during this critical phase of the day/night cycle

218 provides information about the photoperiod and hence season. Alternatively, peaks in clock
219 gene activity might shift over the season following either sunset or sunrise and the phase
220 difference between these peaks would provide another measure of photoperiodic time
221 measurement (internal coincidence model, [29]). The seasonal life cycle of many insects is
222 affected by photoperiod [47] as too are various aspects of copepod biology, including
223 diapause, reproduction, activity, and feeding [49]. As with many of its congeners, *C.*
224 *finmarchicus* undergoes seasonal diapause fueled by its large lipid reserves [20] where lipid
225 content, food availability and temperature are considered important regulators of this resting
226 phase [50]. However, a clear understanding of the mechanisms initiating and terminating
227 *Calanus* diapause is still missing leading to the tantalizing suggestion that this critical life-
228 history transition maybe underpinned by a circadian clock as an integral part in the timing of
229 *C. finmarchicus*' annual cycle.

230

231 Conclusions

232 Our results provide a detailed description of clock gene expression in an ecologically
233 important marine species combined with measurements of DVM and metabolic activity. *C.*
234 *finmarchicus* shows robust clock gene cycling in the wild and endogenous 24 h oscillations in
235 the laboratory. The persistence of circadian rhythms in DVM and respiration under constant
236 conditions suggests circadian clock involvement in the regulation of these processes. So far,
237 the mechanistic link between clock rhythmicity and phenology remains elusive where
238 functional analyses of the clock machinery and its output pathways is now required. DVM has
239 previously been shown to occur in the high Arctic during the polar night, in the aphotic depths
240 beyond 1000 m and spontaneously as midnight sinking, all of which contradict the
241 assumption of DVM being driven by purely exogenous cues. Given the ecological benefits
242 offered by endogenous timekeeping it seems likely that circadian clocks are extant in the
243 regulation of vertical migration patterns. Furthermore, investigations of clock systems and

244 DVM in marine phytoplankton and cyanobacteria [5,51] have led to the suggestion that
245 circadian DVM could exist even in these primordial organisms [52]. Our study provides a
246 basis for better understanding the mechanisms of DVM and also for exploring the adaptive
247 advantages of ancestral clock systems, which are hypothesized to have originated in the
248 aquatic environment [53].

249 Author contributions

250 N.S.H.: principle investigator, study design, fieldwork, laboratory experiments, gene
251 expression analysis, video analysis, rhythm analysis, interpretation, manuscript preparation
252 and review. B.M.: study design, interpretation, and manuscript review. K.S.L.: fieldwork,
253 acoustic data analysis, interpretation, and manuscript review, D.W.P.: fieldwork and
254 manuscript review. L.H.: fieldwork, laboratory experiments, and video analysis. M.T.: study
255 design, interpretation, and manuscript review.

256

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- 428

429 Figure Legends

430

431 Figure 1. Backscatter (Sv) profile at Bonawe deep, Loch Etive in May 2015. DVM rhythms
432 had periods (τ) of 23.9 h and 24.0 h at 25 m and 90 m, respectively (TSA Cosinor analysis,
433 4th-11th May). Color bars indicate local sunrise/sunset. 28 h field sampling is indicated by
434 white box. The sharp backscatter change at ~38 m is a measuring artefact caused by the two
435 acoustic profilers. Sampling site and water column characteristics are detailed in Figures S1
436 and S2, respectively.

437

438 Figure 2. DVM and respiration rhythms in the laboratory. (A): DVM. Depth of *C.*
439 *finmarchicus* CV stages in 90 cm “DVM-columns” is shown. Data derived from video
440 recordings. Mean values (n = 4) \pm standard error of means (SEM) are shown. (B):
441 Respiration. Mean values (n = 6) for each time point are shown. Due to the high sampling rate
442 (5 min), error bars were removed for the sake of clarity. Color bars indicate (subjective) day
443 and night. For both phenotypes, the first day with natural light/dark cycle (LD, photoperiod =
444 16 h) and the two following days in constant darkness (DD) were analyzed separately, as
445 indicated by the dashed grey line. Asterisks (*) indicate significant 24 h rhythmicity.
446 Sinusoidal curves (red) were fitted to illustrate the partially damped but still highly significant
447 rhythms. For exact p-values see Table S1.

448

449 Figure 3. Diel expression patterns of core clock genes in the laboratory (A-H) and in the field
450 (I-P). Expression patterns were recorded in *C. finmarchicus* CV stages and the investigated
451 genes were: *clock* (*clk*), *cycle* (*cyc*), *period1* (*per1*), *period2* (*per2*), *timeless* (*tim*),
452 *cryptochrome2* (*cry2*), *clockwork orange* (*cwo*), and *vri* (*vri*). Color bars indicate
453 (subjective) day and night. In the laboratory experiments (A-H), rhythm analysis was done
454 separately for LD (photoperiod = 16 h) and DD intervals as described in Fig.2. Per time point

455 n = 10 replicates were pooled from two identical experimental runs. In the field (I-P), samples
456 from 5-50 m (shallow) and 50-140 m (deep) were investigated (photoperiod = 16 h). n = 5
457 replicates per time point. Both, laboratory and field data was analyzed for rhythmic
458 expression using the R-package “RAIN”. Asterisks (*) indicate significant 24 h rhythmicity.
459 Mean values \pm standard error of means (SEM) are shown. For exact p-values see Table S2.

460 STAR★METHODS

461

462 CONTACT FOR REAGENT AND RESOURCE SHARING

463 Further information and requests for resources and reagents, including the video material of
464 the DVM experiment, the sequences of custom Taqman[®] probes/primers and the RAIN
465 rhythm analysis script, should be directed to and will be fulfilled by the lead author, Sören
466 Häfker (shaefker@awi.de).

467

468 EXPERIMENTAL MODEL AND SUBJECT DETAILS

469 All investigations were performed on CV life stages of the copepod *Calanus finmarchicus*
470 (Gunnerus, 1770). Copepods were collected at the sampling site Bonawe deep in Loch Etive,
471 Scotland (Fig.S1) and laboratory experiments were performed at the Scottish Association for
472 Marine Science (SAMS) at *in situ* temperature (10°C). During the transfer to the laboratory
473 (max. 1.5 h) the copepods were kept dark and at *in situ* temperature. For the laboratory
474 experiments filtered and UV-treated seawater was used that was pumped in from below a
475 beach next to the institute. The water was adjusted to a salinity of 27.5 by adding Milli-Q
476 water to match the conditions at the sampling site in ~50 m depth.

477 Laboratory copepods were exposed to an *in situ* photoperiod of 16 h with a gradual
478 change in light intensity and spectral compositions to simulate the natural conditions at
479 Bonawe deep in a depth of ~50 m. From 4:00 (sunrise) on light intensity increased to ~5.5
480 Lux at noon measured right above the water surface. During this time color temperature
481 shifted from initial 15460 K to 13780 K at noon. The decrease in the afternoon mirrored the
482 morning increase resulting in complete darkness at 20:00 (sunset). To create these light
483 conditions, a programmable LED-system was used (Mitras Lightbar oceanic blue / ProfiLux
484 3.1T control unit, both GHL Advanced Technology GmbH, Germany).

485

486 METHOD DETAILS

487

488 Study site characteristics

489 Loch Etive is a sea loch at the western coast of Scotland, UK (56°45'N, 5°18'W). It is
490 connected to the open ocean by a sill with a width of 200 m and ~7 m water depth and has
491 another sill with ~13 m depth further up the loch [54]. Beyond the second sill there is the
492 upper main basin with the deepest point of the loch (Bonawe deep, ~145 m) where all
493 samplings were done (Fig.S1). The sills limit the water exchange leading hypoxic conditions
494 in the deeper layers of the upper basin. Turnover events occur during the strongest spring
495 tides in spring/autumn, but are irregular and only happen every few years [54].

496 During the sampling of the 28 h field time series at Bonawe deep (6th/7th May 2015) the
497 water column parameters salinity, temperature, oxygen concentration and Chlorophyll a (Chl
498 a) fluorescence were recorded by a conductivity-temperature-depth (CTD) profiler (SBE
499 19plus V2 SeaCAT Profiler, Sea-Bird Electronics, USA). The water column was
500 characterized by an approx. 5 m thick surface layer with a low salinity ≤ 20 psu (Fig.S2). From
501 5 m on salinity gradually increased to 27 at ~50 m and showed only a minor increase below
502 this depth. Temperature from the surface to 26 m depth ranged between 8.3°C and 8.9°C.
503 Below 26 m temperature sharply rose to a maximum of 12.2°C at 50 m depth before gradually
504 decreasing to 10.4°C at 90 m and below (Fig.S2). The deeper layers of Bonawe deep were
505 hypoxic during the sampling. From the surface to 26 m depth oxygen concentrations was ≥ 8.5
506 mg O₂*L⁻¹ before sharply decreasing to 3.6 mg O₂*L⁻¹ at 40-43 m depth (Fig.S2). Oxygen
507 concentration then continued to gradually decreased to values ≤ 1.6 mg O₂*L⁻¹ in ~80 m depth
508 and below. Chl a fluorescence was high in the upper 10 m (4-16 mg*m⁻³), showed a second,
509 much smaller maximum at ~25 m and then quickly diminished with depth (Fig.S2). The

510 conditions were similar in spring 2016 when animals for laboratory experiments on DVM and
511 respiration were collected (data not shown).

512

513 Vertical migration in the field

514 A mooring was deployed close to Bonawe deep (depth: ~135 m) in March 2015 (Fig. S1).
515 The mooring was equipped with two acoustic Doppler current profilers (ADCPs) pointing
516 upwards at 120 m and 45 m depth. The RDI 300 kHz ADCPs have been employed
517 successfully in making biological observation of zooplankton migrations [14,15]. ADCP data
518 were checked for quality using the RDI correlation index (a measure of signal to noise ratio)
519 and absolute volume backscatter (S_v , measured in decibels, dB) was derived from echo
520 intensity following the method described in Deines [55] with derived acoustic mean volume
521 backscattering strength (MVBS). Acoustic data was analyzed via population mean TSA
522 Cosinor analysis for backscatter rhythmicity in 25 m and 90 m depth (time series analysis
523 [TSA] Cosinor 6.3 package). For the period 4th to 11th May 2015 significant backscatter
524 rhythmicity could be detected in both, the shallow (45 m, $\tau = 23.9$ h, % model fit = 49.6) and
525 the deep layer (125 m, $\tau = 24.0$ h, % model fit = 33.3). Tests on tidal (~12 h) and lunar (24.8
526 h) rhythms did not produce any significant rhythmicity.

527

528 Field time series

529 Samples were collected at Bonawe deep on the 6th/7th May 2015 starting at 11:00 and
530 continuing in 4 h intervals until 15:00 of the next day, resulting in a total of eight time points
531 over a period of 28 hours. At each time point a WP2-net (200 μ m mesh size, Hydro-Bios
532 GmbH, Germany) was towed vertically through the water column to collect animals from 5-
533 50 m depth and 50-140 m depth, respectively. Generally, the upper 5 meters of the water
534 column were excluded to avoid hypoosmotic stress for the copepods. Upon retrieval of the
535 net, the sample was immediately (within 1 min) transferred into RNAlater[®] stabilizing

536 solution (Ambion, UK) for later gene expression analysis (see below). A possible sample
537 contamination by the congener species *C. helgolandicus* is unlikely due to its limited
538 tolerance to low salinities and the brackish conditions in the loch [25].

539

540 DVM experiment

541 To investigate the diel vertical migration (DVM) behavior, copepods were incubated in four
542 so-called DVM-columns made out of acrylic glass (10*8*90 cm lxxh, 7.2 L). Animals were
543 collected on the 3rd June 2016, sorted, and per column 50 *C. finmarchicus* CV stages were
544 incubated for a total of three days (LD-DD-DD, photoperiod = 16 h). The columns were
545 vertically divided into six 15 cm increments and each layer was filmed with a surveillance
546 cameras equipped with filters excluding visible light (SK-B140XP/SO, Sunkwang
547 Electronics, South Korea). Infrared lights were used to illuminate the columns without
548 disturbing the animals. Copepod abundance per layer was then counted by three different
549 persons from the recorded video material at 1 h intervals. For every column, there was a
550 certain fraction of copepods which was inactive and never left the bottom layer of the column.
551 These animals were excluded from statistical analysis by determining the lowest number of
552 copepods in the bottom layer over the course of the experiment for each column, respectively.
553 This number was then defined as zero for the respective column.

554 Copepods were not fed during the DVM experiments to avoid particle accumulation at the
555 bottom, which could have affected vertical distribution. At the end of the experiment a
556 vertical oxygen profile was recorded using an oxygen tipping probe (PreSens GmbH,
557 Germany). There was a weak (<6%), gradual decrease in oxygen from 9.27 mg O₂*L⁻¹ near
558 the surface to 8.75 mg O₂*L⁻¹ close to the bottom.

559

560 Respiration experiment

561 Copepods collected on the 23rd June 2016 and sorted for *C. finmarchicus* CV stages were
562 distributed to six glass bottles (305 mL) with filtered (0.2 µm) and UV-treated seawater which
563 had been air-equilibrated for >1 h (10 animals per bottles). Two additional bottles without
564 animals served as controls. Bottles were closed tightly without any air bubbles inside and
565 incubated for three days (LD-DD-DD, photoperiod = 16 h). Oxygen content was measured
566 using oxygen-sensitive sensor spots and monitoring equipment (OXY-4, PreSens GmbH,
567 Germany). A moving average over 12 h was calculated to remove the trend of gradually
568 decreasing oxygen within the bottles and to reveal underlying rhythmic oscillations. A simple
569 inverse correlation between oxygen content and animal oxygen consumption was assumed.
570 As the moving average is based on comparing O₂-levels between time points, the resulting
571 relative change in oxygen consumption is dimensionless. Data was binned to 1 h intervals for
572 rhythm analysis (see below).

573

574 Gene expression experiment

575 Copepods were collected on the 22nd May 2015 in 10-60 m depth. In the laboratory the
576 animals were evenly distributed to 19 buckets filled with 20 L seawater. At midnight the
577 sampling started by pouring the animals from the first, randomly chosen bucket through a
578 sieve and fixing them in RNAlater[®]. Every 4 h another bucket was sampled accordingly
579 resulting in a total of 19 time points over a period of three days (72 h). On the first
580 experimental day (0-24 h) the animals were exposed to a natural light/dark regime (LD, see
581 above) while they were kept in constant darkness (DD) on the second and third day (24-72 h).
582 Copepods were fed with phytoplankton (Shellfish Diet 1800, Reed Mariculture Inc., USA) in
583 4 h intervals. A constant food concentration of ~200 µg C*L⁻¹ was maintained to avoid
584 starvation effects while not introducing a new *Zeitgeber*. The experiment was repeated in the
585 same way (LD-DD-DD) with copepods collected on the 29th May 2015 and the data of both
586 runs was pooled.

587

588 Gene expression analysis

589 Gene sequences were taken from an Illumina transcriptome of *C. finmarchicus* [23]. Core
590 clock and associated genes had been previously annotated by Christie et al. [56].
591 Housekeeping genes were newly annotated from the respective transcriptome. All gene
592 annotations were verified via blastn against NCBI database (see Tab.S2 for accession
593 numbers). They were then investigated for common protein domains via blastx and were
594 checked for palindromic sequences and repeats via Oligoanalyzer 3.1
595 (<http://eu.idtdna.com/calc/analyzer>) and RepeatMasker 3.0 ([http://www.repeatmasker.org/cgi-](http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker)
596 [bin/WEBRepeatMasker](http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker)). Binding regions for probes and primers were placed in sequence
597 intersects that were specific for the respective genes (checked via blastn).

598 To measure gene expression, copepods were sorted in cooled RNAlater[®] (4°C) using
599 dissecting microscopes. *C. finmarchicus* CV stages were pooled in groups of 15 copepods and
600 RNA was extracted using the RNeasy[®] Mini kit (Qiagen, Netherlands). β-mercaptoethanol
601 was added to the lysis buffer (0.14 M) as recommended for lipid-rich samples. DNA residues
602 were removed with the TURBO DNA-free kit (Life Technologies, USA) and RNA was
603 checked for concentration and purity (Nanodrop 2000 Spectrophotometer, Thermo Fisher
604 Scientific, USA) as well as possible degradation (2100 Bioanalyzer / RNA 6000 Nano Kit,
605 Agilent Technologies, USA). RNA was then converted to cDNA using RevertAid H Minus
606 Reverse Transcriptase (Invitrogen GmbH, Germany). Gene expression was analyzed by real-
607 time quantitative PCR (ViiA[™] 7, Applied Biosystems, USA) using custom-designed
608 Taqman[®] low-density array-cards (Applied Biosystems, USA). The list of investigated genes
609 included eight core clock genes, five clock-associated genes, one gene involved in clock
610 entrainment via light, and 3 housekeeping genes (see Tab.S2). Gene expression levels were
611 normalized against the geometric mean of the housekeeping genes *elongation factor 1 α*, *RNA*
612 *polymerase* and *actin* using the $2^{-\Delta\Delta CT}$ -method developed by Livak & Schmittgen [57].

613 Housekeeping genes were chosen based on expression stability over the 24 h cycle,
614 expression level relative to other investigated genes and the findings of previous studies [58].
615 For both experimental runs, five replicates were analyzed per time point. As there were no
616 visible differences between the first and the second run, the datasets were pooled and treated
617 as one resulting in $n = 10$ replicates per time point. For the 28 h field time series, $n = 5$
618 replicates were analyzed per time point and depth. Shallow and deep samples were
619 normalized against housekeeping genes together to ensure comparability of expression levels
620 between depths.

621

622 QUANTIFICATION AND STATISTICAL ANALYSIS

623 Datasets of DVM, respiration and gene expression were investigated for 24 h rhythmicity in
624 RStudio (version 0.99.442, [59]) using the RAIN-package. RAIN was specifically designed to
625 detect (circadian) rhythms in biological datasets independent of waveform by using a non-
626 parametric approach [60]. For the 28 h field time series from May 2015, each depth
627 (shallow/deep, $n = 5$, respectively) was analyzed separately as one dataset. In the laboratory
628 experiments ($n = 10$), the first 24 h interval (LD) was analyzed separately from the following
629 48 h interval (DD). The time point at midnight between the two intervals (LD/DD) was used
630 in both analyses. Due to the limited computing capacity of RAIN and the large amount of data
631 from the DVM ($n = 4$) and respiration experiments ($n = 6$), the mean values were used to
632 analyses rhythmicity for the 48 h DD interval of these experiments. Thus, to increase the
633 confidence in the obtained results, each DD day in the DVM and respiration experiment was
634 also analyzed individually using the respective replicates (see Tab.S1).

635 For the analyses of DVM and respiration data, an α of 0.05 was used (Tab.S1). For the
636 gene expression analyses, a p-value <0.001 was considered significant to account for the
637 testing of multiple genes (Tab.S2). Graphs were created with SigmaPlot (v. 12.5).

638

639 DATA AND SOFTWARE AVAILABILITY

640 The mRNA sequences of the investigated genes can be found via the accession numbers
641 summarized in Tab.S2. For the video material of the DVM experiment, the sequences of
642 custom Taqman® probes/primers and the RAIN rhythm analysis script, please contact the
643 lead author (shaefker@awi.de). Data of the DVM experiment (abundance counts), the
644 respiration experiment (moving averages) as well as the gene expression data of the
645 laboratory experiment and the field time series (raw CT-values) are accessible via PANGAEA
646 (<https://doi.org/10.1594/PANGAEA.875739>).