The contraction-expansion behaviour in the demosponge *Tethya wilhelma* is light-controlled and follows a diurnal rhythm

Sarah B. Flensburg[1], Anders Garm[2], & Peter Funch[1,*]

[1]Department of Biology, Aarhus University, Ny Munkegade 114-116, 8000 Aarhus C, Denmark
[2]Marine Biological Section, University of Copenhagen, Universitetsparken 4, 2100 Copenhagen Ø, Denmark

[*]Corresponding author: Peter Funch
Contact details: funch@bio.au.dk

Summary statement

A diurnal pattern regulated by light is demonstrated in the contraction-expansion behaviour of the demosponge *Tethya wilhelma* even though sponges lack both nerves and opsins.

Abstract

Sponges (phylum Porifera) are metazoans which lack muscles and nerve cells, yet perform coordinated behaviours such as whole-body contractions. Previous studies indicate diurnal variability in both the number of contractions and expression of circadian clock genes. Here, we show that diurnal patterns are present in the contraction-expansion behaviour of the demosponge *Tethya wilhelma*, by using infrared videography and a simulated night/day-cycle including sunset and sunrise mimics. In addition, we show that this behaviour is at least strongly influenced by ambient light intensity and therefore indicates light-sensing capabilities in this sponge species. This is supported by our finding that *T. wilhelma* consistently contracts at sunrise, and that this pattern disappears both when the sponge is kept in constant darkness and when in constant light.

Keywords: Porifera, pre-nervous system, light reception, behavioural control, diurnal rhythmicity

Introduction

Sponges (phylum Porifera) are aquatic animals with a global distribution, found at all water depths. The group comprises over 9,500 accepted species across four extant classes, of which class Demospongiae is the most diverse (De Voogd et al., 2021; Van Soest et al., 2012). The sponge body is organized around a complex system of cavities and canals allowing water to flow through the sponge (Asadzadeh et al., 2020). Sponges possess a number of different cell types, but lack muscle and nerve cells (Lavrov and Kosevich, 2014; Musser et al., 2021); however, they can perform coordinated behaviours such as whole-body contractions, and some even have the ability to move (Bond, 2013; Nickel, 2006). These contractions occur spontaneously, but can also be initiated by a range of different chemical stimuli (Elliott and Leys, 2010; Ellwanger et al., 2007; Ellwanger and Nickel, 2006; Goldstein et al., 2020) and mechanical stimuli locally applied to the exopinacoderm, which result in a contraction wave spreading across the surface of the sponge (Elliott and Leys, 2007; Nickel et al., 2011). This is seen in the freshwater sponge *Ephydatia muelleri* where both shaking and exposure to high loads of ink particles lead to a contraction, the latter resulting in the expulsion of waste particles from its water canal system (Elliott and Leys, 2007). The mechanism initiating this behaviour is unknown, although primary cilia located in the oscular area are suggested to function as mechanoreceptors involved in water flow regulation and sponge body contractions (Ludeman et al., 2014). Despite the lack of nerve cells, stimulation of one part of the sponge often results in behavioural changes throughout the entire sponge, suggesting coordination which is possibly facilitated by chemical messengers (Kealy et al., 2019). Light also influences sponge behaviours such as the release of larvae, which in some species is correlated with the lunar cycle (Amano, 1986; Amano, 1988; Hoppe and Reichert, 1987; Neely and Butler, 2020). Likewise, some sponge larvae respond to changes in light conditions, suggesting that they possess a light-sensory system (Leys et al., 2002). However, genetic data suggest that sponges lack the photopigment opsin, most often involved in animal light reception, and larval studies of the demosponge *Amphimedon queenslandica* indicate that this function is fulfilled by cryptochromes instead (Mah and Leys, 2017; Rivera et al., 2012). In addition to performing behaviours normally associated with a nervous system, sponges also possess many of the genes associated with the nervous system in other animals. This has led to the hypothesis that sponges, while not having a nervous system *per se*, either possess nerve-like elements resembling a pre-nervous system or possess remnants of a nervous system, able to perform some of the functions normally only seen in proper nerve cells (Leys, 2015; Musser et al., 2021).

Diurnal rhythms are observed in many different types of organisms. In animals, a typical pattern consists of alternating periods of inactivity, i.e. sleep, and an active phase (Siegel, 2008). These patterns also occur in animals without a conventional brain (for a definition, see Richter et al. (2010)) such as the cnidarian polyp *Hydra vulgaris* (Kanaya et al., 2020) and the jellyfish *Cassiopea* sp. (Nath et al., 2017). However, studies of diurnal rhythms in animals without nerve cells are very limited. In a long term *in situ* study Reiswig (1971) showed a synchronized diurnal cycle of body contractions and expansions of the demosponge *Tectitethya crypta*. Another demosponge, *Tethya wilhelma*, also seems to exhibit diurnal variations in body contractions, as indicated by two specimens tested under laboratory conditions with an artificial 12:12 h light:dark cycle (Nickel, 2004). The control mechanisms behind the diurnal rhythm were not tested; however, molecular work on *Amphimedon queenslandica* revealed a diurnal cycle in the expression of two metazoan circadian clock genes, suggesting that it could be at least partly intrinsically regulated (Jindrich et al., 2017). The detection of diurnal rhythms appears to differ between species and possibly also experimental conditions; for example, an *in situ* study on the demosponge *Aplysina aerophoba* did not suggest any diurnal rhythms to pumping activity (Pfannkuchen et al., 2009), whereas an *ex situ* study on the sponge *Cliona orientalis* indicated diurnal periodicity in both pumping rates and oscula contractions (Strehlow et al., 2016).

In this study, we assessed the diurnal rhythm in the body contraction-expansions of the sponge *T. wilhelma* indicated from previous studies, and tested the potential influence of ambient light intensity by manipulations of the light regime. We confirmed the presence of diurnal rhythms in the contractions, and also found evidence for the rhythm to, at least largely, be controlled by ambient light levels.

Materials and methods

Culturing of sponges

Specimens of *Tethya wilhelma* Sarà et al., 2001 were collected at the National Aquarium of Denmark, the Blue Planet, (Copenhagen, Denmark) in a coral reef display tank with sea water of 24°C and 33 psu. The specimens had a diameter of 0.5-1.0 cm. They were transported to Aarhus University where they were transferred to individual wells of a six-well culture plate. Each well had a 2.5 cm diameter black plastic disk placed at the bottom for attachment. The well plate was then placed in a 25-litre glass tank with seawater collected near Lemvig, Denmark, adjusted to 32-34 psu using artificial sea salt (Instant Ocean, Aquarium Systems, France). The water temperature was kept at 22°C, controlled by the room

temperature. The tank water was filtered and circulated using an EHEIM eXperience 150 external filter pump, which included a biological filter (EHEIM GmbH & Co. KG, Germany). Light was supplied by a Fluval Plant Nano Bluetooth LED light (Rolf C. Hagen Inc., Canada), programmed to simulate sunrise with a linear increase in light intensity from 6:00 AM to 7:00 AM, daylight from 7:00 AM to 5:00 PM, sunset with a linear decrease in light intensity from 5:00 PM to 6:00 PM, and dark from 6:00 PM to 6:00 AM. The light intensity and emission profile was measured with a RAMSES radiometer (TriOS Mess- und Datentechnik GmbH, Germany) (Fig. S1A). During daytime, the light had an intensity of $0.8 \cdot 10^{17}$ photons m^{-2} s^{-1}, corresponding to normal room light intensities. Approximately one third of the water in the tank was exchanged every three weeks throughout the experimental period.

Experimental setup

The sponges were transferred to a submerged clear acrylic box (12/17/4.8 cm, H/W/D) inside the holding tank. Light could enter directly from above and from the sides, except the back which was covered with black fabric to provide a uniform dark background for video recordings. A weight was placed on top of the box to prevent it from moving as a result of the water currents in the tank during video recordings. The sponges were filmed from the outside of the tank to document changes in the sponge body volume, measured as changes in the projected area. Recordings were made with a Panasonic HC-VX980 digital camcorder (Panasonic Corp., Japan) using the night mode setting. Two infrared LED light sources (peak emission at 940 nm) were used for illumination during the night (Fig. S1B,C).

Test for diurnal rhythm

To characterize the contraction pattern of *T. wilhelma* under simulated natural dark/light conditions, six specimens were filmed one at a time 72 hours each. The sponge to be filmed was transferred to the recording chamber at least three hours before the start of the recording session to minimize the influence of the handling. All recordings began at 6:00 PM on the first day and after 72 hours of recordings, the sponge was transferred back into the well plate. The influence of the light regime on contraction cycles was investigated by subjecting six new specimens to 72 hours of constant light and 72 hours of constant dark. These two treatments were separated by a seven-day recovery period with a 12:12 h dark:light cycle. During the recovery period, the sponges were placed in another tank. Three sponges were

tested at the same time and they were again transferred to the recording chamber at least three hours before the start of the experiment. Because the experimental period started at 6:00 PM, a simulated sunset was included at the onset of the constant darkness experiments but not the constant light experiment. To ensure complete control of the light environment in the experimental tank, it was covered by a light proof box also including the camera and light sources.

Processing of video material

Single frames were extracted from the videos with a time resolution of 100 s and transferred to the software program ImageJ (Rasband, 2020) as an image stack. Here, a scale bar was added using the plate the sponges were attached to as a reference. The images were converted into 8-bit greyscale, cropped, and the brightness and contrast adjusted to optimize the contrast between sponge and background. Due to the contrast between the bright sponge and the dark background, the part of the image containing the sponge could be selected by converting the image to a binary image by setting an appropriate threshold and applying it to the image stack. The projected area of the sponge was then measured using a "Measure Stack" macro in ImageJ (Fig. S1D).

Analysis of contraction patterns

The area measurements of each sponge were normalized by the maximum area during the 72-hour period to obtain a relative projected area (RPA) (see Goldstein et al. (2020)). The mean RPA for each 12-hour period (6:00 AM to 6:00 PM and 6:00 PM to 6:00 AM) was calculated with a time resolution of 100 s. The number of fully contracted states, identified as RPA minima, were also counted for each 12-hour time period. The mean contraction amplitude was calculated for each 12-hour time period as the mean change in RPA between the initiation of the contractions and the fully contracted states during that time period. The mean contraction area was calculated as the mean RPA of the fully contracted states during each 12-hour period. Lastly, the minimum and maximum RPA during each 12-hour time period was identified. If a contraction spanned both dark and light periods then it was assigned to the period where the contraction was initiated. The sponges are constantly making small changes of their volume and accordingly a 7% reduction in RPA was set as the threshold before it was considered a contraction. This threshold was chosen as contractions exceeding a 7% reduction in RPA displayed the typical shape of a contraction, with a relatively fast contraction, followed by a slower expansion phase. A combined graph was produced for each

of the three experiments, combing the individual specimens (N=5 or 6) and the individual days (N=3) into one graph to reveal possible consistent patterns in the timing of the individual contractions within the 24-hour light cycle. Here, 95% confidence intervals were reported.

Statistical analyses

The statistical analyses were performed in RStudio version 2022.02.1 (RStudio Team, 2022), using the R packages "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017). Plots were made using the package "ggplot2" (Wickham, 2016) and Microsoft Excel. Linear mixed-effect models (LMEM's) were fitted, which met the assumptions of normal distribution, homoscedasticity, and independence of residuals. Response variables used were: mean RPA, mean contraction amplitude, mean RPA of contractions, minimum RPA, and maximum RPA. Explanatory variables were included as follows: day period (either day or night) was added as a fixed factor, and sponge ID and day number were added as random effects. For the analysis of the number of fully contracted states, a generalized linear mixed model (GLMM) with poisson distribution was fitted to the data. This model met the same assumptions and contained the same explanatory variables and random effects as the LMEMs. In the comparisons between experiments (dark/light, constant dark, and constant light), experiment was added as a fixed factor instead of period, and *post hoc* tests were performed by multiple comparisons of means using Tukey contrasts and single-step method for adjusting p-values using the 'glht'-function from the R package "multcomp" (Hothorn et al., 2008). The critical p-value was set to 0.05 for all tests. The standard deviations (± s.d.) are calculated based on number of specimens with the experimental days pooled together.

Results

Diurnal rhythms

When sponges were kept under normal light conditions (N=6), the mean RPA was 0.873 (±0.026) for the night periods and 0.803 (±0.050) for the day periods, which is a significant difference (LMEM, t=3.6, p=0.0011) (Fig. 1A). The mean number of fully contracted states also differed significantly between night and day periods (GLMM, z=-2.4, p=0.016) with a mean of 2.22 (±0.78) during night periods and 3.61 (±0.77) during day periods (Fig. 1B). The relative contraction amplitude was 0.214 (±0.076) during the night and 0.190 (±0.058) during the day (Fig. 1C). This difference was not significant (LMEM, t=1.2, p=0.239) and neither

was the difference in the mean contraction RPA which were 0.705 (±0.073) and 0.676 (±0.083) for night periods and day periods, respectively (LMEM, t=1.6, p=0.135) (Fig. 1D). The mean minimum RPA was 0.702 (±0.062) during the night period and 0.645 (±0.087) during the day period, and this difference was significant (LMEM, t=2.3, p=0.028) (Fig. 1E). Finally, the maximum RPA did not differ between night period, 0.941 (±0.032), and day period, 0.933 (±0.020) (LMEM, t=0.4, p=0.684) (Fig. 1F).

Influence of light environment

Under constant darkness (N=5) and constant light conditions (N=6), no significant differences were found between night and day periods in any of the six examined parameters (Table 1, Fig. 1). When comparing the actual day-time between the experiments, the mean RPA was significantly higher in the constant dark experiment compared to the dark/light according to the linear mixed-effect model (LMEM, t=-2.3, p=0.040), but in the multiple comparisons test this trend is not significant (Tukey comparisons *post hoc*: p=0.054) (Fig. 1A). Also, minimum RPA was significantly higher in the constant dark experiment compared to the dark/light experiment according to the linear mixed-effect model (LMEM, t=-2.3, p=0.047), but again this trend is not significant according to the multiple comparisons test (Tukey comparisons *post hoc*: p=0.055) (Fig. 1E). The constant light experiments did not differ significantly from the two other experiments for any of the six parameters (Fig. 1). When comparing the actual night-time between the three experiments, the number of fully contracted states was significantly higher in constant dark experiment compared to the dark/light experiment (Tukey comparisons *post hoc*: p<0.025) (Fig. 1B), and the mean relative contraction amplitude was significantly lower in the constant dark experiment compared to the dark/light experiment (Tukey comparisons *post hoc*: p=0.028) (Fig. 1C). Again, the constant light experiment did not differ significantly from the two other experiments for any of the parameters (Fig. 1).

Furthermore, the summarized contractions-expansion diagrams showed a consistent pattern of one contraction happening shortly after the time of sunrise in the simulated dark/light-experiment (Fig. 2A, Fig. S2). This pattern was observed in neither the constant dark, nor the constant light experiments (Fig. 2B,C, Fig. S3A,B).

Discussion

Diurnal rhythm

The present study shows a diurnal rhythm in the contraction patterns of *Tethya wilhelma*, whereby the body is more expanded during the night and more actively contracted diurnally. These findings support another study on this species by Nickel (2004), where projected area measurements were also used to compare the number of contractions during day and night. However, the study by Nickel (2004) was limited to two specimens; therefore, the present study is the first to statistically prove this diurnal rhythm occurs in *T. wilhelma*. In addition, our results show that this diurnal rhythm is not restricted to the number of contractions alone but is also present in two additional parameters: minimum and mean relative projected area. Nevertheless, it should be noted that these three parameters are not completely independent, since more contractions equal more minima, and more time spent in a contracted state leads to lower mean RPA. For functional considerations of the contractions in sponges, it is important to note that although contractions result in changes of the overall volume of the sponge, these changes mainly concern the volume of the water canal system (Nickel et al., 2011). Sponge contractions have been suggested to serve different functions, including as a cleaning mechanism to expel unwanted particles by flushing the water canal system (Elliott and Leys, 2007; Nickel, 2004). In this case, diurnal periodicity in contraction-expansion behaviour could be related to maintenance of the water canal system. Further studies are needed to explore the relationship between diurnal rhythms in activity patterns and individual elements of the water canal system, and to test the hypothesis that *T. wilhelma* is more actively filtering during daytime and thus needs more flushing in this period.

Influence of light environment

Our study shows the absence of diurnal rhythms in *T. wilhelma* under constant dark and constant light conditions, implying that contraction behaviour is, at least in part, regulated directly by ambient light intensity. These results suggest that this sponge has light sensing capabilities. This is supported by our finding that a contraction occurs shortly after simulated sunrise, which may indicate that sunrise serves as a cue of when to increase activity, i.e. contractions, potentially flushing the canal system prior to increased daytime filtration. Such light sensing might have influenced the previously mentioned study by Nickel (2004), wherein an external flash was used to capture images of the contraction states of *T. wilhelma*. Further studies are needed to determine a possible behavioural impact of exposure to short flashes of light during the night and clarify if this could influence the natural contraction

patterns, such as by initiating a contraction similar to those observed at sunrise. In this study, filming was conducted under infrared light, removing the potential problem. Also, our inclusion of a simulated sunrise and sunset in the light cycle results in more natural changes of light levels. This may explain that the contraction initiated by sunrise suggested in our results was not reported by Nickel (2004), who did not include a sunrise and sunset phase in the 12:12 h light:dark cycle.

Interestingly, we found that under constant darkness the animals did not display the night-time behaviour of the animals in dark/light experiments. Under constant darkness, the number of fully contacted states and the amplitude of the contractions were similar to those of normal daytime behaviour. We have no clear indications of why this is so, but it could indicate a high level of stress during unnatural light conditions. It should be noted that the multiple comparisons *post hoc* tests failed to show differences among experiments in mean RPA and minimum RPA during the timewise day periods. This is likely due to low statistical power caused by unequal sample sizes between experiments, and as the differences in means show, effects sizes are clearly large and confirm that the sponges are more expanded during darkness, than during light (Fig. 1A,E).

The diurnal rhythm in *T. wilhelma* supports genomic studies on another demosponge *Amphimedon queenslandica*. Here, diurnal variations were found in the expression of two circadian clock genes, but the variations became less pronounced when the sponge was subjected to constant darkness (Jindrich et al., 2017). This suggests at least some endogenous control of diurnal rhythms acting alongside the light control shown by our results. Many sponge species have a diverse microbiome, which includes photosynthesizing symbionts (Bickmeyer et al., 2019; Thomas et al., 2016). The diurnal rhythm of the sponge could be influenced by this microbiome, as the associated decrease in oxygen levels inside the sponge at night could account for such behavioural changes. However, this is contradicted by the very similar patterns observed under constant darkness and constant light. As the sister group to Bilateria, cnidarians are amongst the simplest animals with neurons; here, light seems to play a similar role in their diurnal rhythms as we see in *T. wilhelma*. Studies of *Hydra vulgaris*, *Tripedalia cystophora*, and *Copula sivickisi* all show behaviours with diurnal rhythms directly influenced by ambient light intensity (Garm et al., 2012; Kanaya et al., 2020). Changes in behaviour in response to light are also observed among the sister group to animals, the choanoflagellates. For example, Brunet et al. (2019) revealed that colonies of the choanoflagellate *Choanoeca flexa* inverted when subjected to changing light levels, showing

that even simple holozoans can modulate their behaviour in response to light. This suggests that such behaviour in sponges is not at all unexpected.

Acknowledgements

We thank the National Aquarium of Denmark, the Blue Planet for providing sponges for the study; Dorthe Due Theilade for transport of sponges to our laboratory; Alexandra B. Flensburg for help with designing the experimental setup; Lars Chresten Lund-Hansen for help with light measurements; Josephine Goldstein and Clémence Rose for help with the statistical analyses, and Alison Irwin for helpful comments on the manuscript. In addition, we want to thank two anonymous reviewers for their suggestions to improve the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.B.F, A.G., P.F.; Methodology: S.B.F, A.G., P.F.; Formal analysis: S.B.F.; Investigation: S.B.F.; Resources: A.G., P.F.; Writing - original draft: S.B.F.; Writing - review and editing: S.B.F, A.G., P.F.; Visualization: S.B.F.; Supervision: A.G, P.F.; Funding acquisition: A.G., P.F.

Funding

This work was funded by a research grant (40834) from VILLUM FONDEN and by the Independent Research Fund Denmark (grant number 8021-00392B).

References

Amano, S. (1986). Larval release in response to a light signal by the intertidal sponge *Halichondria panicea*. *Biological Bulletin* **171**, 371-378.

Amano, S. (1988). Morning release of larvae controlled by the light in an intertidal sponge, *Callyspongia ramosa*. *Biological Bulletin* **175**, 181-184.

Asadzadeh, S. S., Kiorboe, T., Larsen, P. S., Leys, S. P., Yahel, G. and Walther, J. H. (2020). Hydrodynamics of sponge pumps and evolution of the sponge body plan. *Elife* **9**.

Bates, D., Machler, M., Bolker, B. M. and Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1-48.

Bickmeyer, U., Thoms, S., Koch, F., Petety Mukagatare, L., Silalahi, R. and Sartoris, F. J. (2019). Enhancement of photosynthesis in *Synechococcus bacillaris* by sponge-derived Ageladine A. *Plos One* **14**, e0213771.

Bond, C. (2013). Locomotion and contraction in an asconoid calcareous sponge. *Invertebrate Biology* **132**, 283-290.

Brunet, T., Larson, B. T., Linden, T. A., Vermeij, M. J. A., McDonald, K. and King, N. (2019). Light-regulated collective contractility in a multicellular choanoflagellate. *Science* **366**, 326-+.

De Voogd, N. J., Alvarez, B., Boury-Esnault, N., Carballo, J. L., Cárdenas, P., Díaz, M.-C., Dohrmann, M., Downey, R., Hajdu, E., Hooper, J. N. A., Kelly, M., Klautau, M., Manconi, R., Morrow, C. C., Pisera, A. B., Ríos, P., Rützler, K., Schönberg, C., Vacelet, J. and Van Soest, R. W. M. (2021). World Porifera Database. Accessed at http://www.marinespecies.org/porifera on November 3, 2022. doi: 10.14284/359

Elliott, G. R. D. and Leys, S. P. (2007). Coordinated contractions effectively expel water from the aquiferous system of a freshwater sponge. *Journal of Experimental Biology* **210**, 3736-3748.

Elliott, G. R. D. and Leys, S. P. (2010). Evidence for glutamate, GABA and NO in coordinating behaviour in the sponge, *Ephydatia muelleri* (Demospongiae, Spongillidae). *Journal of Experimental Biology* **213**, 2310-2321.

Ellwanger, K., Eich, A. and Nickel, M. (2007). GABA and glutamate specifically induce contractions in the sponge *Tethya wilhelma*. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **193**, 1-11.

Ellwanger, K. and Nickel, M. (2006). Neuroactive substances specifically modulate rhythmic body contractions in the nerveless metazoon *Tethya wilhelma* (Demospongiae, Porifera). *Frontiers in zoology* **3**, 1-14.

Garm, A., Bielecki, J., Petie, R. and Nilsson, D. E. (2012). Opposite patterns of diurnal activity in the box jellyfish *Tripedalia cystophora* and *Copula sivickisi*. *Biological Bulletin* **222**, 35-45.

Goldstein, J., Bisbo, N., Funch, P. and Riisgard, H. U. (2020). Contraction-expansion and the effects on the aquiferous system in the demosponge *Halichondria panicea*. *Frontiers in Marine Science* **7**.

Hoppe, W. F. and Reichert, M. J. M. (1987). Predictable annual mass release of gametes by the coral-reef sponge *Neofibularia nolitangere* (Porifera, Demospongiae). *Marine Biology* **94**, 277-285.

Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal* **50**, 346-363.

Jindrich, K., Roper, K. E., Lemon, S., Degnan, B. M., Reitzel, A. M. and Degnan, S. M. (2017). Origin of the animal circadian clock: diurnal and light-entrained gene expression in the sponge *Amphimedon queenslandica*. *Frontiers in Marine Science* **4**.

Kanaya, H. J., Park, S., Kim, J. H., Kusumi, J., Krenenou, S., Sawatari, E., Sato, A., Lee, J., Bang, H., Kobayakawa, Y., Lim, C. and Itoh, T. Q. (2020). A sleep-like state in *Hydra* unravels conserved sleep mechanisms during the evolutionary development of the central nervous system. *Science Advances* **6**.

Kealy, R. A., Busk, T., Goldstein, J., Larsen, P. S. and Riisgard, H. U. (2019). Hydrodynamic characteristics of aquiferous modules in the demosponge *Halichondria panicea*. *Marine Biology Research* **15**, 531-540.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* **82**, 1-26.

Lavrov, A. I. and Kosevich, I. A. (2014). Sponge cell reaggregation: Mechanisms and dynamics of the process. *Russian Journal of Developmental Biology* **45**, 205-223.

Leys, S. P. (2015). Elements of a 'nervous system' in sponges. *Journal of Experimental Biology* **218**, 581-591.

Leys, S. P., Cronin, T. W., Degnan, B. M. and Marshall, J. N. (2002). Spectral sensitivity in a sponge larva. *Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology* **188**, 199-202.

Ludeman, D. A., Farrar, N., Riesgo, A., Paps, J. and Leys, S. P. (2014). Evolutionary origins of sensation in metazoans: functional evidence for a new sensory organ in sponges. *Bmc Evolutionary Biology* **14**.

Mah, J. L. and Leys, S. P. (2017). Think like a sponge: The genetic signal of sensory cells in sponges. *Developmental Biology* **431**, 93-100.

Musser, J. M., Schippers, K. J., Nickel, M., Mizzon, G., Kohn, A. B., Pape, C., Ronchi, P., Papadopoulos, N., Tarashansky, A. J. and Hammel, J. U. (2021). Profiling cellular diversity in sponges informs animal cell type and nervous system evolution. *Science* **374**, 717-723.

Nath, R. D., Bedbrook, C. N., Abrams, M. J., Basinger, T., Bois, J. S., Prober, D. A., Stemberg, P. W., Gradinaru, V. and Goentoro, L. (2017). The jellyfish *Cassiopea* exhibits a sleep-like state. *Current Biology* **27**, 2984-2990.

Neely, K. L. and Butler, C. B. (2020). Seasonal, lunar, and diel patterns in spawning by the giant barrel sponge, *Xestospongia muta*. *Coral Reefs* **39**, 1511-1515.

Nickel, M. (2004). Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera : Demospongiae). *Journal of Experimental Biology* **207**, 4515-4524.

Nickel, M. (2006). Like a 'rolling stone': quantitative analysis of the body movement and skeletal dynamics of the sponge *Tethya wilhelma*. *Journal of Experimental Biology* **209**, 2839-2846.

Nickel, M., Scheer, C., Hammel, J. U., Herzen, J. and Beckmann, F. (2011). The contractile sponge epithelium sensu lato - body contraction of the demosponge *Tethya wilhelma* is mediated by the pinacoderm. *Journal of Experimental Biology* **214**, 1692-1698.

Pfannkuchen, M., Fritz, G. B., Schlesinger, S., Bayer, K. and Brummer, F. (2009). In situ pumping activity of the sponge *Aplysina aerophoba*, Nardo 1886. *Journal of Experimental Marine Biology and Ecology* **369**, 65-71.

Rasband, W. S. (2020). ImageJ: U.S. National Institutes of Health, Bethesda, Maryland, USA.

Richter, S., Loesel, R., Purschke, G., Schmidt-Rhaesa, A., Scholtz, G., Stach, T., Vogt, L., Wanninger, A., Brenneis, G., Doring, C., Faller, S., Fritsch, M., Grobe, P., Heuer, C. M., Kaul, S., Moller, O. S., Muller, C. H. G., Rieger, V., Rothe, B. H., Stegner, M. E. J. and Harzsch, S. (2010). Invertebrate neurophylogeny: suggested terms and definitions for a neuroanatomical glossary. *Frontiers in zoology* **7**.

Rivera, A. S., Ozturk, N., Fahey, B., Plachetzki, D. C., Degnan, B. M., Sancar, A. and Oakley, T. H. (2012). Blue-light-receptive cryptochrome is expressed in a sponge eye lacking neurons and opsin. *Journal of Experimental Biology* **215**, 1278-1286.

RStudio Team. (2022). RStudio: Integrated Development for R: RStudio, Inc., Boston, Massachusetts, USA.

Sarà, M., Sarà, A., Nickel, M. and Brümmer, F. (2001). Three new species of *Tethya* (Porifera: Demospongiae) from German aquaria. *Stuttgarter Beiträge zur Naturkunde (A, Biologie)* **631**, 1-15.

Siegel, J. M. (2008). Do all animals sleep? *Trends in Neurosciences* **31**, 208-213.

Strehlow, B. W., Jorgensen, D., Webster, N. S., Pineda, M. C. and Duckworth, A. (2016). Using a thermistor flowmeter with attached video camera for monitoring sponge excurrent speed and oscular behaviour. *Peerj* **4**.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., Olson, J. B., Erwin, P. M., López-Legentil, S. and Luter, H. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nature Communications* **7**, 1-12.

Van Soest, R. W. M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N. J., Santodomingo, N., Vanhoorne, B., Kelly, M. and Hooper, J. N. A. (2012). Global diversity of sponges (Porifera). *Plos One* **7**.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York: Springer.

Figures and Table

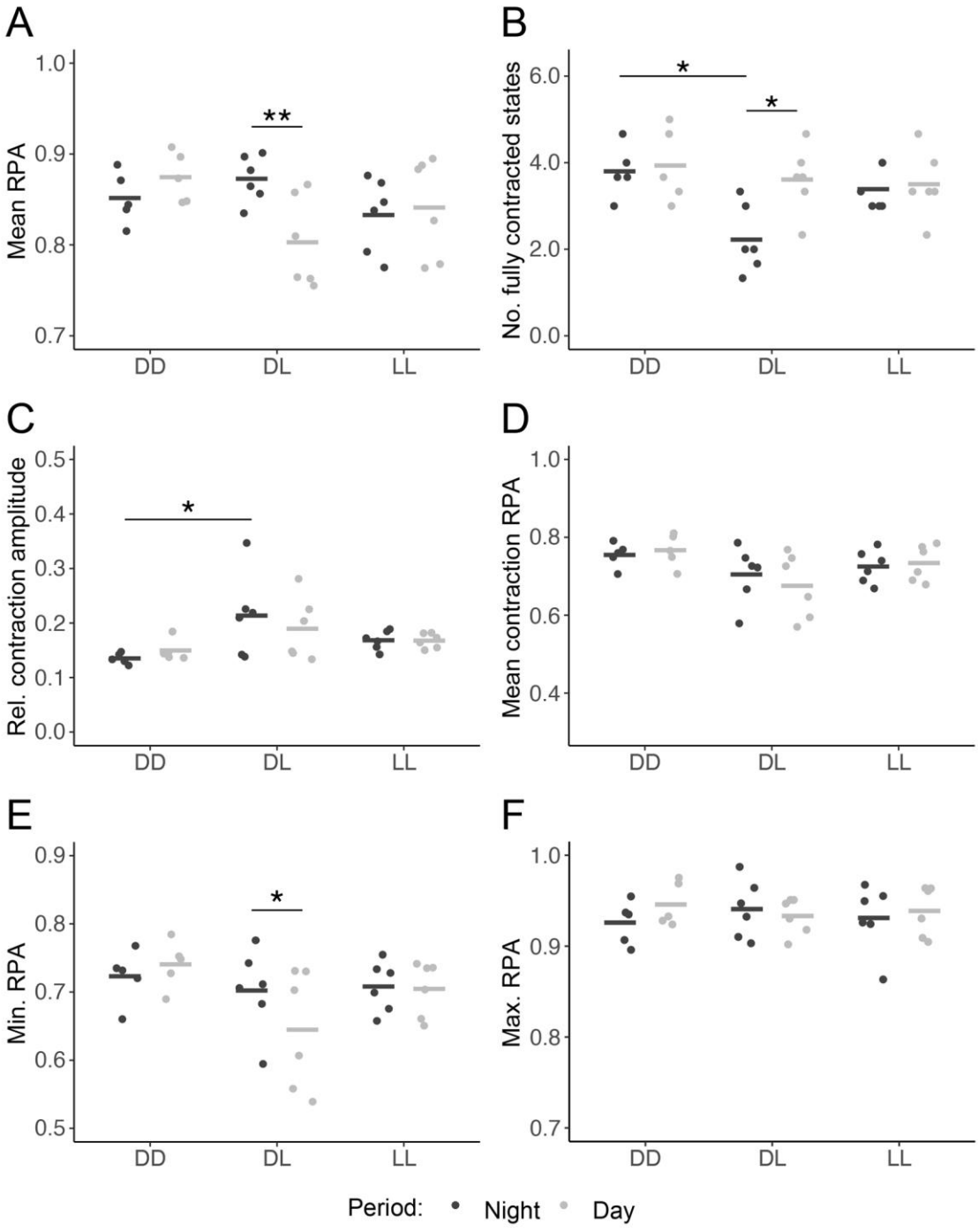

Fig. 1. Influence of light regime on contraction behaviour of *T. wilhelma*. Differences between experiments, and between night period (dark grey) and light period (light grey) in mean RPA (A), number of fully contracted states (B), relative contraction amplitude (C), mean contraction RPA (D), minimum RPA (E), and maximum RPA (F). Asterisks indicate a significant difference; *=p<0.05, **=p<0.01. Points are individual specimens (N=5 for DD, N=6 for DL and LL), bars are means. DD = constant dark, DL = dark/light, and LL = constant light.

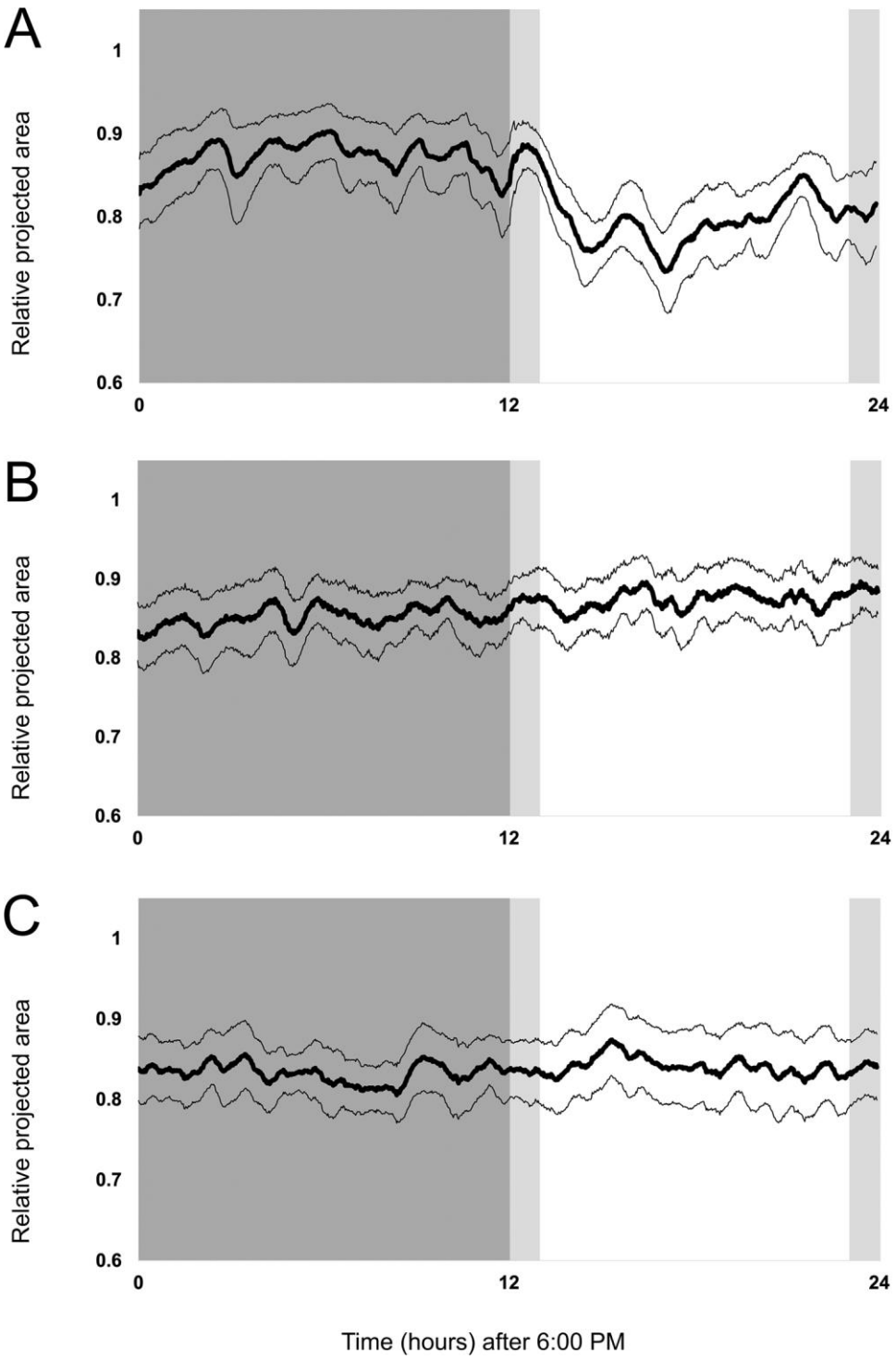

Fig. 2. Summarized contraction-expansion graphs of specimens of T. wilhelma. Mean RPA over three days for all specimens are shown with thick lines and 95% confidence interval with thin lines. A) Simulated night/day-cycle (N=6), B) constant dark (N=5) and C) constant light (N=6). Dark grey indicates night period, light grey indicates periods with sunrise/sunset.

Table 1. Results from dark/light, constant dark, and constant light experiment in *T. wilhelma*.

Simulated dark/light experiment				
	Night period	**Day period**	***t (z)***	***p***
Mean RPA	0.873 (±0.026)	0.803 (±0.050)	3.6	0.001
No. Fully contracted states	2.22 (±0.78)	3.61 (±0.77)	-2.4	0.016
Rel. contraction amplitude	0.214 (±0.076)	0.190 (±0.058)	1.2	0.239
Mean contraction RPA	0.705 (±0.073)	0.676 (±0.083)	1.6	0.135
Min. RPA	0.702 (±0.062)	0.645 (±0.087)	2.3	0.028
Max. RPA	0.941 (±0.032)	0.933 (±0.020)	0.4	0.684
Constant darkness experiment				
	Night period	**Day period**	***t (z)***	***p***
Mean RPA	0.859 (±0.031)	0.878 (±0.026)	-1.4	0.168
No. Fully contracted states	3.17 (±0.89)	3.5 (±1.31)	0.3	0.792
Rel. contraction amplitude	0.154 (±0.010)	0.163 (±0.017)	0.7	0.483
Mean contraction RPA	0.754 (±0.033)	0.766 (±0.037)	-1.0	0.352
Min. RPA	0.730 (±0.039)	0.753 (±0.035)	-1.6	0.117
Max. RPA	0.933 (±0.028)	0.952 (±0.027)	-1.1	0.281
Constant light experiment				
	Night period	**Day period**	***t (z)***	***p***
Mean RPA	0.833 (±0.041)	0.841 (±0.055)	-0.5	0.652
No. Fully contracted states	3.06 (±0.61)	3.00 (±0.42)	0.1	0.887
Rel. contraction amplitude	0.181 (±0.014)	0.177 (±0.014)	0.4	0.698
Mean contraction RPA	0.722 (±0.034)	0.728 (±0.048)	-0.5	0.612
Min. RPA	0.708 (±0.037)	0.705 (±0.040)	0.3	0.746
Max. RPA	0.931 (±0.037)	0.939 (±0.028)	-0.4	0.668

Mean (± s.d.) values for each of the six examined parameters during the night periods and the day periods. For the comparisons between periods, t- and p-values are presented, except for no. fully contracted states, where z- and p-values are presented.

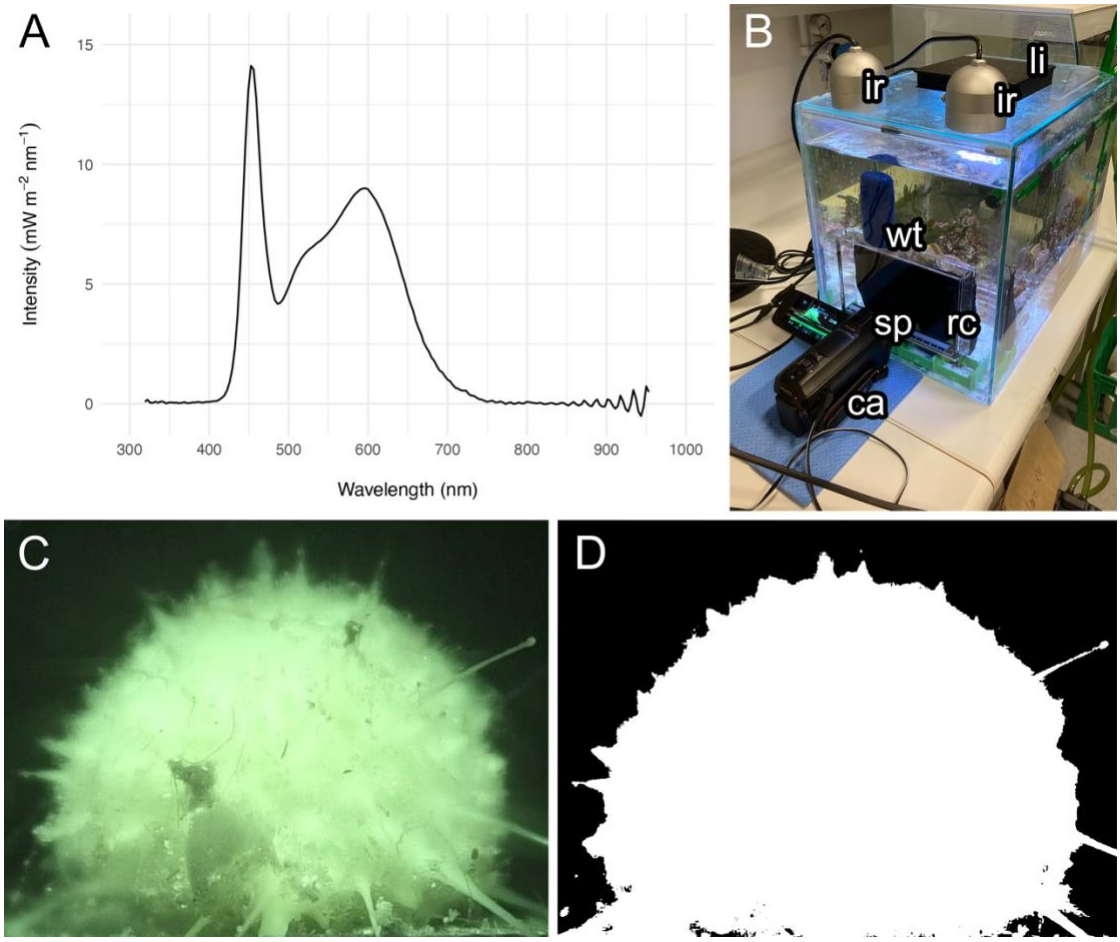

Fig. S1. Light conditions and experimental setup. Emission spectrum of the light that the sponges were exposed to during daytime (A). Experimental setup with recording equipment (B). Camera view of a sponge during recording (C). Image processing of the picture seen in C. The sponge is separated from the background, and the projected area of the sponge is measured as the white part (D). ca = camera, sp = sponge, rc = recording chamber, wt = weight, ir = infrared light sources, li = light source for daytime illumination.

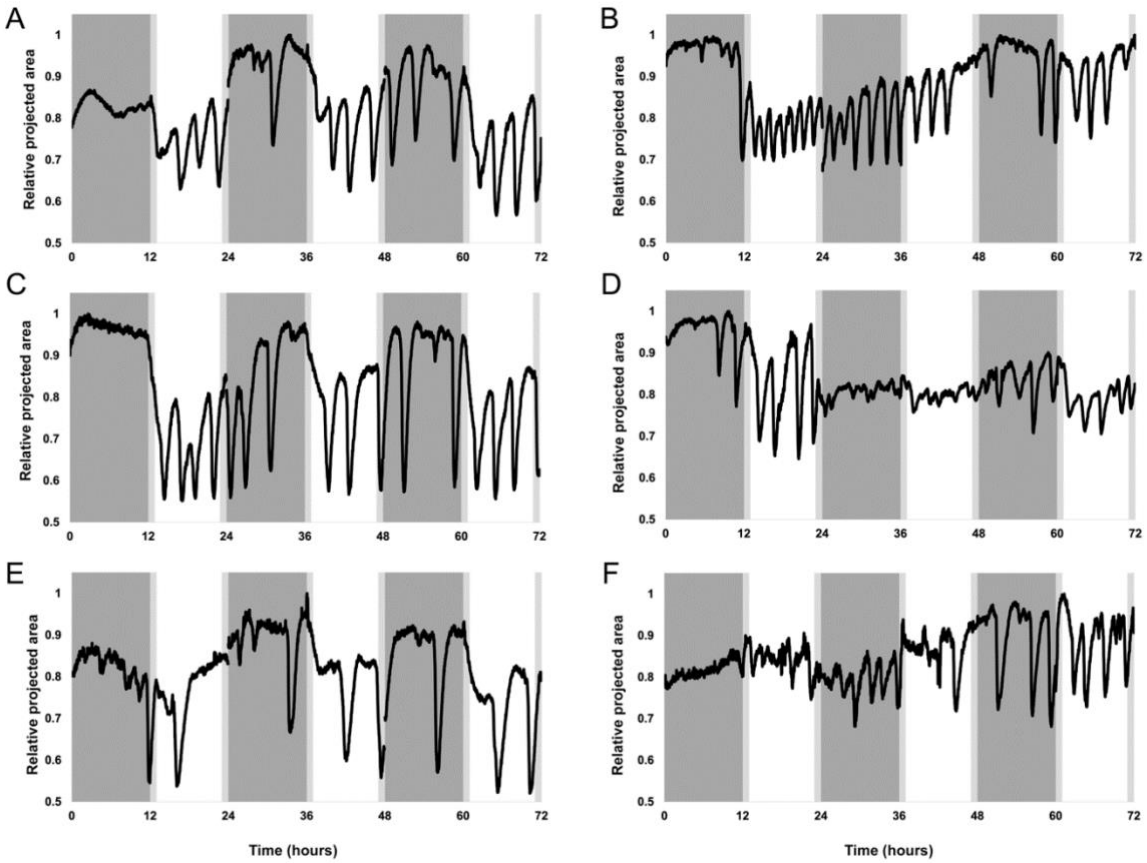

Fig. S2. Contraction-expansion behaviour of *Tethya wilhelma* (N=6) during the dark/light (DL) experiment. Contractions are shown as relative changes in projected area. Dark grey areas indicate 12 h periods in darkness, light grey areas indicate 1 h periods with either sunrise or sunset, and white areas indicate 10 h day periods in light.

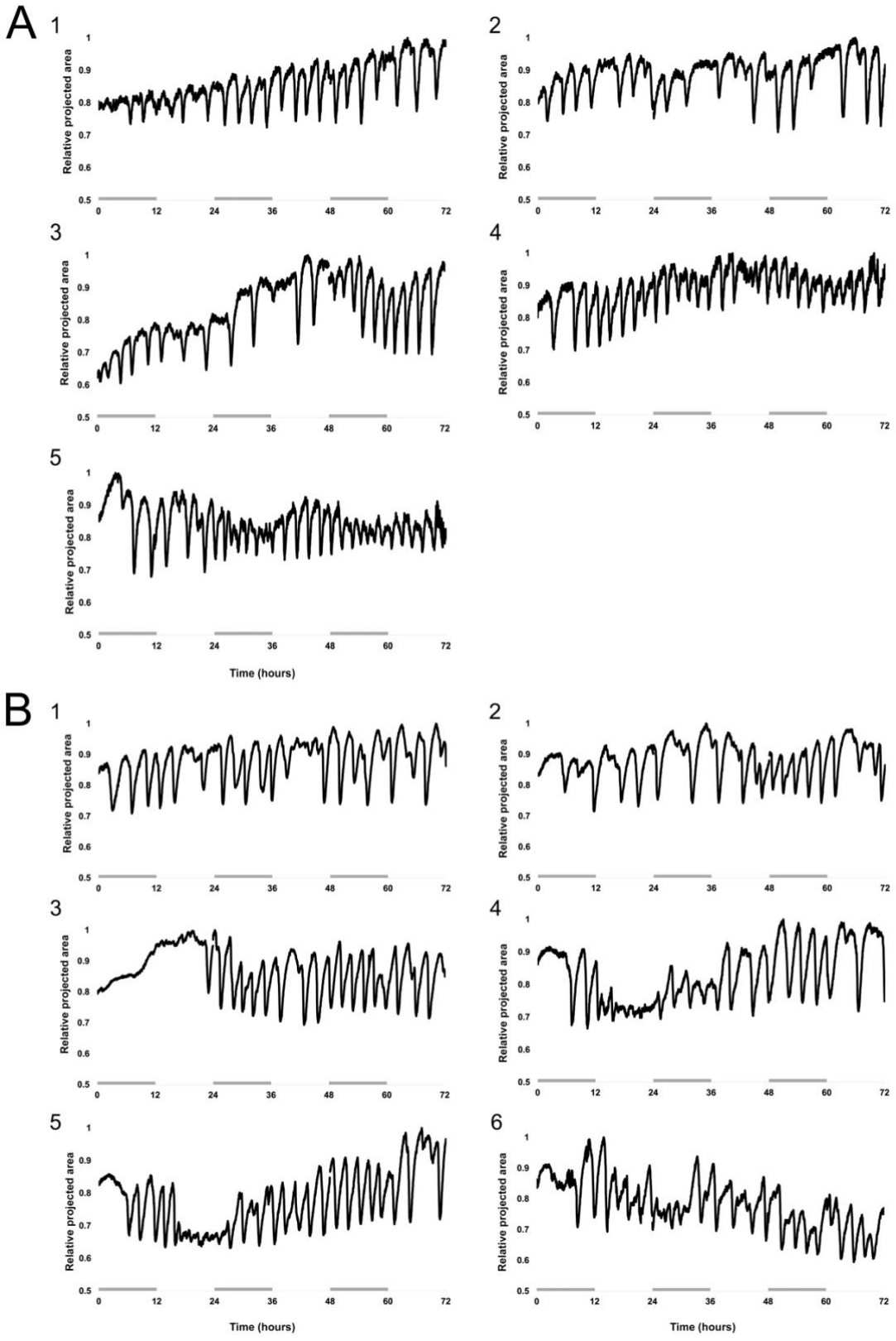

Fig. S3. Contraction-expansion behaviour of *T. wilhelma* in constant light environments. In constant darkness the sponges (N=5) lose their diurnal rhythm (A) and the same happens in constant room light intensities (B, N=6). Dark grey lines along x-axis indicate night periods between 0 and 12 h, 24 and 36 h, and 48 and 60 h.