The Journal of Experimental Biology 207, 4515-4524
Published by The Company of Biologists 2004
doi:10.1242/jeb.01289



# Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae)

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*In memoria* Martha Fritz (1925–2004)

*Accepted 15 September 2004*

## Summary

**Sponges of the species *Tethya wilhelma* display rhythmic body contractions, which were analyzed by digital time-lapse imaging and semi-automated image analysis. For the first time, differential, quantitative data on sponge behaviour could be obtained. The sponges are able to reduce their body volume by up to 73.3% during regular contractions. Each contraction cycle follows a characteristic pattern of four phases, permitting analysis of the kinetics of contraction and expansion. Long-term observations (for >7 days) reveal that the sponge contractions display a day–night periodicity in which contraction cycles are significantly longer during the dark hours. The contractions seem to be mediated by the pinacoderm; they are triggered locally and spread over the sponge surface at 12.5 μm $s^{-1}$. If two individuals of a clone are fused, the individual contraction rhythm of both sponges persists for several days, until a single new individual sponge is formed with a synchronized rhythm. The reported results and techniques establish *T. wilhelma* as a model organism for research on the development of aneural signal transduction and integration during early Metazoan evolution.**

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/207/26/4515/DC1

Key words: contraction, kinetics, diurnal rhythm, time-lapse imaging, coordination, behaviour, sponge, *Tethya wilhelma*.

## Introduction

Sponges (Porifera) are of simple organisation at first sight, and are therefore generally regarded as ‘primitive’. The foundations of sponge biology are sound (Simpson, 1984), but many important aspects of cell biology such as cell differentiation, morphogenesis and inter- and intracellular signalling, have not been fully investigated. Recent work has shown that sponges are indeed primordial Metazoa, but have a huge gene repertoire so are highly organized and complex (Breter et al., 2003; Müller, 2003). They possess many features, such as controlled behaviour, which are usually only attributed to higher Metazoa.

Sponges are generally regarded as sedentary organisms with no striking degree of behaviour or irritability. Nevertheless, since the time of Aristotle (384–322 BC) it has been well known, at least among sponge scientists, that sponges can contract (see Aristotle, 1498; Lieberkühn, 1859; Schmidt, 1866; Weissenfels, 1990), react to external stimuli (Emson, 1966; Leys and Mackie, 1997; McNair, 1923; Pavans de Ceccatty, 1979) and even move (Bond, 1992; Bond and Harris, 1988; Fishelson, 1981; Jones, 1957; Kilian, 1967; McNair, 1923). The integration and coordination of this behaviour has been widely discussed over the last 50 years. The main foci of these discussions have been whether or not sponges possess a nervous system (Jones, 1962; Lentz, 1968; Mackie, 1979, 1990; Pantin, 1952; Parker, 1910, 1919; Pavans de Ceccatty, 1960; Perovic et al., 1999) and other possible mechanisms of integration (Jones, 1962; Pavans de Ceccatty, 1974, 1979; Weyrer et al., 1999). This polarizing discussion concluded with the statement that sponges do not possess a nervous system, but did not explain the mechanisms underlying coordination in the aneural Porifera. Much more effort is needed on this topic, which is directly linked to basic questions about the evolution of multicellularity, and for which experimental model sponge systems are needed.

Recently we described three new species of the genus *Tethya* (Sarà et al., 2001), which have the potential to serve as model organisms for integrative research on behaviour, signal transduction and the underlying basal molecular mechanisms. One of these species, *T. wilhelma*, is especially interesting as a model system because it displays strong, rhythmical body contractions and is able to expand and retract body extensions and even to move (Nickel, 2001, 2003; Nickel and Brümmer, in press). In addition it is possible to cultivate *T. wilhelma*.

The present report characterizes the contraction behaviour of *T. wilhelma*, its endogenous short- and long-term rhythms, and detailed kinetics of the contraction cycles, taking into

account any endogenous and exogenous triggers involved in contraction regulation. The aim of the study was to assess sponge behaviour precisely for the first time, both qualitatively and quantitatively. We used *T. wilhelma* as a model system for quantitative physiological and pharmacological studies on signal triggering, transmission and integration from organism level down to tissue, cellular and molecular levels, respectively.

## Materials and methods

### *Animals*

Specimens of the sponge *Tethya wilhelma* Sarà et al. 2001 (Tethyidae, Hadromerida, Demospongiae) were obtained from the type location in the aquarium of the zoological-botanical garden 'Wilhelma' in Stuttgart (Sarà et al., 2001). For experiments the sponges were maintained in a 180 l aquarium, at 26°C, using running artificial seawater (Nickel et al., 2001), under a light:dark cycle of 12 h:12 h. Sponges were fed regularly using commercial liquid invertebrate food (Marine de Luxe Coralfood Extra, H+S GmbH, Herdecke, Germany). Seawater was exchanged at a rate of 5–10% of the total aquarium volume each week.

### *Experimental manipulations*

For the experiments the sponges were allowed to attach on a black plastic carrier slide for 24–48 h in the aquarium. After attachment they were transferred to stages inside the observation chambers. For one experiment, two sponges were allowed to fuse together, and then their contractile behaviours recorded.

### *Observation chambers*

Long-term observations were either performed directly in the aquarium, or in open and closed observation chambers. The open glass chamber had a volume of 3.5 l and was connected to the aquarium by a pump cycle. The closed system had a total volume of 0.25 l, and consisted of an aerated experimental reactor, based on the principles of airlift reactor design, connected to a temperature regulation unit (F25, Julabo, Seelbach, Germany). Oxygen level and temperature were monitored using a multi-sensor system (P4, WTW, Weilheim, Germany), controlled by a computer-software (MultiLab Pilot 3.0, WTW). A built-in optical glass filter (Ø 49 mm, D.K. Enterprises, India) allowed proper imaging.

### *Time-lapse imaging*

Digital images of the sponges were taken at a resolution of 2048×1536 pixels at regular intervals of 30–200 s, depending on the experiment. A Nikon Coolpix 990E digital camera in manual macro focus and exposure mode was used to acquire greyscale images. The camera was connected to a Nikon SB 24 flash unit, set to manual mode (24 mm, output 1/16). The camera was controlled by a PC, using USB connection cable and the software DC_RemoteShutter V 2.3.0 in conjunction with DC_TimeTrigger V. 1.0 (Madson, 2003). Images were downloaded, saved on the PC and erased on the CF-card of the camera immediately after being taken. A reference image including a scale bar placed next to the sponge was taken for each experimental series, for scaling. In all cases a black background was used to maximize contrast.

### *Image analysis and statistics software*

Image analysis was performed using ImageJ 1.30 and 1.31 (NIH, Washington, USA), based on built in functions (Rasband, 1997–2004; http://rsb.info.nih.gov/ij/). Excel 2000 (Microsoft, Redmond, USA) was used to prepare activity diagrams. The SPSS software package (V. 11.5, SPSS Inc, Chicago, USA) was used to perform statistical analysis.

### *Projected area measurement*

The projected area measurement was based on the contrast difference between sponge (whitish) and background (black). All images were scaled using the reference image. A threshold value between 50 and 90 was applied to the 8-bit images and the absolute projected area of the sponge was measured using ImageJ's built-in measurement tool. All time-lapse series were loaded as image stacks into ImageJ. A macro was programmed to measure semi-automatically. Measurement results were written to a text file and further computed using Excel 2000. For the aquarium-based time-lapse series a manual image control and correction was performed in ImageJ prior to measurement. In this way false measurements were avoided in cases where errant organisms (snails, polychaets or amphipods) were crawling on the sponge surface.

For the measurement of two fused sponges, the method described was slightly modified. The outer half of each sponge was used for projected area measurement. A compensation of the shift of the areas during contraction was applied manually. For projected area calculations the values of the measurements were doubled to estimate the area of each individual.

### *Segment measurement*

A measurement method based on image segmentation was developed to obtain temporally resolved information on contraction waves running over the sponge body. A grid of 1 $mm^2$ fields was projected over the image and selected fields were measured separately, as described for the whole sponge projected area above.

### *Contraction kinetics*

For calculation of the kinetics of an average contraction cycle, values of 12 sequential cycles of a time-lapse series were analysed. For maximum contraction (area minimum), time was set to zero. For each cycle, relative contraction values were calculated by setting the starting non-contracted state (area maximum) of each cycle to 1. The relative projected area values of each cycle were calculated in relation to the antecedent maximum. In this way the influence of changes in the body extension on the projected area was minimized. The average contraction including standard deviation (S.D.) was calculated for each relative time point. Contraction kinetic

diagrams were plotted for two independent datasets. Subcontractions, small but incomplete events, were not used when calculating contraction cycle kinetics.

### *Statistical analysis*

For statistical analysis of the long-term rhythm, cycle durations were measured as time between maximum contractions (area minimum). A *t*-test (Welch-test; assuming equal distribution, but no equal variances; $N$=80) was performed on two alternative hypotheses: $H_0$: no difference in cycle length between day and night, *vs* $H_1$: difference in cycle length between day and night. Since two datasets ($N$=36 and $N$=44) were combined for this analysis, the same test was applied to make sure there was no significant difference between them.

For comparison of different contraction states (full *vs* partial contractions) the contraction extent was compared using relative projected area values (see above). A *t*-test (Welch-test; assuming equal distribution, but no equal variances; $N$=53) was performed on two alternative hypotheses: $H_0$: only one class of contraction extent, *vs* $H_1$: two classes of contraction extent.

## Results

### *Contraction cycle duration and frequency*

*T. wilhelma* contraction has been observed to occur regularly at intervals between 60 and 600 min, with frequencies of $2.8\times10^{-4}$ to $2.8\times10^{-5}$ Hz, respectively. This broad range of contraction cycle duration can occur in one sponge specimen over a period of a week under natural or natural-like conditions in the aquarium, where many factors influence the sponge (see below). In a closed experimental system, specimens of *T. wilhelma* tend to display very regular contraction patterns over periods of hours and days. As an example, two different specimens displayed cycle durations of 83.3±11 min ($\cong2.0\times10^{-4}$ Hz; $N$=12; specimen Tw1) and 169.0±28 min ($\cong9.9\times10^{-5}$ Hz; $N$=6; specimen Tw2) during a period of 18 h (Fig. 1). If cycle duration is increased, only the cycle phase of maximum expansion of the sponge is prolonged. No difference is observed in either the duration of the contraction phases ($\Delta t^C$) or in the duration of the expansion phases ($\Delta t^E$) (Table 1). A contraction event can be triggered without a following full contraction (Fig. 1A). In this case local contractions that take place on the sponge surface have hardly any influence on the body size.

Table 1. *Comparison of durations of contraction and expansion phases in the two specimens of* T. wilhelma *of Fig. 1*

| Specimen | $N$ | $\Delta t^C$ (min) | $\Delta t^E$ (min) |
|---|---|---|---|
| Tw 1 | 12 | 21.6±4 | 48.0±12 |
| Tw 2 | 5 | 23.3±3 | 53.8±7 |

$\Delta t^C$, duration of contraction phase; $\Delta t^E$, duration of expansion phase.

Values are means ± S.D.

### *Long-term rhythm of contraction*

The contraction rhythms of two specimens were recorded inside the aquarium (Fig. 2A) and an open chamber (Fig. 2B) for 177 h and 169 h, respectively, under a light:dark cycle of 12 h:12 h. When conditions in the aquarium were natural-like, *T. wilhelma* displayed 44 contraction cycles during the observation period, compared to 36 in the open chamber, where there were no other organisms and very stable current conditions. The duration of the contraction cycles varied between 73.3 min ($\cong2.3\times10^{-4}$ Hz) and 609.0 min ($\cong2.7\times10^{-5}$ Hz).

The average relative contraction extent, measured as relative reduction of the projected sponge area, differed significantly between the two sponges. In contrast no significant difference

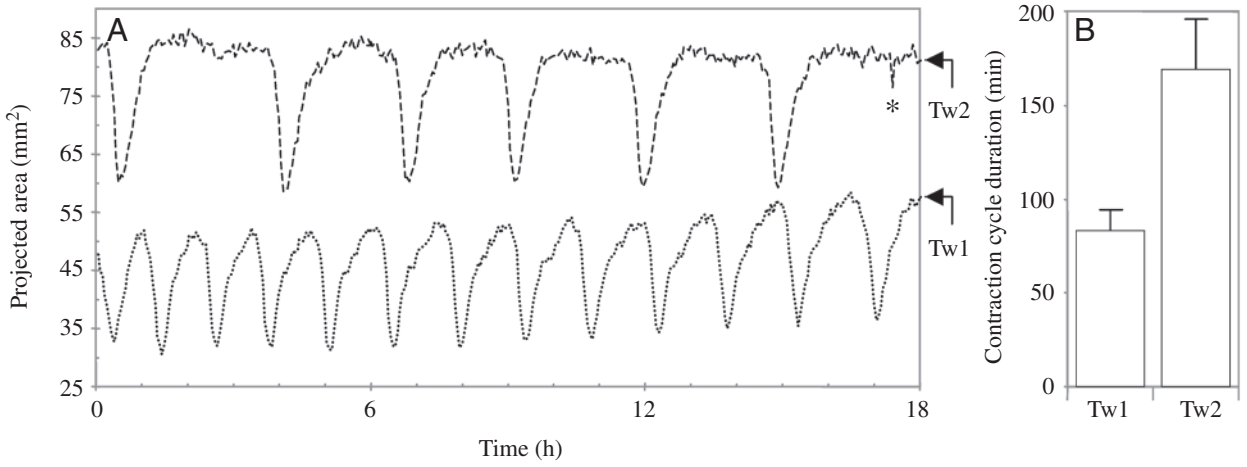


Fig. 1. (A) Contraction patterns of two specimens (Tw1 and Tw2) of *T. wilhelma*, representing the changes in projected areas over an experimental period of 18 h. Tw1 was placed in the aquarium, Tw2 in a closed experimental reactor. Note the subcontraction (asterisk) of Tw2 at a time point when a regular contraction should have taken place if the rhythm of the former contraction series had continued; dotted lines, Tw1; broken lines, Tw2. (B) Comparison of average contraction cycle duration of Tw1 (83.3±11 min; $N$=12) and Tw2 (169.0±28 min; $N$=6).

could be found for the average duration of the contraction cycles between the two datasets. Consequently both datasets were combined for analysis of a day–night cycle. During the day, sponges contracted every 215.7±92 min ($\cong 7.7\times10^{-5}\pm2\times10^{-4}$ Hz; $N$=44), in comparison to a cycle length of 274.5±149 min ($\cong 6.1\times10^{-5}\pm2\times10^{-4}$ Hz; $N$=36). A significant difference was found between the average cycle durations of day and night ($P$=0.042, $\alpha$=0.05; Fig. 3).

In addition to the regular full contraction (movie S1 in supplementary material) several subcontractions were observed, especially in the aquarium specimen (Fig. 2A, movie S2 in supplementary material). The subcontractions led to a reduction in the relative projected sponge area by 0.158±0.04 ($N$=9) in comparison to 0.442±0.06 ($N$=44) for regular full contractions in the same experiment. The maximum reduction of projected area observed was 0.58, with minimum 0.30, representing the extremes for regular contractions. The difference in contraction extent between full contraction and subcontractions was highly significant ($P$<0.00001, $\alpha$=0.05).

In aquarium conditions, several periods of irregular contractions occurred that are a direct reaction of the sponge to organisms such as snails, polychaets and crustaceans crawling on its surface or even feeding on the sponge or epibiontic algae. In one case, *T. wilhelma* showed a series of strong, irregular contractions when attacked by an amphipod (Fig. 2A, movie S3 in supplementary material). Comparable irregular contraction patterns were not observed in the open chamber, which was virtually free of errant organisms.

### *Kinetics of contraction*

Two consensus diagrams were calculated and plotted, from

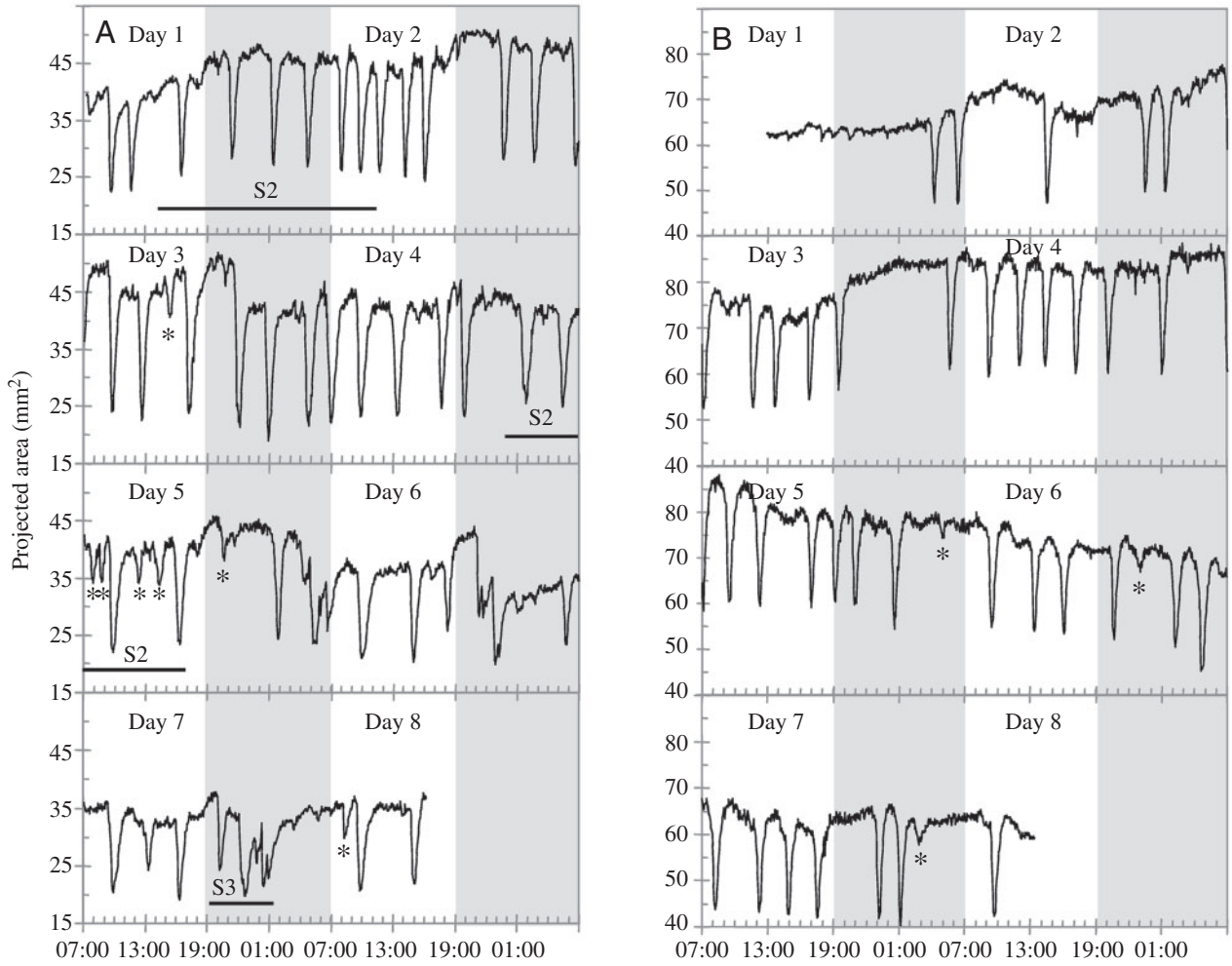


Fig. 2. Long-term recording over more than 7 days of the changes in projected area during contraction cycles of two specimens of *T. wilhelma* in the aquarium (A) and the open glass chamber (B). Light and dark periods are represented by white and grey backgrounds, respectively. Subcontractions, which differ significantly from regular contractions, are marked by asterisks. Experimental periods S1, S2 and S3 are represented by movies in the supplementary material. S1 represents a regular contraction series, S2 shows a series of subcontractions between regular contractions, S3 demonstrates the reaction of the sponge on a mechanical stimulation (amphipod attack). Note the adaptation phase in B after settling the sponge in the observation chamber.

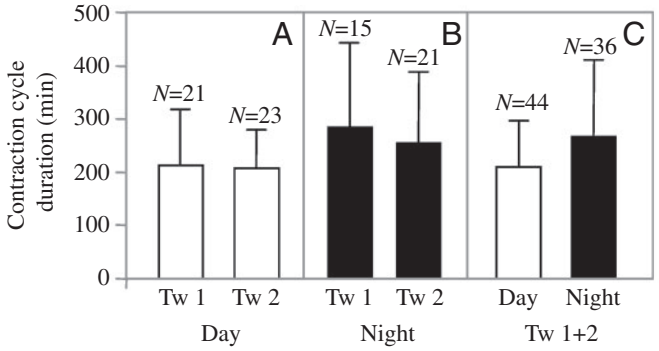


Fig. 3. Comparison of average contraction cycle durations during day and night for two specimens of *T. wilhelma* (Tw 1 and Tw 2) and for the combined datasets (Tw 1+2). Contraction cycle duration differs significantly between day and night ($P$=0.042).

two independent datasets ($N$=12; Fig. 4). The principle kinetics are the same in both cases, though there is variability in extent of contraction, depending on the sponge specimen. Maximum contraction differs by means of relative projected area in the range of 0.15 (0.71±0.03, $N$=12 *vs* 0.56±0.03, $N$=12). The maximum relative rates of contraction ($v^{C}_{max}$) calculated by change in projected area per unit time varies between $-42\times10^{-3}$ s$^{-1}$ and $-63\times10^{-3}$ s$^{-1}$, respectively, whereas the maximum speed of expansion calculated as change in projected area per unit time is very similar in both cases, $19\times10^{-3}$ s$^{-1}$ and $22\times10^{-3}$ s$^{-1}$, respectively. The general kinetics of the contraction cycle of *T. wilhelma* can be subdivided into four phases (Fig. 5): contraction (size reduction), contracted state, expansion (size increase) and expanded state. Contraction is a faster process than expansion ($v^{C}_{max} > v^{E}_{max}$).

### *Local contractions, spreading*

In time-lapse movies prepared from the image series the propagation and spreading of local contractions over the sponge surface can be observed (movie S4 in supplementary material). By measuring the local projected area changes of two sponge sectors of 2 and 3 mm$^2$, respectively, at a distance 3 mm apart, the speed of propagation was quantified. The local contraction spread along the pinacoderm at a rate of 750 μm min$^{-1}$ (=12.5 μm s$^{-1}$; Fig. 6). The first local contractions may propagate faster (Fig. 6B), but the main contraction clearly represents a wave running over the sponge.

### *Contraction in fused sponge specimens*

Since *T. wilhelma* reproduces mainly by budding, most specimens in our aquariums are clones, which may be fused to form larger sponge masses. Sponges undergo a characteristic morphological reorganisation during fusion, resulting in the final loss of individual skeletal structures of the progenitor sponges by forming one larger sponge. In the early phase of reconstruction the progenitors can still be recognised as individuals by their contraction pattern, which is not synchronised at the beginning. In a fusion experiment this

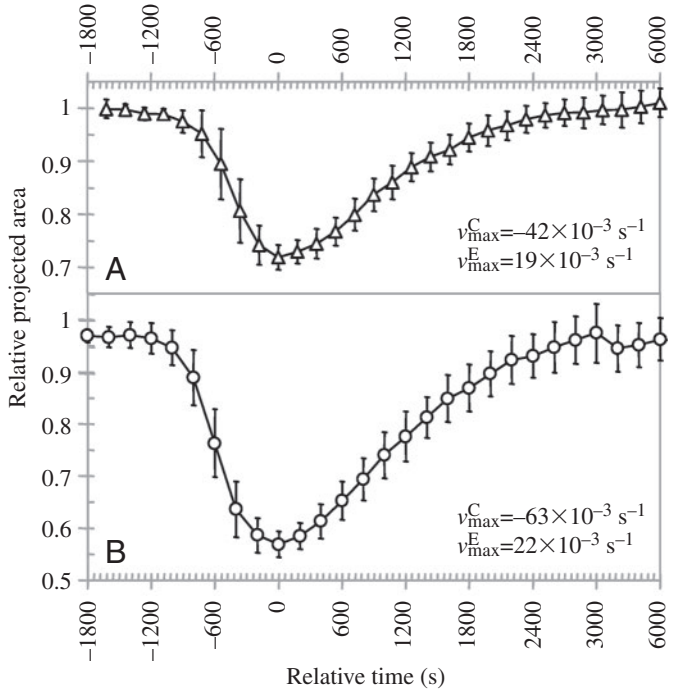


Fig. 4. Contraction kinetics of two independent datasets from two specimens of *T. wilhelma* (A and B), each representing the average of 12 contraction cycles. For calculations, relative contraction extent was used in order to minimize the influence on changes in the projected area by expansion and retraction of body extensions. For each contraction cycle included, time $t$=0 was set at the state of maximum contraction. In both cases the absolute values of maximum contraction rates $|v^{C}_{max}|$ are higher than the absolute values of maximum expansion rates $|v^{E}_{max}|$.

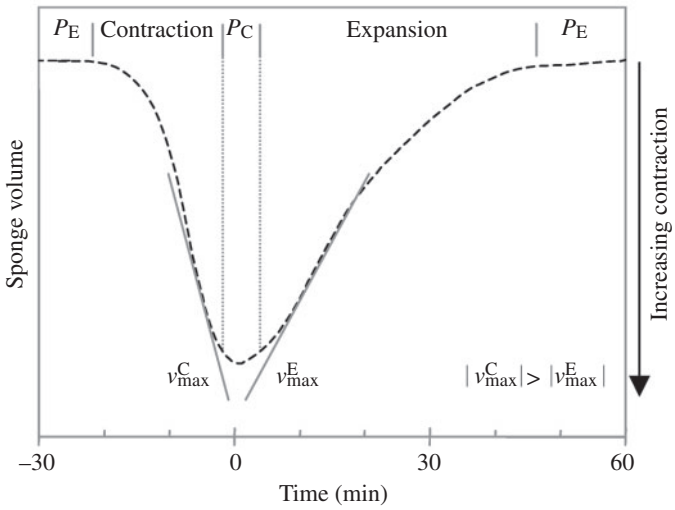


Fig. 5. Schematic representation of contraction kinetics and the four resulting phases of a contraction cycle in *T. wilhelma*. The contraction phase is shorter than the expansion phase, resulting in a higher absolute value for the maximum contraction rate $|v^{C}_{max}|$ than the absolute values of maximum expansion rates $|v^{E}_{max}|$. The phase of maximum contraction ($P_C$) is shorter than phase of maximum expansion ($P_E$), see also Table 1. Variations in contraction cycle length are solely due to differences in $P_E$.

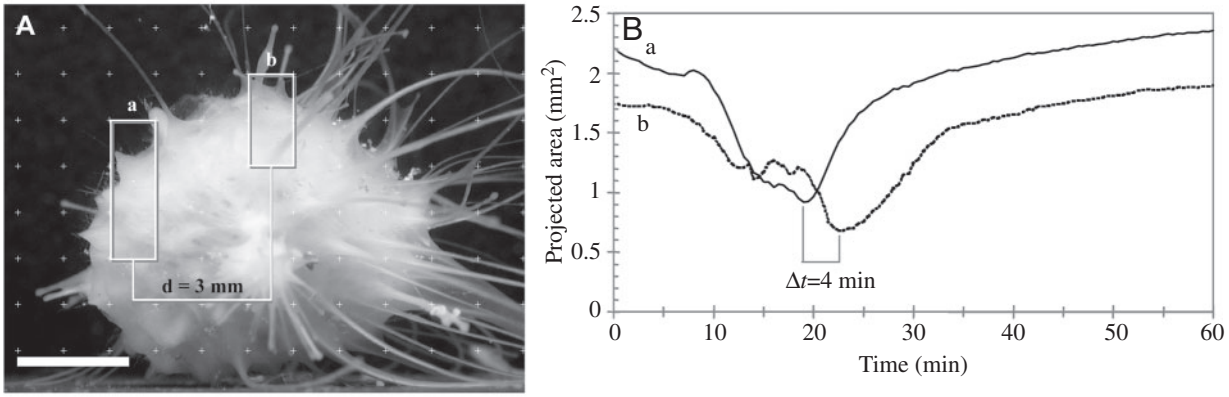


Fig. 6. (A) Local fields used to measure the spreading of local contractile waves over the sponge body. Field a represents an measurement area of 3 $mm^2$, field b represents 2 $mm^2$; both are not completely filled by sponge, to record changes due to contraction; distance *d* between a and b is 3 mm; bar, 2.5 mm. (B) Changes in the projected areas of fields a and b during a contraction event. The maximum contraction spreads as a wave over the sponge surface, taking 4 min to traverse the 3 mm distance, a speed of 750 µm $min^{-1}$ (=12.5 µm $s^{-1}$). The contraction used for this measurement is shown in movie S4 in supplementary material.

individuality could be demonstrated (movie S5 in supplementary material). By measuring the projected area of the outer parts of each progenitor the contraction pattern of both parts of the fused sponge could be monitored independently (Fig. 7). The resulting contraction pattern is not as precise as in the case of non-fused sponges, since contraction of one progenitor always indirectly influences the measurement area of the other progenitor. It is methodologically impossible to compensate for this completely. Nevertheless the independence of contraction of both parts can be monitored. It becomes obvious that two asynchronous contraction pattern are overlain, resulting in the mixture of four possible states: both expanded, one contracted and the other expanded (and *vice versa*), and both contracted. The contraction patterns of both sponges influence each other. In many cases a contraction of one sponge is followed by one of the other within 15 min. However, neither of the two sponges attains a consistent leading position, triggering the contraction of the other sponge.

In contrast to body contraction the formation and retraction of filaments is synchronised (movie S5 in supplementary material). It is unclear whether one of the sponges or both

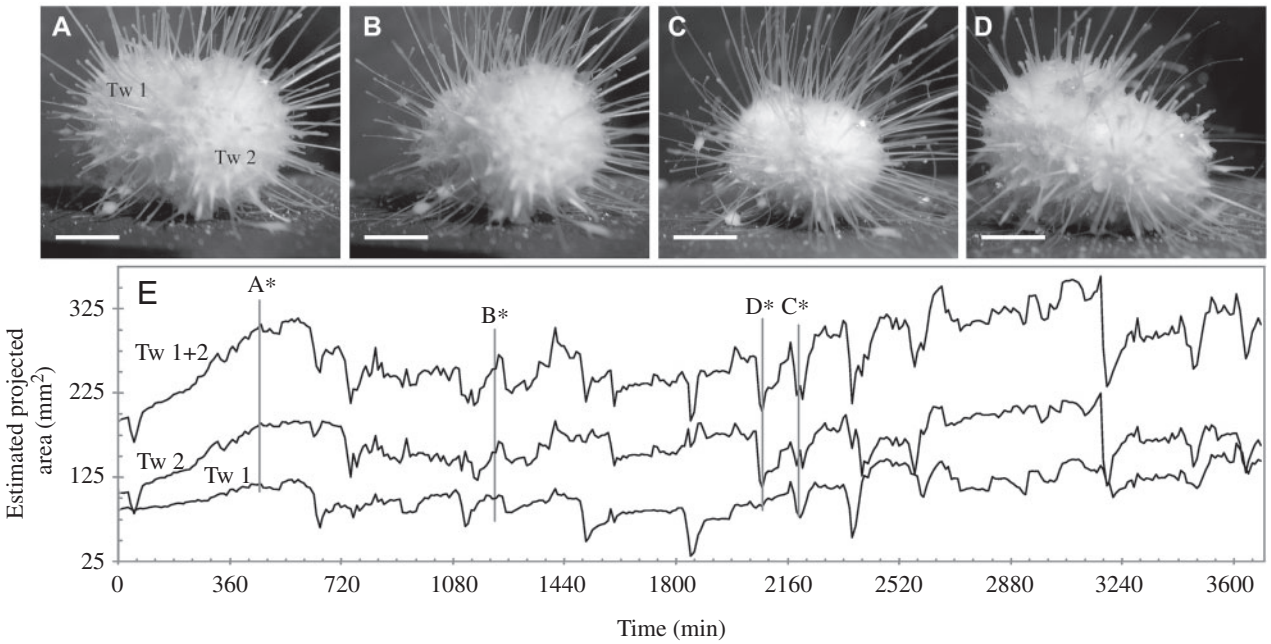


Fig. 7. Contraction pattern and main contraction states of two fused individuals (Tw 1 and Tw 2) of *T. wilhelma*. (A) Both individuals in expanded phase. (B) Tw 1 in contracted phase, Tw 2 in expanded phase. (C) Both individuals in contracted phase. (C) Tw 1 in expanded phase, Tw 2 in contracted phase. Bars, 5 mm. (E) Contraction patterns of Tw 1 and Tw 2 and both together (Tw 1+2) over 24 h. A*–D* are contraction states represented by images A–D. The time-lapse series used for this measurement is shown in movie S5 in supplementary material.

Table 2. *Comparison of the two extremes of contraction cycles of the same individual of* T. wilhelma *(Tw 1) during the long-term experiment presented in Fig. 1A*

| Cycle | Area $A$ (mm$^2$) | | | Radius $r$ (mm) | | Volume $V$ (mm$^3$) | | |
|---|---|---|---|---|---|---|---|---|
| | $A_{max}$ | $A_{min}$ | $\Delta A$ (%) | $r_{max}$ | $r_{min}$ | $V_{max}$ | $V_{min}$ | $\Delta V$ (%) |
| A | 51.36 | 29.29 | 58.5 | 4.04 | 2.60 | 276.21 | 73.62 | 73.3 |
| B | 34.96 | 24.31 | 30.5 | 3.34 | 2.78 | 156.07 | 90.00 | 42.3 |

*A*, projected area; max, maximum; min, minimum.

Cycle A represents the maximum contraction extent, cycle B the minimum during the experiment.

$r$ estimated from $r=\sqrt{A/\pi}$; $V$ estimated from $V=4/3\pi r^3$; $\Delta A$ and $\Delta V$: relative changes in measured projected area and estimated volume, respectively.

sponges together trigger the signal for extension and retraction of the filaments.

## Discussion

### *Rhythm and kinetics of contraction*

*Tethya wilhelma* is one of the behaviourally most active sponges known so far. In addition to its ability to crawl at speeds up to 2 mm h$^{-1}$ (Nickel and Brümmer, in press) and to produce filamentous body extensions (Nickel, 2001), it has an extraordinary body contraction behaviour. The whole body is able to contract both rhythmically and upon receipt of external stimuli. We used digital time-lapse imaging to record changes in the projected area of the sponge. Assuming a spherical shape for the body of *T. wilhelma*, a maximum reduction in the projected area by 58.5% represents a volume reduction of 73.3% (Table 2). A comparable degree of contraction has not previously been reported for a sponge, even though it has long been known that several species of the genus *Tethya* are able to contract (Lieberkühn, 1859; Reiswig, 1971; Sarà and Manara, 1991; Schmidt, 1866). The extent or amplitude of the contraction is variable, but usually very stable over a period of several cycles (Fig. 1). Subcontractions occur when the body only partly contracts. The average resulting reduction in the projected area of 15.8% corresponds to a volume reduction of 22.6%. A major difference between regular full contraction and the subcontractions can be observed in the time-lapse movies: regular contractions involve the whole body contracting more or less at once, whereas during subcontractions only parts of the body contract, or if the whole body contracts then some parts contract more strongly than others. Even when such irregularities are also observed as contractile waves during regular contractions (see below), unequal body contraction is a major characteristic of subcontractions.

The rhythm or frequency of contraction cycles is not necessarily disturbed if subcontractions occur. In some cases subcontractions replace regular contractions (Fig. 1), even though the majority of subcontractions occur irregularly. Distinct contraction types in sponges have not been reported before, and this is the first time that different contraction behaviours have been discovered in a sponge.

The frequency of contraction is usually quite variable over a restricted range in long-term experiments (Fig. 2). Nevertheless, for periods of hours or even days the contraction cycle frequency can be very stable (Fig. 1), raising the question of an intrinsic, endogenous timing or triggering mechanism. Similar regular repeated contractions have been shown for oscules of *Spongia officinalis* (Pavans de Ceccatty, 1971) and for whole individuals of the marine species *Tethya crypta* (Reiswig, 1971) as well as for the freshwater sponge *Ephydatia fluviatilis* (Kilian and Wintermann-Kilian, 1979; Weissenfels, 1984, 1990). Even though the existance of an endogenous rhythm in *E. fluviatilis* has been questioned (De Vos and Van De Vyver, 1981), re-examination of this data points towards endogenous periodicity (Weissenfels, 1990).

Two independent long-term experiments show a significant difference in the contraction cycle frequency between day and night (Fig. 3), suggesting a diurnal rhythm, as has also been proposed for the related species *T. crypta* (Reiswig, 1971). In addition, Reiswig found that artificial illumination during darkness disturbs the rhythm, a result that seems not to be same for *T. wilhelma*. However, further experiments need to be performed under constant darkness and constant light conditions, in order to determine if the diurnal rhythm is endogenous or coupled to light sensitivity.

In *T. wilhelma* the difference in contraction cycle duration is only given by the variable duration of the fully expanded phase. Variabilities in the durations of the contraction, the contracted phase and the subsequent expansion are negligible (Table 1). Contraction kinetics calculated from two independent datasets (Fig. 4), revealed variation only in the amplitude of the contraction, and not in the duration of the cycle phases. The contracted state itself lasts only a few minutes. The schematic representation demonstrates that the contraction phase is shorter than the expansion phase (Fig. 5), indicating a difference in the mechanism. This is underlined by the fact that the absolute value of the maximum contraction rate $|v^C_{max}|$ is higher than that of maximum expansion rate $|v^E_{max}|$. An active expansion mechanism by contraction of antagonistic mesohyle cells has been discussed (Wilson, 1910), but seems unlikely to occur in light of the difference between contraction and expansion kinetics in *T. wilhelma*. Furthermore, histological details of the appearance of dilating tissue and canals are not consistent with an active expansion mechanism (Jones, 1962). It seems more likely that expansion follows an increasing hydrostatic pressure inside the aquiferous system.

*Mechanisms and functions of contractions*

There have been many discussions concerning the nature of the contractile tissue in sponges (Jones, 1962). The two hypothetical principles are: (1) the contraction of the mesohyle is due to a contractile cell type called myocytes (Bagby, 1966; Pavans de Ceccatty, 1960, 1974; Sollas, 1888) or more recently actinocytes (Boury-Esnault and Rützler, 1997); (2) the contraction of the pinacoderm is due to the pinacocytes themselves (Bagby, 1970; Pavans de Ceccatty, 1986; Wilson, 1910). Neither of the two hypotheses can be excluded on the basis of present knowledge, but many observations point towards the latter mechanism, or both mechanisms working in conjunction. The presence of actinocytes in the mesohyle of the cortex has been shown in *T. wilhelma* (Nickel, 2001), but there is no direct evidence for their contractile nature. Both cell types contain actin filaments and networks (Bagby, 1966, 1970; Matsuno et al., 1988; Pavans De Ceccatty, 1981) and myosin has also been demonstrated in actinocytes and other sponge cells (Lorenz et al., 1996; Nickel, 2001). Hence it can be assumed that contraction of sponge cells is mediated by an actin–myosin mechanism.

The results presented here strongly support the contractile pinacoderm mechanism. Contractile waves on the surface of the sponge can be recorded (Fig. 6), indicating that direct contraction of the pinacoderm occurs. The extent of contraction discussed above is another piece of evidence: the cortex of *T. wilhelma* is very rich in endopinacoderm (canals and lacuna), whereas the mesohyle of the cortex is of low cellular density (Nickel, 2001; Sarà et al., 2001). The extent of contraction can be easily attributed to the distinctive endopinacoderm, taking into account the physiological and ecological value of an increased water exchange due to a contraction of the endopinacocytes. The main volume change affects the volume of the aquiferous system (canal and lacuna) and not the volume of the mesohyle. The volume of the mesohyle is not necessarily reduced during contraction in this model, only the shape of the mesohyle, which is possible due to the loose organization of the cells and the extracellular matrix. In this case, every contraction cycle is accompanied by an enormous exchange of water whereby nutrient- and oxygen-depleted water, which may also be loaded with waste products, is discarded. Regular rhythmic contraction is therefore a concomitant factor in the continuous water exchange provided by the currents produced by choanocytes.

Experiments simulating strong sedimentation events, similar to those in the natural environment of many tropical *Tethya* species (i.e. the reef top or shallow lagoons), indicate that contraction plays an additional ecological role in unloading sediment from the sponge body (data not shown). This is in conjunction with the observation that sponges can use reverse currents for ‘backwashing’ of blocked canals (Simpson, 1984; Storr, 1979). Since the flow direction during contraction has not yet been determined for *T. wilhelma*, we cannot exclude the occurrence of backwashing during contraction.

For other sponge groups, e.g. *Aplysina*, *Spongia* or *Tedania*, contractions are usually thought to be limited to the oscular region (Pavans de Ceccatty, 1971; Prosser et al., 1962). By applying digital time-lapse imaging to other sponges, it can be demonstrated that at least the whole sponge cortex or exopinacoderm region is able to contract to various degrees (data not shown), depending on the morphology of the sponge species. The oscular regions of many sponges are less rigid and resemble more-or-less the lacunar cortex of *T. wilhelma*: they are characterized by a low density of framework-building spicules, a distinct mesohyle part, a high degree of canals and cavities, and therefore a dominant number of pinacocytes. Subsequently, I assume that oscular contractions in most, if not all sponges follow the same mechanism than body contraction in *T. wilhelma*.

*Coordination of contraction*

In 350 BC, in his history of the animals, Aristotle wrote in chapter 1 of book one and in chapter 16 of book five that sponges are animals endowed with a certain sensibility (Aristotle, 1498). It has taken more than 2000 years to accept his view that sponges are true animals (Müller and Müller, 2003) but whether they are able to react directly to mechanical stimuli is still a question of debate. Our results clearly show that *T. wilhelma* directly responds to external stimuli, e.g. the attack of an amphipod (Fig. 2A, movie S3 in supplementary material). The contraction helps the sponge to protect its tissue from mechanical damage. A dense layer of tylasters can be found close to the surface in sections of *T. wilhelma* (Sarà et al., 2001). Contraction condenses this layer of micrasters, consequently enhancing its mechanical stability, resulting in a robust, but flexible protective coating, resembling chain mail.

Many authors have discussed the controversial question of whether sponges possess a nervous system (Jones, 1962; Lentz, 1968; Mackie, 1979, 1990; Pantin, 1952; Parker, 1910, 1919; Pavans de Ceccatty, 1974, 1979). The question itself seems to be more of philosophical quality than of biological evidence. Obviously, sponges do not possess exactly what we call a nervous system in higher organisms, since no true neurons have yet been found in sponges. The far more interesting question is: which elements of the nervous systems of higher animals can be found in sponges? Some elements of nervous systems, like neurotransmitters and their specific receptors, have been reported from unicellular Protozoa (Walker et al., 1996; Walker and Holden Dye, 1991), so we can also expect to find such elements in the Porifera, which evolved early in the lineage of the Metazoa. Indeed, there have been many hints that sponges react to neuromodulating substances or possess elements of their accompanying signal transduction pathways, such as neurotransmitters, enzymes needed for their synthesis or degradation, or their specific receptors (Emson, 1966; Jones, 1962; Lendenfeld, 1889; Lentz, 1966; Pavans de Ceccatty, 1971; Perovic et al., 1999; Weyrer et al., 1999). Even electrical propagation and action potentials have been shown, though so far only in Hexactinellida (Leys and Mackie, 1997, 1999; Leys et al., 1999; Mackie et al., 1983). On the other hand all these results remain sketchy and patchy: no conclusive, comprehensive hypothetical model on coordination in sponges

has been developed; no model sponge has been used to test the hypothesis that sponges are capable of integrating a variety of signals, both chemical and electro-chemical, through several differentiated signalling pathways. However, there are at least two sponge models suitable for comprehensive studies, and these could combine behavioural, physiological, pharmacological, histological, cell and molecular biological studies: the freshwater sponges, which have been used for many investigations (De Vos and Van De Vyver, 1981; Kilian and Wintermann-Kilian, 1979; McNair, 1923; Wintermann, 1951) and the marine sponges of the genus *Tethya*, especially *T. wilhelma* as reported here. Both systems exhibit contractions that are triggered endogenously and by external events (e.g. mechanical stimulation). The results from fused individuals of *T. wilhelma*, reported here, indicate that the coordination system in sponges must have reached a certain complexity: while the contraction patterns of the two individuals are not synchronized at first, the expansion and retraction of body extensions are. In conclusion, at least two independent means of triggering and controlling these behaviours are necessary. Moreover, contractile waves were observed, spreading at $12.5\ \mu m\ s^{-1}$ over the sponge surface, implying that contractions are triggered locally and spread consequently, following a diffusing (possibly chemical) signal. The existence of subcontractions, significantly weaker than regular contractions, again indicates that the sponge is able to control this behaviour by means of integrating various internal (physiological) and external (environmental) information. Preliminary results of our ongoing research indicate that several neuroactive substances are involved in the coordination of contraction in *T. wilhelma* (Ellwanger et al., 2004).

The detailed analysis of the kinetics and rhythm of *T. wilhelma* reported here is unique for sponges, allowing for the first time a differential, quantitative characterization of sponge behaviour. These results provide the basis for establishing a new sponge model system for the investigation of the integration signals and coordination of behaviours in an aneural organism. Since it has been pointed out that early Eumetazoa were also probably aneural (Mackie, 1990), studies on *T. wilhelma* and other sponge models may provide valuable information about the early evolution of metazoan signalling systems. Further work on this topic is in process, including the development of a tissue-culture system for experiments on the cellular level, as well as molecular work.

I would like to thank Franz Brümmer and Hans-Dieter Görtz (Stuttgart University) for supporting this work, Isabel Koch (Wilhelma Stuttgart) for providing sponges, Kornelia Ellwanger and Marco Vitello (Stuttgart University) for work and discussions on the topic and also Isabel Heim and Carsten Wolf (Stuttgart University) for maintaining the aquariums. I would also like to thank two anonymous referees, who improved the manuscript with suggestions and comments. The Federal Ministry of Education, Science and Technology (BMB+F), Germany, supported a part of this study through the Center of Excellence *BIOTEC*marin (F 0345D).

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