The following supplements accompany the article

Silicon utilization by sponges: an assessment of seasonal changes

María López-Acosta, Aude Leynaert, Valérie Coquille, Manuel Maldonado*

*Corresponding author: maldonado@ceab.csic.es

Marine Ecology Progress Series 605: 111–123 (2018)

Supplement 1: Intended versus assayed DSi concentrations

There were slight deviations between intended and assayed DSi concentrations at both experiments (Table S1). These deviations were imposed by two logistic constrains. First, the water from the bay of Brest was the base to prepare the experimental DSi solutions used over the experiments, being DSi concentration in the seawater daily changed while the experiments lasted. During the summer experiment (July 2015), the concentration decreased from 2.16 to 0.68 µM DSi; during the autumn experiment (mid-October – mid-November 2016), DSi availability raised from 3.63 to 4.30 µM. Second, there were small transfers of water from one experimental DSi step (at low DSi concentration) to the following one. When a given DSi concentration step finished, we carried each sponge within a "transferring container" to the next one. To avoid any exposure of the sponge to air during the transfer, a small volume of water (120 ± 31 mL) was transferred with each sponge. These two reasons were responsible of a slight deviation of 6.5 ± 3.4 % in summer and 7.6 ± 5.0 % in autumn between the intended and the finally assayed DSi concentrations.

Table S1. Intended and mean (±SD) assayed DSi concentration (µM) at each experiment.

Intended DSi concentration	Assayed DSi concentration			
	Tethya citrina		*Hymeniacidon perlevis*	
	Summer	Autumn	Summer	Autumn
Field values	2.24 ± 0.07	4.17 ± 0.09	2.32 ± 0.07	4.30 ± 0.04
75	72.08 ± 1.58	74.78 ± 0.85	72.83 ± 0.90	75.61 ± 0.74
150	143.16 ± 2.61	137.42 ± 1.11	144.36 ± 3.12	139.43 ± 0.45
200	194.63 ± 1.73	188.86 ± 1.94	193.37 ± 3.80	191.45 ± 0.41
250	238.79 ± 1.67	239.47 ± 1.44	238.54 ± 4.83	242.11 ± 0.61

Supplement 2: DSi consumption in the laboratory

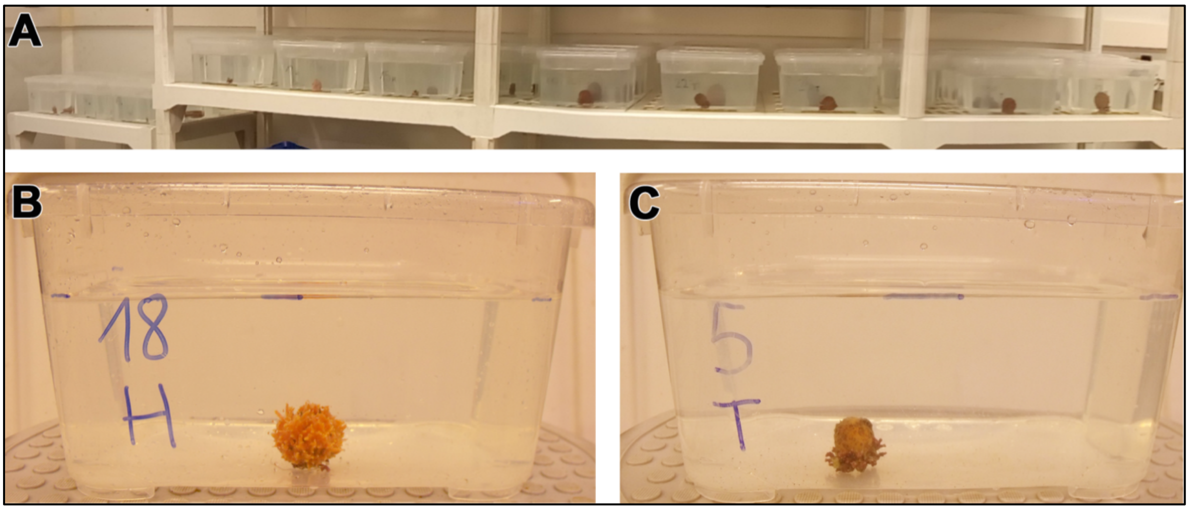

Fig. S1. (A) General view of part of the experimental setup in the laboratory to investigate Si consumption kinetics in sponges. (B-C) Individual of *Hymeniacidon perlevis* (B) and *Tethya citrina* (C) in an experimental aquarium. Aquarium size: 22 x 15 x 13 cm.

Empirical detection of DSi consumption by sponges in experimental aquaria involves a tradeoff between using aquaria with seawater volumes as large as possible to make every cultured sponge comfortable according to its body-size needs and as small as possible to be able to detect changes in DSi concentration over relatively short periods of time (Fig. S1). After some preliminary trials, we selected for individuals of a body size that could be maintained without problems over the course of the experiments and using a relatively small seawater volume that still made possible to detect the DSi consumption over a 72h incubation. Due to these constraints, the assayed size range did not represent the entire size range in the natural populations, being the largest individuals of the wild population intentionally excluded. Yet, the DSi consumption by some of the assayed individuals of *Hymeniacidon perlevis* at the lowest DSi concentration of the summer experiments (i.e., 2 µM) fell right at the analytical detection limit of our approach, producing some artefactual cases of negative DSi consumption.

To prevent formation of nutrient and/or oxygen gradients in the aquaria over the course of the 72h incubations, we mixed manually the water of each aquarium using a plastic rod three times a day. There were no casualties in any of the experiments during the 15 days that they lasted.

Supplement 3: R script of ANOVA-like tests and Brunner-Munzel tests

```
### R script of ANOVA-like test ###
# Hymeniacidon perlevis #
# factor 1 = group = season (summer, autumn)
# factor 2 =  time = [DSi] (Cnat, C75, C150, C200, C250)
data_Hym_seasonality <- read.table(header = TRUE, text = "
                              resp  group     time  subject
                               -0.001  Summer     Cnat  1
                                0.001  Summer     Cnat  2
                               -0.002  Summer     Cnat  3
                               -0.001  Summer     Cnat  4
                                0.000  Summer     Cnat  5
                                0.000  Summer     Cnat  6
                               -0.004  Summer     Cnat  7
                                0.001  Summer     Cnat  8
                               -0.001  Summer     Cnat  9
                                0.003  Autumn     Cnat  10
                                0.010  Autumn     Cnat  11
                                0.014  Autumn     Cnat  12
                                0.006  Autumn     Cnat  13
                                0.006  Autumn     Cnat  14
                                0.008  Autumn     Cnat  15
                                0.008  Autumn     Cnat  16
                                0.005  Autumn     Cnat  17
                                0.004  Autumn     Cnat  18
                                0.137  Summer     C75   1
                                0.035  Summer     C75   2
                                0.090  Summer     C75   3
                                0.046  Summer     C75   4
                                0.065  Summer     C75   5
                                0.110  Summer     C75   6
                                0.041  Summer     C75   7
                                0.054  Summer     C75   8
                                0.050  Summer     C75   9
                                0.096  Autumn     C75   10
                                0.088  Autumn     C75   11
                                0.104  Autumn     C75   12
                                0.091  Autumn     C75   13
                                0.128  Autumn     C75   14
                                0.070  Autumn     C75   15
                                0.063  Autumn     C75   16
                                0.085  Autumn     C75   17
                                0.115  Autumn     C75   18
                                0.167  Summer     C150  1
                                0.027  Summer     C150  2
                                0.131  Summer     C150  3
                                0.058  Summer     C150  4
                                0.082  Summer     C150  5
                                0.137  Summer     C150  6
                                0.053  Summer     C150  7
                                0.096  Summer     C150  8
                                0.070  Summer     C150  9
                                0.139  Autumn     C150  10
                                0.139  Autumn     C150  11
                                0.128  Autumn     C150  12
                                0.121  Autumn     C150  13
                                0.130  Autumn     C150  14
                                0.141  Autumn     C150  15
                                0.082  Autumn     C150  16
                                0.125  Autumn     C150  17
                                0.113  Autumn     C150  18
                                0.223  Summer     C200  1
                                0.022  Summer     C200  2
                                0.149  Summer     C200  3
                                0.074  Summer     C200  4
                                0.123  Summer     C200  5
                                0.120  Summer     C200  6
                                0.040  Summer     C200  7
```

```
                                        0.114   Summer      C200   8
                                        0.104   Summer      C200   9
                                        0.157   Autumn      C200   10
                                        0.126   Autumn      C200   11
                                        0.129   Autumn      C200   12
                                        0.130   Autumn      C200   13
                                        0.102   Autumn      C200   14
                                        0.134   Autumn      C200   15
                                        0.064   Autumn      C200   16
                                        0.120   Autumn      C200   17
                                        0.120   Autumn      C200   18
                                        0.229   Summer      C250   1
                                        0.044   Summer      C250   2
                                        0.140   Summer      C250   3
                                        0.081   Summer      C250   4
                                        0.079   Summer      C250   5
                                        0.089   Summer      C250   6
                                        0.047   Summer      C250   7
                                        0.076   Summer      C250   8
                                        0.081   Summer      C250   9
                                        0.159   Autumn      C250   10
                                        0.074   Autumn      C250   11
                                        0.140   Autumn      C250   12
                                        0.130   Autumn      C250   13
                                        0.081   Autumn      C250   14
                                        0.133   Autumn      C250   15
                                        0.065   Autumn      C250   16
                                        0.095   Autumn      C250   17
                                        0.129   Autumn      C250   18
                                          ")

# Convert variables to factor
resp_H <- data_Hym_seasonality[,"resp"]
group_H <- data_Hym_seasonality[,"group"]
time_H <- data_Hym_seasonality[,"time"]
subject_H <- data_Hym_seasonality[,"subject"]

# nparLD package
require("nparLD") || {install.packages("nparLD")
  library ("nparLD")}

# F1 LD F1 model
exH.f1f1 <- f1.ld.f1(y=resp_H, time=time_H, group=group_H,
subject=subject_H, w.pat=NULL, w.t=NULL, w.g=NULL,
time.name="Conc", group.name="Season", description=TRUE,
time.order=c("Cnat","C75","C150","C200","C250"),
group.order=c("Summer","Autumn"), plot.RTE=TRUE, show.covariance=FALSE,
order.warning=TRUE)
exH.f1f1$ANOVA.test


### R script of Brunner-Munzel test ###
require("lawstat") || {install.packages("lawstat")
  library ("lawstat")}

## Investigating between DSi concentration treatment differences
Hnat <- c(-0.001, 0.001,      -0.002,     -0.001,     0.000,      0.000,
      -0.004,     0.001,      -0.001,     0.003,      0.010,      0.014,
      0.006,   0.006,   0.008,      0.008,      0.005,      0.004)
H75 <- c(0.137,   0.035,      0.090,      0.046,      0.065,      0.110,
      0.041,      0.054,      0.050,      0.096,      0.088,      0.104,
      0.091,   0.128,   0.070,      0.063,      0.085,      0.115)
H150 <- c(0.167,  0.027,      0.131,      0.058,      0.082,      0.137,
      0.053,      0.096,      0.070,      0.139,      0.139,      0.128,
      0.121,   0.130,   0.141,      0.082,      0.125,      0.113)
H200 <- c(0.223,  0.022,      0.149,      0.074,      0.123,      0.120,
      0.040,      0.114,      0.104,      0.157,      0.126,      0.129,
      0.130,   0.102,   0.134,      0.064,      0.120,      0.120)
```

```
H250 <- c(0.229,  0.044,      0.140,      0.081,      0.079,      0.089,
      0.047,      0.076,      0.081,      0.159,      0.074,      0.140,
      0.130,   0.081,   0.133,      0.065,      0.095,      0.129)

## Ordering from lower to higher AVERAGE rate
#Cnat       0.003
#C75       0.081
#C250       0.104
#C150       0.108
#C200       0.114

#Hymeniacidon - C200
brunner.munzel.test(Hnat, H200)
brunner.munzel.test(H75, H200)
brunner.munzel.test(H250, H200)
brunner.munzel.test(H150, H200)

#Hymeniacidon - C150
brunner.munzel.test(Hnat, H150)
brunner.munzel.test(H75, H150)
brunner.munzel.test(H250, H150)

#Hymeniacidon - C250
brunner.munzel.test(Hnat, H250)
brunner.munzel.test(H75, H250)

#Hymeniacidon - C75
brunner.munzel.test(Hnat, H75)

## Investigating between seasons (summer vs autumn) differences within
each DSi treatment
Hnat_sum <- c(-0.001,   0.001,      -0.002,     -0.001,     0.000,
      0.000,      -0.004,     0.001,      -0.001)
Hnat_fall <- c(0.003,   0.010,      0.014,      0.006, 0.006,     0.008,
      0.008,      0.005,      0.004)

H75_sum <- c(0.137,     0.035,      0.090,      0.046,      0.065,
      0.110,      0.041,      0.054,      0.050)
H75_fall <- c(0.096,    0.088,      0.104,      0.091, 0.128,     0.070,
      0.063,      0.085,      0.115)

H150_sum <- c(0.167,    0.027,      0.131,      0.058,      0.082,
      0.137,      0.053,      0.096,      0.070)
H150_fall <- c(0.139,   0.139,      0.128,      0.121, 0.130,     0.141,
      0.082,      0.125,      0.113)

H200_sum  <- c(0.223,   0.022,      0.149,      0.074,      0.123,
      0.120,      0.040,      0.114,      0.104)
H200_fall <- c(0.157,   0.126,      0.129,      0.130, 0.102,     0.134,
      0.064,      0.120,      0.120)

H250_sum <- c(0.229,    0.044,      0.140,      0.081,      0.079,
      0.089,      0.047,      0.076,      0.081)
H250_fall <- c(0.159,   0.074,      0.140,      0.130, 0.081,     0.133,
      0.065,      0.095,      0.129)

#Comparisons for factor: season within Cnat
brunner.munzel.test(Hnat_sum, Hnat_fall)

#Comparisons for factor: season within C75
brunner.munzel.test(H75_sum, H75_fall)

#Comparisons for factor: season within C150
brunner.munzel.test(H150_sum, H150_fall)

#Comparisons for factor: season within C200
brunner.munzel.test(H200_sum, H200_fall)

#Comparisons for factor: season within C250
```

```
brunner.munzel.test(H250_sum, H250_fall)


### R script of ANOVA-like test ###
# Tethya citrina #
# factor 1 = group = season (summer, autumn)
# factor 2 =  time = [DSi] (Cnat, C75, C150, C200, C250)
data_Tethya_seasonality <- read.table(header = TRUE, text = "
resp  group time  subject
                                  0.001    Summer     Cnat  1
                                  0.002    Summer     Cnat  2
                                  0.003    Summer     Cnat  3
                                  0.001    Summer     Cnat  4
                                  0.004    Summer     Cnat  5
                                  0.005    Summer     Cnat  6
                                  0.003    Summer     Cnat  7
                                  0.001    Summer     Cnat  8
                                  0.021    Autumn     Cnat  9
                                  0.006    Autumn     Cnat  10
                                  0.004    Autumn     Cnat  11
                                  0.008    Autumn     Cnat  12
                                  0.008    Autumn     Cnat  13
                                  0.005    Autumn     Cnat  14
                                  0.008    Autumn     Cnat  15
                                  0.008    Autumn     Cnat  16
                                  0.112    Summer     C75   1
                                  0.092    Summer     C75   2
                                  0.157    Summer     C75   3
                                  0.070    Summer     C75   4
                                  0.153    Summer     C75   5
                                  0.329    Summer     C75   6
                                  0.180    Summer     C75   7
                                  0.092    Summer     C75   8
                                  0.362    Autumn     C75   9
                                  0.113    Autumn     C75   10
                                  0.178    Autumn     C75   11
                                  0.181    Autumn     C75   12
                                  0.118    Autumn     C75   13
                                  0.134    Autumn     C75   14
                                  0.291    Autumn     C75   15
                                  0.144    Autumn     C75   16
                                  0.148    Summer     C150  1
                                  0.122    Summer     C150  2
                                  0.215    Summer     C150  3
                                  0.114    Summer     C150  4
                                  0.210    Summer     C150  5
                                  0.387    Summer     C150  6
                                  0.180    Summer     C150  7
                                  0.127    Summer     C150  8
                                  0.518    Autumn     C150  9
                                  0.185    Autumn     C150  10
                                  0.293    Autumn     C150  11
                                  0.294    Autumn     C150  12
                                  0.301    Autumn     C150  13
                                  0.184    Autumn     C150  14
                                  0.458    Autumn     C150  15
                                  0.237    Autumn     C150  16
                                  0.155    Summer     C200  1
                                  0.124    Summer     C200  2
                                  0.223    Summer     C200  3
                                  0.121    Summer     C200  4
                                  0.197    Summer     C200  5
                                  0.304    Summer     C200  6
                                  0.157    Summer     C200  7
                                  0.100    Summer     C200  8
                                  0.461    Autumn     C200  9
                                  0.195    Autumn     C200  10
                                  0.303    Autumn     C200  11
                                  0.372    Autumn     C200  12
```

```
                                0.310    Autumn      C200  13
                                0.190    Autumn      C200  14
                                0.577    Autumn      C200  15
                                0.243    Autumn      C200  16
                                0.139    Summer      C250  1
                                0.142    Summer      C250  2
                                0.276    Summer      C250  3
                                0.099    Summer      C250  4
                                0.242    Summer      C250  5
                                0.275    Summer      C250  6
                                0.155    Summer      C250  7
                                0.082    Summer      C250  8
                                0.343    Autumn      C250  9
                                0.182    Autumn      C250  10
                                0.285    Autumn      C250  11
                                0.328    Autumn      C250  12
                                0.286    Autumn      C250  13
                                0.159    Autumn      C250  14
                                0.445    Autumn      C250  15
                                0.270    Autumn      C250  16
                                ")

# Convert variables to factor
resp_T <- data_Tethya_seasonality[,"resp"]
group_T <- data_Tethya_seasonality[,"group"]
time_T <- data_Tethya_seasonality[,"time"]
subject_T <- data_Tethya_seasonality[,"subject"]

# nparLD - Brunner
require("nparLD") || {install.packages("nparLD")
  library ("nparLD")}

# F1 LD F1 model
exT.f1f1 <- f1.ld.f1(y=resp_T, time=time_T, group=group_T,
subject=subject_T, w.pat=NULL, w.t=NULL, w.g=NULL,
time.name="Conc", group.name="Season", description=TRUE,
time.order=c("Cnat","C75","C150","C200","C250"),
group.order=c("Summer","Autumn"), plot.RTE=TRUE, show.covariance=FALSE,
order.warning=TRUE)
exT.f1f1$ANOVA.test


### R script of Brunner-Munzel test ###
require("lawstat") || {install.packages("lawstat")
  library ("lawstat")}

## Investigating between-season (summer vs autumn) differences
Tsum <- c(0.001,  0.002,      0.003,      0.001,      0.004,      0.005,
      0.003,      0.001,   0.112,   0.092,      0.157,      0.070,
      0.153,      0.329,      0.180,      0.092,     0.148, 0.122,
      0.215,      0.114,      0.210,      0.387,      0.180,      0.127,
0.155,      0.124,      0.223,      0.121,      0.197,      0.304,
      0.157,      0.100,   0.139,   0.142,      0.276,      0.099,
      0.242,      0.275,      0.155,      0.082)
Tfall <- c(0.021, 0.006,      0.004,      0.008,      0.008,      0.005,
      0.008,      0.008,   0.362,   0.113,      0.178,      0.181,
      0.118,      0.134,      0.291,      0.144,   0.518,   0.185,
      0.293,      0.294,      0.301,      0.184,      0.458,      0.237,
0.461,      0.195,      0.303,      0.372,      0.310,      0.190,
      0.577,      0.243,    0.343,  0.182,      0.285,      0.328,
      0.286,      0.159,      0.445,      0.270)


##Ordering from lower to higher AVERAGE rate
#Season     AVRG
#summer     0.137
#autumn     0.225
brunner.munzel.test(Tsum, Tfall)
```

```
## Investigating between DSi concentration treatment differences
Tnat <- c(0.001,  0.002,      0.003,      0.001,      0.004,      0.005,
      0.003,      0.001,      0.021,      0.006,      0.004,      0.008,
      0.008,   0.005,   0.008,      0.008)
T75 <- c(0.112,  0.092,      0.157,      0.070,      0.153,      0.329,
      0.180,      0.092,      0.362,      0.113,      0.178,      0.181,
      0.118,   0.134,   0.291,      0.144)
T150 <- c(0.148,  0.122,      0.215,      0.114,      0.210,      0.387,
      0.180,      0.127,      0.518,      0.185,      0.293,      0.294,
      0.301,   0.184,   0.458,      0.237)
T200 <- c(0.155,  0.124,      0.223,      0.121,      0.197,      0.304,
      0.157,      0.100,      0.461,      0.195,      0.303,      0.372,
      0.310,   0.190,   0.577,      0.243)
T250 <- c(0.139,  0.142,      0.276,      0.099,      0.242,      0.275,
      0.155,      0.082,      0.343,      0.182,      0.285,      0.328,
      0.286,   0.159,   0.445,      0.270)

# Ordering from lower to higher AVERAGE rate
#Cnat       0.005
#C75       0.169
#C250       0.232
#C150       0.248
#C200       0.252


#Tethya - C200
brunner.munzel.test(Tnat, T200)
brunner.munzel.test(T75, T200)
brunner.munzel.test(T250, T200)
brunner.munzel.test(T150, T200)

#Tethya - C150
brunner.munzel.test(Tnat, T150)
brunner.munzel.test(T75, T150)
brunner.munzel.test(T250, T150)

#Tethya - C250
brunner.munzel.test(Tnat, T250)
brunner.munzel.test(T75, T250)

#Tethya - C75
brunner.munzel.test(Tnat, T75)

## Investigating between seasons (summer vs autumn) differences within
each DSi treatment

Tnat_sum <- c(0.001,    0.002,      0.003,      0.001,      0.004,
      0.005,      0.003,      0.001)
Tnat_fall <- c(0.021,   0.006,      0.004,      0.008,      0.008,
      0.005,      0.008,      0.008)

T75_sum <- c(0.112,     0.092,      0.157,      0.070,      0.153,
      0.329,      0.180,      0.092)
T75_fall <- c(0.362,    0.113,      0.178,      0.181,      0.118,
      0.134,      0.291,      0.144)

T150_sum <- c(0.148,    0.122,      0.215,      0.114,      0.210,
      0.387,      0.180,      0.127)
T150_fall <- c(0.518,   0.185,      0.293,      0.294,      0.301,
      0.184,      0.458,      0.237)

T200_sum  <- c(0.155,   0.124,      0.223,      0.121,      0.197,
      0.304,      0.157,      0.100)
T200_fall <- c(0.461,   0.195,      0.303,      0.372,      0.310,
      0.190,      0.577,      0.243)

T250_sum <- c(0.139,    0.142,      0.276,      0.099,      0.242,
      0.275,      0.155,      0.082)
```

```
T250_fall <- c(0.343, 0.182, 0.285,      0.328,      0.286,      0.159,
      0.445,      0.270)

#Comparisons for factor: season within Cnat
brunner.munzel.test(Tnat_sum, Tnat_fall)

#Comparisons for factor: season within C75
brunner.munzel.test(T75_sum, T75_fall)

#Comparisons for factor: season within C150
brunner.munzel.test(T150_sum, T150_fall)

#Comparisons for factor: season within C200
brunner.munzel.test(T200_sum, T200_fall)

#Comparisons for factor: season within C250
brunner.munzel.test(T250_sum, T250_fall)
```