

Temperature and size associated differences in the skeletal structures and osculum cross-sectional area influence the pumping rate of contractile sponge *Cinachyrella* cf. *cavernosa*

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Abstract

Sponges alter the proportion of structural and morphological components such as spicule skeleton and number of oscula in response to environment, development, and growth. Such mechanisms indicate morphological and physiological variations within populations. We measured the pumping rate of different sized specimens of contractile sponge *Cinachyrella* cf. *cavernosa* present in the intertidal rockpools, during morning and afternoon low tides along the central west coast of India. Additionally, we measured the exhalant opening (oscular cross-sectional area- OSA), tissue density, spicule skeleton weight and the abundance of choanocyte chambers to assess the morphological variations. Water temperature and spongesize showed a significant positive effect on pumping rate. OSA and the number of oscula increased with sponge size, but the sponge-volume specific pumping rate did not increase ($0.029 \pm 0.020 \text{ ml s}^{-1} \text{ ml}^{-1}$). Histological sections showed the abundance of choanocyte chambers increased marginally with the sponge size, but the contraction state affected the count. Both tissue density and spicule skeleton weight were positively correlated with sponge size, particularly the increase in the size and number of spicule bundles radiating from the center indicated a denser core in larger specimens. The subcortical lacunae and the peripheral choanosome with high abundance of canals appear to be the major volume reduction zones during contraction events. Our results indicate that the size and number of spicule bundles increase with sponge volume and the variation in temperature, contraction state, and size and number of oscula influence the pumping rate of *C. cf. cavernosa*.

Key Words: Porifera; pumping; spicules; sponge; contraction; rock pools; temperature; intertidal; osculum.

1 Introduction

Sponges have a wide distribution in both latitudes and depth with moderate to high abundance in various marine and freshwater ecosystems (Taylor, 2007) and there is growing evidence that sponges are particularly important benthic suspension feeders (DeGoeij et al., 2013; Khan et al., 2015). Most sponges are filter feeders and can pump a water volume corresponding to their own volume several times per minute (Riisgård et al., 2016, Ludeman et al., 2017). Sponge pumping activity is not constant and varies significantly within and between species (Reiswig, 1971; McMurray et al., 2014; Ludeman et al., 2017). Various factors like water temperature (Riisgård et al., 1993), suspended sediment concentration (Gerrodette & Flechsig, 1979; Strehlow et al., 2016; Grant et al., 2018), epizoic zoanthids (Lewis and Finelli, 2015), microbial abundance (Weisz et al., 2008), tissue density (Turon et al., 1997), sponge shape (McMurray et al., 2014), osculum contraction (Kumala and Riisgård, 2017; Strehlow et al., 2016; Goldstein et al., 2019), canal system (Reiswig, 1975), and number of choanocyte chambers (Massaro, 2012) are known to influence sponge pumping rate.

Sponges pump water through an aquiferous system, which mainly constitutes of ostia (incurrent pores), choanocyte chambers (pumping units), a network of incurrent and excurrent canals, and oscula (exhalant openings). A system of choanocyte chambers and aquiferous canals associated with a single osculum is considered as an "aquiferous module" (Fry, 1970 & 1979), and number and size of aquiferous modules determine the sponge volume (Ereskovsky, 2003). As the sponge grows in size, a new system of canals develops de novo in the specific zones (Ereskovsky, 2003), and new aquiferous modules are added (Wiens et al., 2005). Individuals of same species with differences in the sponge size and the number of oscula may show vast differences in the aquiferous system as the network of inhalant and exhalant canals change considerably with the number of oscula (Giano et al., 1995) and thus may influence the pumping capacity of different sized individuals within a species.

The pores and the canals of the aquiferous system are supported by an endoskeleton made of gelatinous matrix called mesohyl and skeleton made of mineral spicules. The proportions of the mesohyl material and the area occupied by the flagellated chambers and canals in the choanosome affects the pumping rate of sponges (Turon et al., 1997). Sponges with high microbial abundance have a denser mesohyl, and the aquiferous system is composed of longer, narrower canals which result in reduced pumping rate (Vacelet & Donadey, 1977; Weisz et al., 2008). Similarly, spicules can make up a significant portion of the sponge mass and volume (Bergquist, 1978). The mineral skeleton of spicules and proteinaceous fibers allows cell organization and establishment of the

aquiferous system (Uriz et al., 2003). Differences in the growth processes and skeletal architecture of sponges with similar morphology and growth forms are accompanied with differences in the aquiferous system (Kaandorp, 1991). For example, sponge *Halichondriapanicea* has characteristic tangential spicule bundles due to which it has a slightly lower cross-sectional area of the ostia compared to the morphologically similar *Haliclona permollis* (Reiswig, 1975). In many Astrophorids, skeletal elements like aster and spongin contribute to the organization of inhalant structures (Uriz, 2002). Spherical sponges with radial skeletons have species specific differences in the arrangement and abundance of spicules in the cortex and choanosome (Bavestrello et al., 2000; Sara & Manara, 1991). Anatomical studies on the rigid and stiff sponge *Tethya aaurantium* and soft and lacunar sponge *Tethya citrina* show that such morphological characteristics correspond with differences in the amount and the arrangement of spicule skeleton structures of these sponges (Bavestrello et al., 2000; Sara & Manara, 1991). It is suggested that these characteristics are related to the ecological requirements of these sponges as the plasticity of the cortex and its ability to contract may function as an adaptive device to control the pumping rate (Bavestrello et al., 2000; Sara & Manara, 1991). This suggests a close relationship between spicule arrangement and aquiferous structures. Dimensions of the aquiferous structures affect the pumping rate of sponges (Ludeman et al., 2017). Moreover, spiculogenesis is a dynamic process and depends on the developmental stage during the life cycle of the sponge (Cao et al., 2007), also the amount of spicule skeleton may vary with the sponge volume (Barthel, 1986; Bavestrello et al., 2000). This indicates differences in the spicule skeleton in different sized sponges may affect the aquiferous system, which in turn would influence the sponge pumping rate.

Choanocyte chambers are the pumping units located between the incurrent and excurrent canals of the aquiferous system. Actively pumping sponges generate the water current by the beating action of the choanocyte flagella in the choanocyte chambers. It has been suggested that pumping rate could be correlated to the abundance of choanocyte chambers (Reiswig 1975; Riisgård et al., 1993). Laboratory experiments to study the effect of thermal stress on feeding behavior of sponge *Rhopaloeides odorabile* reported a reversible reduction in the density of choanocyte chambers, filtration efficiency, and pumping rate, additionally, a strong correlation in pumping rate and choanocyte chamber abundance was also observed (Massaro et al., 2012). Sponge *Xestospongia muta* shows isometric scaling between the sponge size and the pumping rate, which was inferred to indicate the proportionality between the number of choanocyte chambers and the sponge tissue volume (Mc Murray et al., 2014). In a recent study, experiments on single-ostium sponge explants (i.e. single aquiferous modules) of the demosponge *Halichondriapanicea* showed that the scaling

relationship of the osculum cross sectional area (OSA), exhalant jet speed (U_o), and pumping rate ($F = OSA \times U_o$) with the sponge volume is similar to the estimates based on the hypothesis that all the choanocyte chambers deliver same volume flow and have a constant density throughout sponge tissue (Goldstien et al., 2019). However, the abundance of the choanocyte chambers within the sponge tissue can change. For example, processes of sexual reproduction like gametogenesis and viviparity can cause reduction in the flagellar activity due to choanocyte loss and cause long-term effects on the pumping rate (Simpson, 1984). Reproductive effort (sexual and asexual) is an integrative indicator of resource allocation (Ereskovsky et al., 2017) and individual sponges undergoing reproduction may be physiologically compromised due to a reduction in the number of choanocytes and, in turn, the pumping rate (Wahab et al., 2017). Thus the number of the choanocyte chambers in the sponge tissue is highly critical for sponge pumping rate, and variation in the abundance of choanocyte chambers in different sized individuals of same species is likely to influence the pumping rate.

The primary aim of this investigation was to assess the variation in morphology and in situ pumping rates of different sized individuals of spherical shaped and contractile sponge *C. cf. cavernosa*. To determine the pumping rate, we measured the osculum cross-sectional area (OSA), and ex-current velocity of different sized specimens present in the intertidal rock pools. Sponge pumping is strongly influenced by contraction mechanisms (Kumala & Riisgård, 2017; Strehlow et al., 2016; Elliot & Leys, 2007). Although, spicules are a non-compressible part of sponges (Hammel & Nickel, 2006), they affect the contractile ability of cortical structures in spherical shaped *Tethyasponges* (Bavestrello et al., 2000). The growth of spherical shaped sponges with a radial skeleton, is constrained along predefined directions and is organized around the central region (Bavestrello et al., 2000). Therefore, in order to assess the morphological differences in the different sized sponges we studied the spicule skeleton structure for characteristics such as the size and the number of the spicule bundles, the percentage of dry mass devoted to spicules and the tissue density. Additionally we also measured the abundance of the choanocyte chambers in different sized sponges.

The intertidal rock pools have high diurnal and seasonal variation in the physical-chemical parameters (Morris & Taylor, 1983, Metaxas & Scheibling, 1996; Firth et al., 2014). During low tide exposure rock pools form isolated water bodies without the effect of waves and current flows (Astles, 1993). The physical-chemical parameters of the rockpools show vast differences between different low tides, depending on the time of occurrence (morning, afternoon or night). Hence we also determined the effect of environmental parameters on sponge pumping rate during morning and afternoon low tides. The cyclic variation in physical-chemical parameters and

the stagnant still water in the rock pools provide an ideal set up to test the sponges pumping rate in the natural habitat with different environmental conditions.

2 Materials And Methods

2.1 Study species

C. cf.cavernosa (sensu Burton 1959) is a spherical shaped and contractile sponge commonly present in the sub-tidal and low intertidal habitats along the central west coast of India (Figure 1 a,b and c).The osculum is usually situated at the top of the sponge, and individuals have several porocalices (rounded depressions/pits), usually on the lateral surfaces, near the base containing porefields, which are mostly inhalant and rarely exhalant (Figure 1c,e and f). Spicule arrangement is highly ordered, and the skeleton consists of radiating bundles of megascleres originating from the central nucleus, which is not always in the center but shifted little towards the basal part of the sponge. When exposed to air, by the natural fall of the tide, the entire sponge contracts and shrinks in size and the osculum closes down and completely occludes the excurrent aperture. Contraction of entire sponge or just osculum or canals are observed in several sponge species (Nickel, 2010) and may be related to various ecological requirements of the species. For example, spherical shaped sponge *Tethyawilhelma* displays rhythmic contractions of entire sponge, local sub contractions and locomotive movement, which is suggested to be induced in response to local environmental conditions (Nickel, 2004; Nickel, 2006). Similarly, detail studies of the entire sponge contractions of fresh water sponge *Ephydatiamuelleri* show that, coordinated peristaltic-like contractions are effective in expelling waste from the aquiferous system (Elliot & Leys, 2007).

2.2 Study sites and sponge morphology

Osculum cross-sectional area (OSA) and morphological dimensions of sponge *C. cf.cavernosa* (n=94) were measured at four intertidal locations: Kunkeshwar (16°20'01.7"N 73°23'27.2"E), Mhapan (15°55'28. 16"N 73°33'26. 69"E), Reddy (15°44'29.1"N 73°39'22.9"E) and Anjuna (15°33'57. 51"N 73° 44'30. 39"E) along the Central West Coast of India (Figure 1a). The data of OSA was grouped into two categories: morning (before 11 am) and afternoon (after 12 noon). Sponge oscula were photographed in situ, with an underwater camera. For sponges with more than one osculum, the largest osculum was photographed to obtain the measure of maximal dilation. The OSA was measured using functions in image editing software GIMP 2.8. The mean diameter of the sponge was calculated by taking multiple readings (4 times per sponge) with a vernier caliper. This value was used to calculate the volume of the sponge using the formula for the volume of a sphere.

2.3 Sponge pumping rate and physical-chemical parameters of the rock pools

Sponge pumping rate and the physical-chemical parameters of the rock pools were measured in February and March 2015 at two locations Anjuna and Kunkeshwar (Figure 1 b and c). During high tides it is difficult to observe the sponge excurrent jet due to high water movement, therefore measurements were made during low tide. At both study sites, three rock pools from the mid intertidal zone were selected based on their accessibility and location, as they experienced sufficient exposure during low tides to complete the experimental work. All the pumping rate measurements were performed during the morning (7.30-9.00 am) and afternoon (2.30-4.30 pm) low tides. At each sampling pool, before measuring the sponge pumping rate the temperature, salinity, pH and dissolved oxygen were measured using a waterproof multi-parametric probe (Oakton PCD 650). The probes were calibrated according to the manufacturer's instructions. Local variations in the physical-chemical parameters can exist within the pool, hence for each rock pool, an average was calculated by taking the measurements along the edge and the center of the pool at the depth ranging from 7 to 15 cm.

Pumping rate of total of 35 different sponges was measured (18 in the morning and 17 in the afternoon). Sponge pumping rate was measured using an in situ dye technique (Weisz et al., 2008). In dye technique, the excurrent velocity is measured from the leading edge of the dye plume. This technique does not give information of the precise characteristics of the flow, like the velocity distribution and the fluid flow profile (plug or parabolic). However, proximate estimates of sponge pumping rate can be obtained using this technique (Weisz et al., 2008; Fiore et al., 2016). In the present study, an underwater video camera was set up directly in front of the sponge osculum, and small amounts (2-3 ml) of a concentrated fluorescein dye solution, was released into the ambient water, near the sponge osculum. The pumping rate of the sponges was determined by video recording the movement of exhalant plumes from the osculum, perpendicular to the plane of the osculum. Video images of dye movement were checked frame-by-frame with reference to a laminated graph paper held in the background directly behind the osculum. Dye speed was calculated as the distance traveled by the sharp edge of the plume, perpendicular to the plane of the osculum, divided by the time in which it traveled that distance. The frame rate was 30 frames per second. For each observation, the travel time of five to ten dye fronts across the first 3-5 centimeters of the excurrent plume was averaged together to obtain the velocity perpendicular to the plane of the osculum opening. This mean value was multiplied by the area of the osculum to obtain the volume of water passing through the osculum per second. For sponges with more than one osculum, the pumping rate of the whole sponge was obtained by adding the pumping rate measured for each

osculum present on that individual. For four individuals with multiple oscula, some of the oscula (total = 6) were not accessible to place the scale and the camera in proper position. In such situations, the pumping rate of the whole sponge was calculated following the methods mentioned by Tsubaki & Kato, 2014. In each of these individuals, an average pumping rate (obtained by measuring minimum three oscula for each specimen) was used for the osculum that could not be measured. No physical contact was made with sponges before and during all excurrent velocity measurements. Following the pumping rate measurements, sponge dimensions were measured to estimate the sponge volume as described earlier.

2.4 Tissue density and spicule skeleton

Sixteen sponges were measured for tissue density and spicule skeleton. Actively pumping sponges, determined by releasing small amount of fluorescein dye near the osculum, were collected from the rock pools and immediately transferred to 7 % formalin for fixation. In the laboratory, sponges were washed in seawater to remove sand and debris from the surface and sponge volume was determined by water displacement method. Sponge density was calculated as the mass (dry weight)/volume ratio. Sponges were dried in an oven at 60° C for more than 48 h to obtain the dry sponge mass. The proportion of spicule skeleton in the sponge dry mass was measured by isolating the spicules by tissue digestion method (Schönberg, 1999). Briefly, dried pieces of sponge tissue were dissolved in test tubes, with repeated washes (5 washes) of aqua regia (1 volumetric unit of 70% nitric acid and 3 units of 70% hydrochloric acid) at 140° C (Schönberg, 1999). After each wash the spicules were allowed to settle overnight, supernatant acid was pipetted out, and the residual acid was allowed to react with 70 % ethanol. Spicules were washed three times with 100 % ethanol and were allowed to settle overnight; the spicule pellet weight was obtained by subtracting the empty test tube weight.

2.5 Histology and scanning electron microscopy (SEM)

Total fifteen specimens of *C. cf. cavernosa* were collected for histological investigations, of which eleven individuals were actively pumping (determined by releasing small amount of fluorescein dye near the osculum), and four individuals were completely contracted (due to low tide exposure). Sponges were fixed in 7 % formalin immediately after collection. The spherically shaped sponges were cut into eight pieces by making three mutually perpendicular cuts (two vertical and one horizontal). Sponge pieces treated with 5 % Hydrofluoric acid did not dissolve the spicules completely so we used a treatment of 20 % Hydrofluoric acid for 8 to 10 h to dissolve the spicules. Desilicified tissue samples were dehydrated through a graded series of ethanol, embedded in paraffin,

sectioned to 5 μm (transverse plain and vertical plain) and stained using Harris's Haematoxylin, and Young's Eosin Erythrosin. Total six sections per sponge and ten fields of view per section were examined at 10X to quantify the number of choanocyte chambers (Luter et al., 2012). Slides with transverse sections were examined for the number of spicule bundles and the number of spicules per bundle. For SEM, sponge pieces ($\sim 10 \times 5$ mm) from four individuals were fixed in a fixative consisting of 2% OsO_4 , 2.5% glutaraldehyde in a filtered seawater. The samples were dehydrated through a graded series of ethanol and fractured in liquid nitrogen. The fractured pieces were critical point dried (Leica EM CPD300) and mounted on stubs with a double-sided tape. Specimens were then sputter coated in a gold:palladium mix. Samples were viewed on a MERLIN Compact VP (Carl Zeiss) scanning electron microscope.

2.6 Data analysis

Multi-variate methods were employed to analyze the relationship between physical-chemical parameters (temperature, pH, salinity and DO) of the rock pools during the morning and afternoon low tides. Principle component analysis (PCA) was conducted on normalized dataset as a method of ordination to visualize multi-variate patterns. The two-factor design consisted of the low tide - morning and afternoon (2 levels) and the month - February and March (2 levels). PCA was computed using similarity matrixes based on Euclidean distance on normalized data. To analyze the relationship between the sponge pumping rate and the physical-chemical parameters (temperature, pH, salinity and DO) stepwise multiple regression analysis was performed. Variables retained in regression models were screened for collinearity via correlation coefficients (i.e., collinear measures with $P > 0.05$ were avoided). The relationship of the OSA (morning and afternoon) with the sponge volume was assessed with a Pearson's correlation and simple linear regression. A Fisher's t -to- z transformation was performed to test for potential differences between the correlation of sponge volume and morning and afternoon OSA. The relationship between sponge volume and pumping rate and sponge volume-specific pumping rate was described using linear regression. The relationship of sponge volume with tissue density and spicule skeleton weight was analyzed with Pearson's correlation and best fit curve was fitted with the quadratic regression model for the curvilinear relationship observed in the scatter plots.

3 Results

3.1 Study sites and sponge morphology

Sponge diameter and osculum cross-sectional area (OSA) was measured at four locations. Small sponges (< 10 ml) had a single osculum situated at the top, medium sponges (10-25 ml) had 2-

4 oscula, and large sponges (>25ml) had 4-9 oscula (Figure 2a). However, different sized individuals with single osculum were also common. Due to the contractile nature of the oscula, specimens of similar size showed high variation in OSA (Figure 2b). The OSA increased with the sponge volume ($r = 0.623$, $n = 94$, $p < 0.01$) and the OSA of the sponges measured in the afternoon was slightly higher compared to the measurements made in the morning (Figure 2b). Both, the morning OSA ($r = 0.72$, $n = 49$ and $p < 0.05$) and the afternoon OSA ($r = 0.6$, $n = 45$, $p < 0.05$) showed significant correlation with the sponge volume. To test for potential difference between the morning and afternoon OSA correlations, the r values were transformed into z -scores using Fisher's r -to- z transformation resulting in z scores of 0.90 and 0.69 for the morning and afternoon OSA, respectively. A z -score based on the difference between these two values and the variance of the difference between the two scores was obtained. Using a one-tailed test of significance, the difference between the morning and afternoon OSA correlations was statistically non-significant, Fisher's $Z = 1.01$, $p > 0.05$. Simple linear regression showed that the variation in the OSA was partially explained by the sponge volume ($R^2 = 0.38$, $n = 94$, $p < 0.01$).

3.2 Sponge pumping rate and physical-chemical parameters of the rock pools

The pumping rate was highly variable between individuals. Oscula on multioscular individuals maintained speeds within 1 cm s^{-1} of each other. The excurrent velocity and the pumping rate increased with the sponge volume (Figure 3 a and b). Measurements of the pumping rate regressed against sponge volume showed that the increase in the pumping rate was explained by the increase in the sponge volume ($F(1,33) = 118.53$, $p < 0.01$, $R^2 = 0.78$). Similarly, significant relationship was found between sponge-volume specific pumping rate and sponge volume ($F(1,33) = 4.74$, $p < 0.01$, $R^2 = 0.12$). However, the regression slope for sponge-volume specific pumping rate (0.002) was very low compared to the regression slope of volume flow (0.45).

The PCA analysis revealed two distinctive groups of the rock pools based on the physical-chemical parameters (Table 1). Two significant components (eigen value >1) were extracted explaining 80.9 % of the total variation. Temperature and salinity had a strong effect on the first component, which accounted for 51.2 % variance. The second component accounted for 29.7 % variance and was affected by pH and DO. The grouping was mainly driven by temperature. Rock pools with the temperature between 24°C - 26°C formed one group, and the other group consisted of rock pools with the temperature between 30°C to 33°C . Stepwise multiple regression was calculated to analyze the relationship of pumping rate and the physical-chemical parameters in the rockpools (Temperature, DO, salinity, pH). Stepwise multiple regressions identified two significant models showing relationships between temperature, DO and pumping rate (Model 1: $R = 0.504$, $R^2 = 0.254$,

Adjusted $R^2=0.232$, $P<0.001$ and Model 2: $R=0.591$, $R^2=0.349$, Adjusted $R^2=0.308$, $p<0.001$). Model 1 showed temperature as the significant contributor (Model 1: $F(1,33) = 11.25$, $p = 0.002$) and explained 25.4 % of the variance in the pumping rate (Coefficient $B= 0.26$, $SE =0.07$, $p =0.002$). Model 2 consisted of Temperature and DO as the significant contributors, and explained 34.9 % of the variance in the pumping rate (Model 2: $F(2,32)=8.57$, $P=0.001$), with temperature (Coefficient $B=0.18$, $SE=0.08$, $p = 0.034$) and DO (Coefficient $B= 1.66$, $SE =0.77$, $p = 0.039$). Since, the multivariate PCA analysis also assembled the rock pools into two groups based on temperature, we examined the sponge excurrent velocity and volume flow rates in these two groups of rock pools. Sponges present in the rock pools with the temperature between 30°C to 33°C ($n=23$) had higher excurrent velocity (Mean= $43.33 \text{ mm sec}^{-1}$, Std. Error=3.29) compared to the excurrent velocity of sponges (Mean= $26.60 \text{ mm sec}^{-1}$, Std Error=4.70) present in rock pools with the temperature between 24°C to 26°C ($n=11$). Similarly, sponges present in the rock pools with the temperature between 30°C to 33°C had higher pumping rate (1.49 ml sec^{-1} , Std. Error=0.36) compared to the pumping rate (0.73 ml sec^{-1} Std Error= 0.25) in the rock pools with the temperature between 24°C to 26°C .

3.4 Spicule skeleton and tissue density

Different sized sponges showed considerable differences in the spicule bundles (Figure 4 a, b and c). The number of spicule bundles and the number of spicules within the bundle increased with the sponge volume (Figure 4 d and e). Within each bundle, the individual spicules were connected to each other by perispicular collagen as well as collagen of the mesohyl. The SEM images of the spicule bundles cut in transverse plane showed the megascleres within the bundles were placed out of phase (Figure 4 f). The measurements of spicule skeletal weight and tissue density increased with sponge volume (Figure 4 g and h). The spicule skeleton weight in small sponges ($< 2 \text{ ml}$) was one-half that of large sponges ($>20 \text{ ml}$). The sponge volume had a significant positive correlation with the tissue density ($r = 0.69$, $n = 16$, $p < 0.01$) and spicule contents ($r = 0.78$, $n = 16$, $p < 0.01$). Quadratic regression model was used to fit the curvilinear relationship of spicule skeleton weight ($R^2=0.95$, $p < 0.01$) and tissue density ($R^2=0.76$, $p < 0.01$) with sponge volume. The number of choanocyte chambers per view field showed a marginal increase with sponge volume and the correlation was not significant (Figure 4i).

3.3 Histology and scanning electron microscopy (SEM)

Expanded specimens showed presence of lacunae below the cortex, at the boundary between the cortex and choanosome (Figure 5). These subcortical lacunae were completely absent in the contracted specimens (Figure 5 a). In the expanded state the elements of the canal system were

inflated and appeared to be dominant in the peripheral choanosome regions (Figure 5 b and c). The inner half of the choanosome, towards the central core, showed presence of densely packed spicule bundles. The choanocyte chambers were circular and randomly distributed in the choanosome (Figure 5 d, e, f). The spicule skeleton consisted of a radiate pattern of spicule bundles originating from the center of the sponge and reaching sponge surface (Figure 5 g and h). All the studied specimens (0.8 ml to 33.5 ml) showed two distinct concentric zones, an outer cortex, and a central choanosome (Figure 5 i).

4 Discussions

Pumping rate of sponge *C. cf. cavernosa* present in the intertidal rock pools was measured during morning and afternoon low tides. The physical-chemical characteristics of intertidal rock pools are described in three ways; 1) diurnal and short-term fluctuations 2) long-term seasonal effects and 3) spatial gradients and discontinuities within the pool (Morris & Taylor, 1983). In the present study, we examined the diurnal variations, which showed significant difference and PCA plot revealed two distinctive groups of the rock pools based on the temperature. Sponges present in the rock pool with the temperature between 30°C-33°C had a higher excurrent velocity and volume flow rates compared to the excurrent velocity and volume flow rates of the sponges present in the rock pools with a temperature between 24°C-26°C (Figure 3). The high temperature in the intertidal rock pools during daytime low tides results in evaporation and salinity differences, with concomitant changes in water properties like density and viscosity. Seawater in the intertidal rockpools can undergo stratification due to salinity and temperature differences (Morris & Taylor, 1983, Kooistra et al., 1989). There is no common temperature dependent factor controlling the pumping rate in the various pump and filter systems of different filter-feeding animals (Riisgård & Larsen, 1995). Temperature variations are known to influence the filtration and pumping activity of sponges (Reiswig, 1971; Riisgård et al., 1993; Massaro et al., 2012). However, experimental studies on sponge *Halicondriapanicea* and energy equations indicate that the temperature driven difference in the sponge pumping activity cannot be explained by a combined effect of a change in viscosity and a change of biological processes such as the beat frequency of the choanocyte flagella (Riisgård & Larsen, 1995). *H. panicea* is a contractile sponge and it is suggested that the acute temperature effect may be partly determined by the factors such as constriction/dilation of inhalant canals and/or choanocyte chambers which may be temperature-dependent sites determining the water flow (Riisgård & Larsen, 1995; Riisgård et al., 2016). In the present study the oscular area of the *C. cf. cavernosa* measured in the afternoon was slightly higher compared to the measurements made in the morning indicating a possible diel pattern in the oscular behaviour. Similarly, diel patterns in

oscular behaviour have been reported for other sponge species such as *Cliona orientalis* and *Clionavarians* (Strehlow et al., 2016) and *Tethyacrypta* (Reiswig, 1971).

The excurrent velocity and the volume of water passing through the entire sponge were positively correlated with the sponge volume. The increase in the number of the oscula with sponge volume indicates a more extensive network of the canal system and a more developed aquiferous system, which may explain the increased pumping rate. Osculum cross-sectional area (OSA) was positively correlated to the sponge volume, which explains the increase in the excurrent velocity with sponge volume. Positive correlation, between OSA and excurrent speeds, are reported in several sponge species such as *Tethyacrypta* (Reiswig, 1971), *Cliona orientalis* (Strehlow et al., 2016), and more recently in single-osculum explants of sponge *Halicondriapanicea* (Goldstein et al., 2019). Similarly, a linear relationship was observed between the osculum cross-sectional area and the pumping rate of the single-osculum explants of sponge *Halicondriapanicea* (Kumala et al., 2017). However, the oscula are known to be contractile in several sponges and the dynamic nature of OSA also affects the sponge pumping rate (Strehlow et al., 2016; Kumala et al. 2017; Goldstein et al., 2019). The OSA and excurrent velocity, both are correlated to the osculum contractions in single osculum explants (Goldstein et al. 2019). Experimental studies show that the osculum with sensory cilia functions as a sensory organ, that responds to the excurrent flow generated by the choanocyte chambers (Ludeman et al. 2014). Moreover, changes in OSA subsequent to change in the excurrent velocities in the demosponge species *Tethyacrypta* (Reiswig, 1971) and *Cliona orientalis* (Strehlow et al., 2016) suggest a possibility that osculum dynamics may follow changes in the beating activity of choanocyte flagella (Reiswig, 1971). However, in a recent study, video microscope recordings show that the contraction of the pinacoderm lining the excurrent canals effectively diminish the excurrent flow (Goldstein et al., 2019).

The water current is generated in the choanocyte chambers and the absolute abundance of the choanocyte chambers is one of the factors related to the sponge excurrent velocities (Reiswig, 1974). In present study, histological sections showed the abundance of choanocyte chambers increased marginally with the sponge volume, but there is a possibility that a change in the contraction state during fixation process may have affected our counts. Theoretical estimates and experimental observations suggest that the choanocyte chambers have a uniform distribution within the sponge tissue and have similar individual pumping rate (Reiswig, 1975; Riisgård et al., 2016; Goldstein et al., 2019) and thereupon these findings also support the volumetric scaling of the sponge pumping rate (Goldstein et al., 2019). It is suggested that one of the possible mechanisms sponges may use to increase/alter the pumping rate and volume filtered is to increase the flagellar beats in the

choanocyte chambers and activate more pumping units (Ludeman et al., 2017). However, unlike Hexactinellids, there is no evidence that cellular sponges can control their flagellar beating, except when the flagellar movement is arrested by contortion of the chambers during contraction (Leys & Meech, 2006). A new possibility is raised by the report of a new flow regulating cell type 'reticuloapopyloocyte' observed in sponge *Tethyawilhelma* (Hammel & Nickel, 2014). These cells cover the apopylar opening of the choanocyte chambers with their fenestrated, grid-like morphology that occurs in opened, intermediate closed state and are probably capable of regulating the flow in individual choanocyte chambers and their connecting canals (Hammel & Nickel, 2014). Similarly, the apopyle in *Tethyacaliforniana* consists of a sieve-plate (Ludeman et al., 2017). The other important factor that strongly influences the excurrent velocities is the shape of the spongocoel as revealed by the studies on the pumping rate of the barrel shaped sponge *Xestospongia muta* (McMurray et al., 2014) and tube shaped sponge *Mycale laxissima* (Mycale sp. in Reiswig 1974). Sponge *C. cf. cavernosa* has a solid spherical morphology and a very shallow spongocoel and the dye technique does not give information of the velocity distribution and the fluid flow profile (plug or parabolic) hence we could not study this factor in the present investigation.

Histological sections of the contracted and non- contracted specimens showed some major differences (Figure 5). The subcortical lacunae and the elements of the canal system were inflated and visible only in the specimens that got fixed in expanded state. The cortex and the choanosome of the specimens in the contracted state appeared completely solid and devoid of any canals or lacunae. Anatomical analysis of expanded and contracted states of a spherical shaped sponge *Tethyawilhelma* showed that during contraction the water is expelled from the aquiferous systems and then the canals collapse. Consequently, the sponge shrinks in size due to a major volume reduction in the canal system and not the mesohyl (Nickel et al., 2011). The pinacoderm cells lining the canals and the sponge surface are the primary pathways in the sponge contraction (Nickel et al., 2011). Histological sections of *C. cf. cavernosa* showed that the aquiferous canals had the highest abundance in the outer choanosome regions (Figure 5). This indicates that the subcortical lacunae and the peripheral choanosome dominated with canals appear to be the major volume reduction zones during contraction events.

Hypothetical biomechanical contraction models of *Tethya* specimens indicate that the skeletal superstructures composed of spicules restrain the physical stress generated during the contraction behaviour (Nickel et al., 2006). Similarly, in *C. cf. cavernosa* the spicule skeleton consists of bundles of megascleres radiating from the center. Histological and SEM images showed that the individual spicules within the bundles are placed out phase and a collagen sheath surrounds and connects

individual spicules within the bundles (Figure 5). This suggests that such arrangement may help the spicules within the bundles to slide along each other and shift along the vertical axis during contraction events. The number of spicule bundles, and the number of spicules within the bundles increased with the sponge size. The number of megascleres within a bundle depends on the profile view and may also change due to the movement of spicules during contraction (Nickel et al., 2006 (b)). The skeletal structures are known to change in different sized specimens of *Tethya* spp. and the pattern differs among different species (Bavestrello et al., 2000). The size-dependent differences in the number and the thickness of the spicule bundles radiating from the center indicate a more dense and developed core in the larger specimens of *C. cf. cavernosa*.

The tissue density and the spicule skeleton weigh in *C. cf. cavernosa* increased with the sponge volume. Sponge pumping is influenced by morphological factors like tissue density (Weisz et al., 2008). Sponges with dense mesohyl have water vascular system composed of longer, narrower canals that necessitate slower filtration rates (Weisz et al., 2008). However, structural analysis of spherical shaped sponge *Tethyawilhelma* revealed a complex and non-uniform inner structure in the aquiferous system (Hammel et al., 2012). In sponges, the proportion between the weight of tissues and the distribution and arrangement of the components of the aquiferous system leads to optimization of some physiological factors (Ereskovsky, 2003). In the present study the increase in the spicule skeleton weight in the large sponges may have affected the tissue density in large sponges. Furthermore, differences in sponge volume and contraction behaviour add to the variability between and within individuals. This may explain the low regression slope between the sponge volume-specific pumping rate and the sponge volume of *C. cf. cavernosa*. The volumetric flow rates of a giant barrel sponge *Xestospongia muta* scaled isometrically with sponge size (Mcmurray et al., 2014). *Theonellawinhoei* has a negative correlation between specific water transport (normalized to the volume of the sponge) and sponge size (Yahel et al., 2003). Sponges are highly diverse and different species have high variability in gross morphology such as shape, size and number of oscula, moreover species also differ in the nature of osculum, which can be rigid or contractile. The pumping activity patterns determined for one species cannot be projected on other, even closely related species (Rieswig 1971). The developmental and growth related changes, together with the phenotypic plasticity and sensitivity to external environmental conditions are some of the factors that bring huge variations in sponge pumping. Sponges have adaptive interspecific variation in structure and efficiency of the filtering systems, which correlates with diverse biological strategies (Turon et al., 1997).

Here we found that the size and the number of spicule bundles in *C. cf. cavernosa* increase with sponge size. Moreover, the maximal cross-sectional area of the dilated osculum also increases with the in *C. cf. cavernosa* sponge volume. Such size associated morphological changes, the contraction state and the water temperature influence the pumping capacity of *C. cf. cavernosa*. Understanding the factors affecting the sponge filtration and pumping activities is important as they mediate several important ecological processes (DeGoeij et al., 2013; Khan et al., 2015). However, knowledge of sponge functioning is still limited to a few species and additional long-term studies are required to improve our understanding of the various factors that affect sponge pumping.

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Compliance with Ethical Standards:

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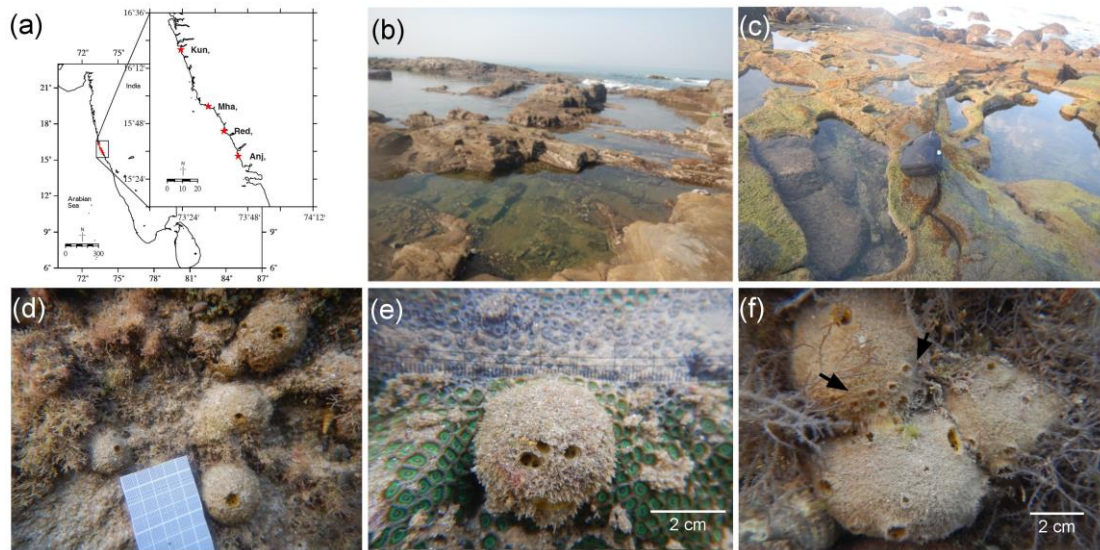


FIGURE 1 a) The study sites Kunkeshwar (Kun),Mhapan (Mha), Reddy (Red), and Anjuna (Anj). b and c) The intertidal rock pools at the Anjuna and Kunkeshwar. e) Different sized individuals of sponge *Cinachyrella* cf. *cavernosa*. f and d) sponges with multiple oscula, black arrows in d) point to the porocalices containing the inhalant porefields.

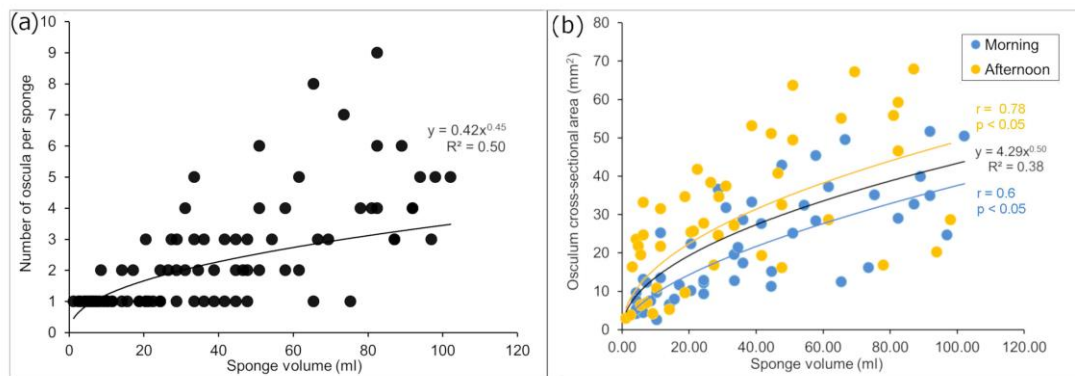


FIGURE 2 The relationship of oscula and sponge volume a) the number of oscula in different sized sponges. b) The osculum cross-sectional area in different sized sponges.

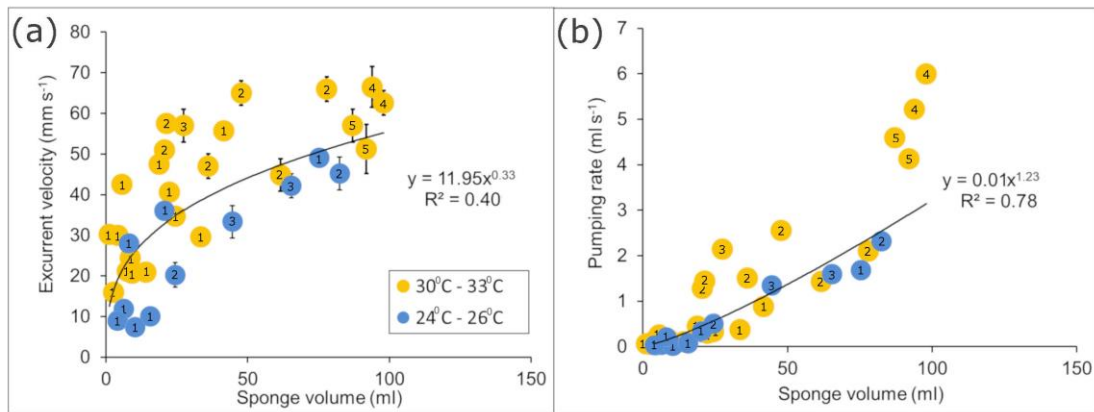


FIGURE 3 a) Relationship between the excurrent velocity and the sponge volume. (Error bars represent the standard deviation between the oscula of that individual) b) Flow rate vs the sponge volume. Blue filled circles are the sponge ex-current velocity measured at 24^oC to 26^oC; Yellow filled circles are the sponge ex-current velocity measured at 30^oC to 33^oC. The numbers within the circles denote the number of oscula for that sponge individual

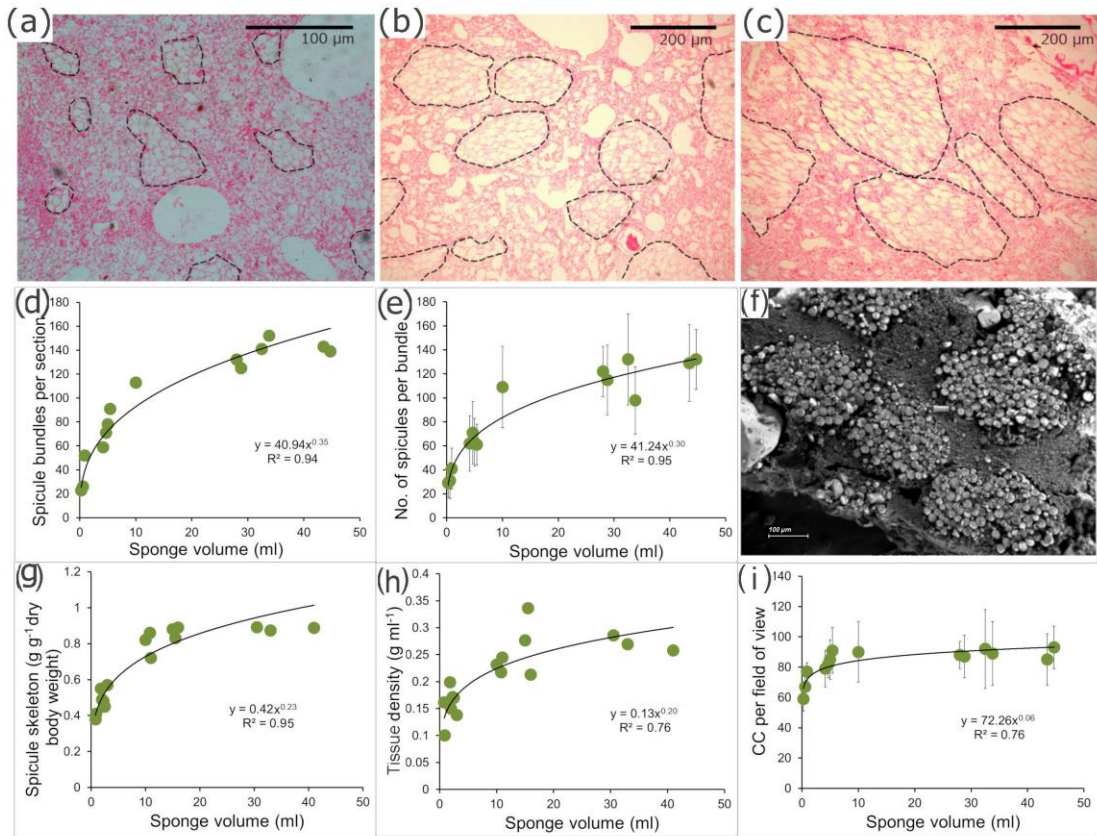


FIGURE 4 Spicule bundles in transverse sections, individual spicules within the bundles are surrounded by a collagen sheath. The number of bundles increased with body size. The number of spicules within the bundles also increased with body size. Sponge size for **a)** <10 ml; **b)** 10 ml–25 ml; **c)** >25 ml. **d)** number of spicule bundles per section representing approximately a quarter of sponge. **e)** number of spicules within the bundles in different sized individuals. **f)** SEM image of the spicule bundle showing the spicules placed out of phase within the bundles. **g** and **h)** The body density and spicule skeleton weight in different sized individuals of sponge *Cinachyrella cf. cavernosa*. **i)** The abundance of choanocyte chambers per field of view in different sized individuals. Error bars are standard deviation ($p > 0.05$).

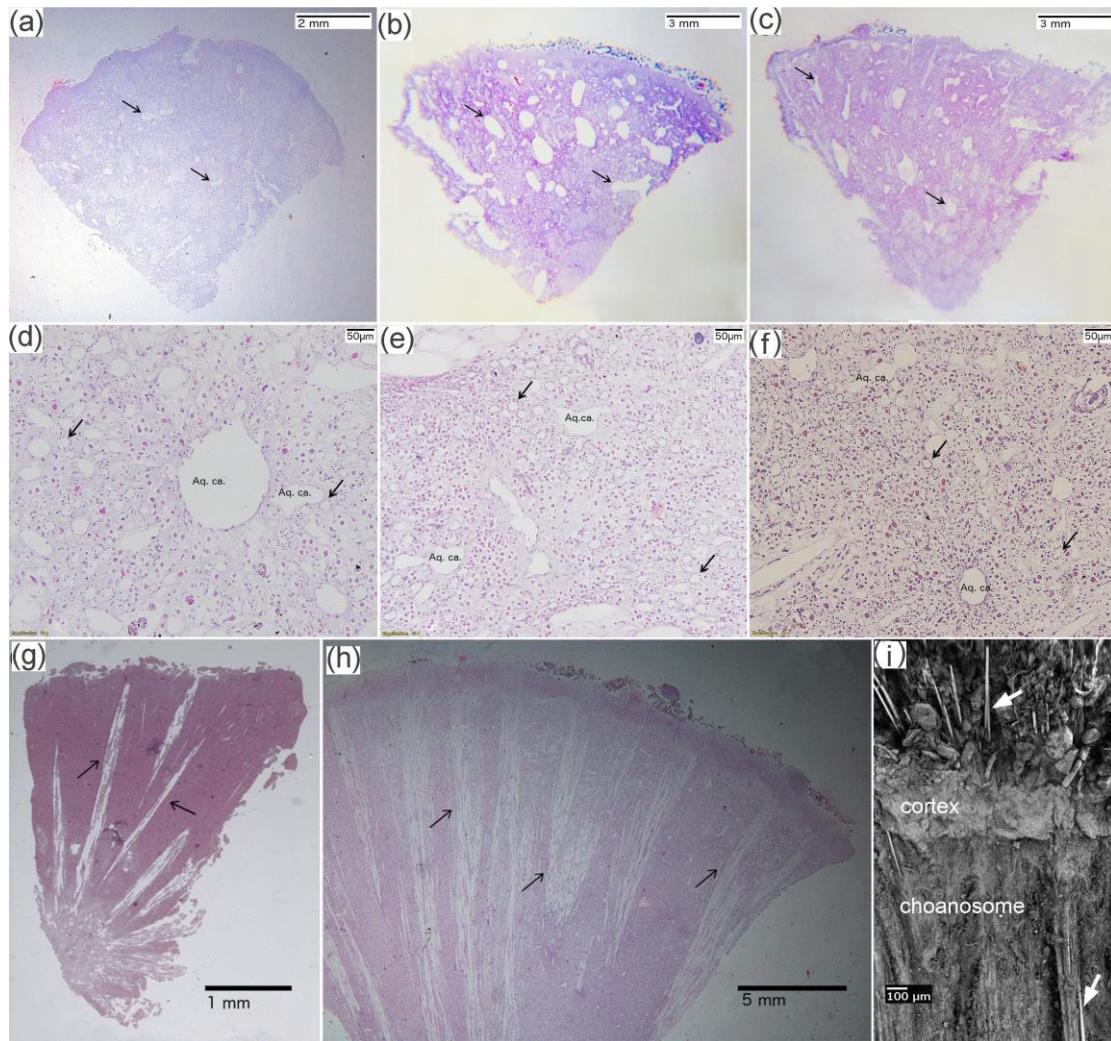


Figure 5 Histological sections of the different sized individuals of sponge *Cinachyrella cf. cavernosa*, **a)** sponge is in contracted state **b** and **c)** in expanded state. Black arrows in **a**, **b** and **c** point to the aquiferous canals. **d**, **e**, **f)** Choanocyte chambers in the different sized individuals **d)** <10 ml; **e)** 10 ml–25 ml; **c)** >25 ml. **f)** Arrows in D,E and F point to the choanocyte chambers. Aq. Ca.= Aquiferous canal. **g&h)** The vertical sections, parallel to the spicule bundles show the spicule bundles radiating from the central core. Sponge volume <10 ml and 10 ml–25, respectively. **i)** SEM image showing the distinct cortex and the choanosome. White arrows point to the spicules within the tissue.

Table 1 The Physical-chemical parameters of the rocks pools measured during low tide exposure. Anj = Anjuna ,Kun= Kunkeshwar (\pm Std dev)

		Morning			Afternoon				
13 Feb 2015 (8.00 am)		Pool 1	Pool 2	Pool 3	13 Feb 2015 (2.30 pm)		Pool 1	Pool 2	Pool 3
KUN	Temperature	25 \pm 0.01	25.6	25.8	KUN	Temperature	32.3 \pm 0.01	32.4	32.5
	pH	7.95	7.99	8 \pm 0.01		pH	8.1 \pm 0.01	8 \pm 0.00	8.11
	Salinity (‰)	34.67	34.5	34.23		Salinity (‰)	33.81 \pm 0.1	34.5	34.23
	DO (mg l ⁻¹)	5.31	5.66	5.5 \pm 0.09		DO (mg l ⁻¹)	6.01 \pm 0.12	5.89	5.97
					17 Feb 2015 (4.30 pm)				
					ANJ	Temperature	31.3 \pm 0.01	31.5	31.9
						pH	8.72 \pm 0.00	8.64	8.55
						Salinity (‰)	33.06	33.0	33.09
						DO (mg l ⁻¹)	5.38 \pm 0.11	5.31	5.33
21 Feb 2015 (7.30 am)					21 Feb 2015 (4.00 pm)				
ANJ	Temperature	24.3	25.4	24.2	ANJ	Temperature	30.9 \pm 0.02	30.4	30.7
	pH	7.81	7.25	7.91		pH	8.26 \pm 0.00	8.43	8.37
	Salinity (‰)	32.5 \pm 0.1	33 \pm 0.07	32.7 \pm 0.2		Salinity (‰)	33.5 \pm 0.11	33.4	33.3
	DO (mg l ⁻¹)	4.27 \pm 0.2	3.82	4.04		DO (mg l ⁻¹)	5.35 \pm 0.01	5.18	5.92
24 Mar 2015 (9.00 am)					19 Mar 2015 (4.00 pm)				
KUN	Temperature	30.2	30 \pm 0.01	30.2	ANJ	Temperature	32.7 \pm 0.01	32.5	31.8 \pm 0.3
	pH	8.24	8.31	8.4 \pm 0.01		pH	8.45 \pm 0.01	8.59	8.76
	Salinity (‰)	33.85	34.05	34.28		Salinity (‰)	32.6 \pm 0.12	32.4 \pm 0.1	32.2
	DO (mg l ⁻¹)	5.21 \pm 0.2	5.33 \pm 0.1	5.81 \pm 0.1		DO (mg l ⁻¹)	5.89 \pm 0.31	5.91	5.46 \pm 0.1