Cell Turnover in Marine Sponges: Insight into Poriferan Physiology and Nutrient Cycling in Benthic Ecosystems

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Summary

Cell Turnover in Marine Sponges: Insight into Poriferan Physiology and Energy Cycling in Benthic Ecosystems

The sponge loop is a major energy and nutrient recycling pathway that has recently been described in Caribbean coral reef ecosystems. The majority of food produced on coral reefs consists of dissolved organic matter (DOM), which is not readily available to heterotrophic multicellular reef inhabitants. Sponges enable the shunt of this energy source to heterotrophs through the conversion of DOM to particulate organic matter (POM), or detritus, which acts as a major food source for higher trophic levels. The main underlying mechanism behind the production of sponge-derived detritus is hypothesized to be a rapid turnover of sponge cells through fast cell proliferation balanced by cell shedding (De Goeij et al. 2013). This thesis aims to gain a deeper understanding of these cellular mechanisms and the effect that the physiological state of sponges has on cell proliferation, shedding, and detritus production.

Rapid cell turnover had previously only been investigated in a single species of tropical reef sponge, *Halisarca caerulea*. We therefore investigated if rapid cell turnover and detritus production are common mechanisms occurring in a range of sponge species from diverse benthic ecosystems, including tropical coral reef (n = 6), tropical mangrove (n = 1), and temperate Mediterranean reef (n = 1) ecosystems. Sponges were investigated under steady-state conditions, i.e. negligible growth, and all species displayed substantial amounts of cell proliferation, predominantly in their choanocytes (filter cells), and less abundant proliferation of their mesohyl cells. The majority of the tropical reef species investigated (*H. caerulea*, *Chondrilla caribensis*, *Scopalina ruetzleri*, *Clathria* sp., *Haliclona vansoesti*), and the Mediterranean species (*Chondrosia reniformis*), had similar rates of choanocyte proliferation: between 16.1 ± 15.9% and 19.0 ± 2.0% (mean ± SD) after 6–10 hours of continuous labeling with the proliferation marker, BrdU. However, two species did not follow this trend. The tropical reef sponge *Monanchora arbuscula* had lower choanocyte proliferation (8.1 ± 3.7%) and the mangrove species *Mycale microsigmatosa* had substantially higher levels of choanocyte proliferation (70.5 ± 6.6%). Apoptosis was investigated as a potential mechanism of cell loss and was found to be negligible. High amounts of detritus were produced in all species investigated (2.5–18% $DW_{detritus}\ DW_{sponge}^{-1}\ d^{-1}$). Cell shedding was observed in seven of the eight species, however choanocyte proliferation rates did not always relate to the abundance of shed cells observed in histological sections. Instead, histological observations of cell shedding were most likely related to differences in the residence time of detritus within canals. Large amounts of cell shedding were observed only in sponge species with low microbial abundances (LMA), which are known to have lower pumping rates than high microbial abundance (HMA) species. As soon as cellular debris was expelled from the sponges as detritus, it quickly degraded, meaning that it was not possible to directly link detritus production to the shedding of 'old' cells. We demonstrated that under steady-state conditions, cell turnover via cell proliferation and cell shedding are common mechanisms for maintaining tissue homeostasis in a variety of sponge species. We suggest that, in addition to tropical Caribbean reef ecosystems, cell-turnover driven sponge loop pathways may also drive energy and nutrient recycling in tropical mangrove and temperate Mediterranean benthic ecosystems.

In addition to rapid cell turnover, sponges also have extremely rapid rates of tissue regeneration, which can occur in response to wound infliction. However, little is known about the involvement of cell proliferation in tissue regeneration, and we therefore investigated cell proliferation at different stages of the regenerative process using the encrusting reef sponge *H. caerulea* as a model species. Again, the predominant population of proliferative cells was the choanocytes. There was no difference in the number of proliferative mesohyl cells in regenerative sponges compared to steady-state sponges, indicating that their proliferation did not play a role in the regenerative process. During the first 8 hours following experimental wound infliction, the choanocyte growth fraction (i.e. the percentage of the choanocyte cell population involved in proliferation) directly adjacent to the wound was significantly lower (7.0 ± 2.5%) than in steady-state, undamaged tissue (46.6 ± 2.6%). However, the cell cycle duration remained similar in regenerative sponges (5.6 ± 3.4 hours) compared to steady-state sponges (5.9 ± 0.4 hours). Choanocyte proliferation rates increased with time and after 6 days of regeneration were comparable to rates previously measured for steady-state tissue. Choanocyte proliferation rates also varied spatially, with less proliferative choanocytes closer to the wound. This implies that the resources required for tissue regeneration are derived predominantly from the tissue closest to the wound. Dense, collagen-rich tissue rapidly occupied the coral rock substrate exposed by the wound. This is a key process in wound repair that is likely to direct energetic resources away from choanocyte renewal. These results show that tissue regeneration and choanocyte renewal are competing and negatively correlated life-history traits, both essential to the ecological success of sponges.

The majority of the experiments in this thesis were carried out in running seawater flow-through aquaria. Within such systems, it is usually assumed that the nutritional quality of the aquaria water matches that of *in situ* seawater. However, we found a discrepancy between the choanocyte proliferation rates for *H. caerulea* (17.6 ± 3.3% in 6 hours) measured during the fieldwork period 2011–2013 compared to proliferation rates estimated approximately 5 years earlier (46.6 ± 2.6% in 6 hours). Measurements taken during all of these experiments used exactly the same methodology in the same running seawater aquaria systems. We hypothesized that this discrepancy may be a consequence of suboptimal food conditions within the flow-through aquaria. Preliminary tests indeed showed that the abundance of heterotrophic bacteria (as proxy for food) in our tropical aquaria system were approximately three times lower (3.0×10^5 mL^{-1}) than for *in situ* reef water at the entrance of the inlet pipe (8.8×10^5 mL^{-1}). We therefore investigated the water quality, i.e. bacterial abundances and inorganic nutrient (nitrate, nitrite, ammonium, phosphate) concentrations along the length of the inlet pipe that fed the flow-through aquaria before and after its replacement. The first 12 meters of the old inlet pipe was extensively colonized by biofouling communities of suspension- and filter-feeding organisms, which included sponges, bivalves, barnacles, and ascidians. Bacterial abundances were significantly higher and inorganic nitrogen species significantly lower in the new pipe, which was free from biofoulers, in comparison to the old pipe, and these patterns were also evident along the length of the old pipe with increasing distance from the pipe inlet. The feeding activity and waste production of the suspension- and filter-feeding communities found explain the changes in bacterial and inorganic nutrient concentrations within the pipes. The proliferation rates of choanocytes in *H. caerulea* also increased from 15.1 ± 1.9% in 6 hours to 20.2 ± 3.8% in 6 hours within only 7 days after replacing the inlet pipe, indirectly suggesting that suboptimal nutritional conditions in the aquaria caused a compromise in the physiological functioning of the sponge. Our study shows the

importance of regularly checking and replacing inlet pipes in order to control biofouling communities and to ensure optimal water quality in aquaria. Unknowingly altering water quality may lead to drawing incorrect conclusions from scientific experiments performed in running seawater systems.

Sponge-derived detritus is a major food source on coral reefs that fuels the transfer of energy and nutrients to higher trophic levels. As previously mentioned, cell turnover is proposed to be the main underlying mechanism behind the production of sponge-derived detritus. Since choanocyte proliferation rates are altered during stressful conditions, such as tissue regeneration in response to wound infliction, we tested whether detritus production rates might also be affected by stress. Detrital dry weight, particulate organic carbon content, particulate total nitrogen content, and nutritional quality were determined during steady-state, regressed, and regenerative conditions for our model species *H. caerulea*. We found that detritus production rates are indeed dependent on the physiological state of a sponge. The highest quantities of detritus were produced by 'normal' steady-state sponges that showed limited amounts of growth. This detritus had the highest nutritional quality, demonstrated by its low carbon to nitrogen ratio. Steady-state sponges also turned over the highest amounts (approximately 60%) of organic carbon and total nitrogen contained in their tissues. Effluxes (output) of detrital particulate organic carbon accounted for 96% of the daily intake of organic carbon by *H. caerulea*, and approached the same order of magnitude as the gross primary production rates of an entire reef ecosystem. The ecological significance of increases or decreases in the abundance of sponge-derived detritus still needs to be determined. Anthropogenic disturbances, such as increased pollution and overfishing, and climatic changes, such as increases in storm intensity, can cause the physiological state of sponges on coral reefs to become altered. Widespread changes in these physiological states may influence the availability of sponge-derived detritus, thereby impacting the cycling of energy and nutrients in coral reef ecosystems.

In conclusion, rapid cell proliferation and cell shedding are important homeostatic mechanisms in sponges from diverse benthic ecosystems, which act to maintain healthy populations of cells. There is growing evidence that cell turnover is the driving force behind energy and nutrient recycling on coral reefs through the sponge loop. However, when sponges are stressed, such as during tissue regression and tissue regeneration, choanocyte proliferation rates become compromised, which subsequently results in lower production rates of sponge-derived detritus that is of lower nutritional quality. Alterations in sponge physiology due to anthropogenic and natural perturbations are likely to have implications for the availability of food to higher trophic levels on coral reefs. This thesis has demonstrated how knowledge of sponge physiology on a cellular level can lead to insight into the functioning of benthic ecosystems. These fundamental advancements in sponge physiology and cell biology have important implications in the improvement of *ex situ* culture regimes for the development of biotechnological products. In order to make further progress in sponge cell kinetics, the development of improved sponge cell-specific molecular markers and sponge-specific antibodies is required, as well as advancements in knowledge of the regulation of cell turnover.