MASTS Small Grant 13

Rationale

Funds were requested to purchase three respiration chambers. These were originally envisaged to be used with *Holothuria forskali* to better understand the energetic of the use of this species in IMTA. However due to logistical constraints they were eventually used for looking at the suitability of Scottish sponge species in an IMTA context.

This work was conducted in conjunction with Philip Nemoy, University of Haifa, Israel with additional funding from the Assemble program.

Introduction

Marine sponges produce bioactive compounds with high biotechnological potential (1). In addition, sponges are known to feed on a wide range of microorganisms and organic matter from seawater, which makes them prospective candidates as biofilters for integrated multi-trophic aquaculture (2). Fish farms release large amount of wastes in the form of feed residues, faeces and excreta, which either suspend or dissolve in the surrounding water. Sponges can be used to filter fish farming wastes from the water, and also provide additional revenue (3).

In this study we examined the potential of Scottish sponges (*Haliclona* and *Halichondria* spp.) to take up nutrients from salmon fish feed, to simulate IMTA. Our goal was to figure out which components of the fish feed are consumed by the sponges. We tested the effects of sponge filtration on particulate and dissolved nutrients leaching from uneaten fish feed under controlled laboratory conditions.

Methods

Sponge handling

The sponges were collected in November 2012 from ~ 6m depth nearby SAMS, Dunstaffnage, and transferred to the laboratory while completely immersed in seawater. In the laboratory, sponges were kept in two 45 lit. flow-through seawater tanks, each species in a separate tank. The water flow was ~ 5 lit/hr, water temperature was between 9-12 °C.

Water sample collection

In order to measure the concentrations of nutrients, two types of sampling were applied:

1. Incubations: individual sponges were transferred from the holding tanks to experimental tanks with respirometer chambers (0.45 lit. Loligo, Denmark). Salmon feed pellets (crude contents: Protein 54%, Fat 18%, Fibre 0.5%, Ash 5.1%, Dana Feed AS) were ground by mortar and pestle, incubated in aerated seawater overnight, let settle for at least 2 hours and then added to the incubation chambers. This resulted in X2 to X5 increases in dissolved and particulate organic carbon and nitrogen, relatively to the background levels in the supplied seawater. One 100 ml water sample was retrieved from the chambers at the beginning of each experiment (before) and another 100 ml

sample was retrieved after 60 minutes of incubation (after). Each incubation experiment included 2 chambers with sponges (same species each time) and 2 control chambers without sponges. The experimental setup was washed with fresh water and refilled with flow-through seawater between the incubations.

2. In-Ex direct sampling: the same organic enrichment as for incubations (0.01 g/L ground fish feed) was added to the tank with 15 *Halichondria* sp. and the water flow through this tank was shut for the duration of sampling. The sampling was performed as described in (4) by two 0.5 mm i.d. Tygon tubes of 130 cm length. Each tube connected the sponge with a collection bottle located 0.7 m below the water level outside the tank. The water from near sponge surface (incurrent, In) and from inside an osculum (excurrent, Ex) was sampled simultaneously at the rate of ~ 1 ml/min, reaching final volume of 100 ml in slightly more than 1 hr. The sampling was performed on active osculae, as detected by Fluorescein dye (4).

Sample treatment and analyses

Sample collection was performed by acid-washed glass syringes and while wearing clean polyethylene gloves. The samples were passed through pre-combusted GF/F filters and refrigerated or freezed until analyses (depending on assay). The concentrations of 7 analytes were measured as follows:

DOC (Dissolved Organic Carbon) = NPOC, Non-Purgeable Organic Carbon by Shimadzu TOC-V analyzer.

DON (Dissolved Organic Nitrogen) = TN (Total dissolved Nitrogen by Shimadzu) - Inorganic N (Ammonium+NOx by Lahat autoanalyzer).

Ammonium by Lahat autoanalyzer.

NOx – Nitrite+Nitrate by Lahat autoanalyzer.

Phosphate by Lahat autoanalyzer.

POC – Particulate Organic Carbon = total Carbon from GF/F filter, by ANCA-GSL GC/MS.

PON – Particulate Organic Nitrogen = total Nitrogen from GF/F filter, by ANCA-GSL GC/MS.

Finally, all the sponges were dry-weighed to normalize the results per unit of sponge biomass.

Results and Discussion

Both sponge species showed significant uptake of POC and PON and excretion of Ammonia and DOC (fig. 1, 2). These results suggest that the studied sponges feed mainly on particulate organic matter (POC and PON) and excrete their wastes as ammonia. Recent publications suggested that tropical and temperate sponges may feed on dissolved organic matter (5, 6). Yet, the studied sponges did not exhibit significant uptake of dissolved organic nutrients. The expected uptake of dissolved organic matter was not observed due to the following possible reasons:

1) low statistical power to detect significant changes in DOM concentrations (low number of replicates);

2) presence of high concentration of particulate organics, which are preferred by sponges due to their higher nutritional value.

Further suggestions: According to Gitai Yahel, the In-Ex method when combined with direct measurements of water flow from osculae (by ADV or videography) is superior to incubation for studying the effects of sponge filtration on dissolved nutrients, since the former it is far less prone to the bias associated with background bacterial activity which is present in incubation chambers.

In incubation experiments, the bias of background bacterial activity can be reduced by conducting the experiments in sterilized seawater. Moreover, particulate nutrients can be removed by GF/F filtering of the water prior to incubation. These practices were not employed in the present study due to the tight schedule.

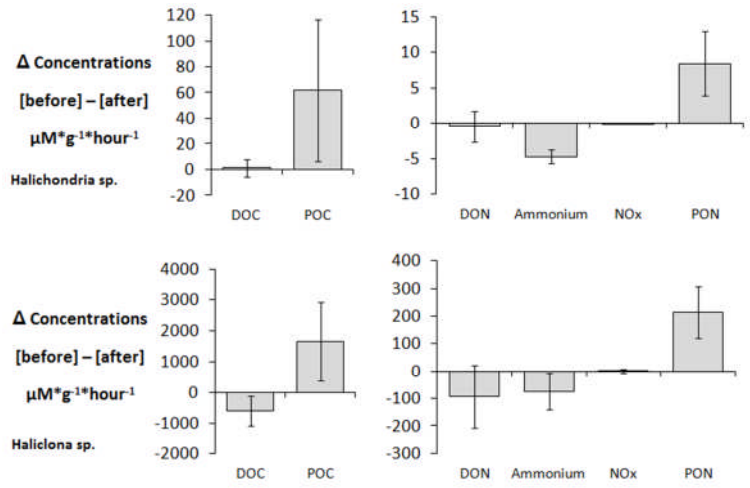

Figure 1: Biomass-corrected changes in analyte concentrations (Average ± 95% CI/gram sponge dry weight/hour) in incubation experiments.

n=10 for *Halichondria* sp.
n= 12 for *Haliclona* sp.

Note the different Y-axes for Carbon and Nitrogen.

Positive values suggest uptake (before>after) and negative values

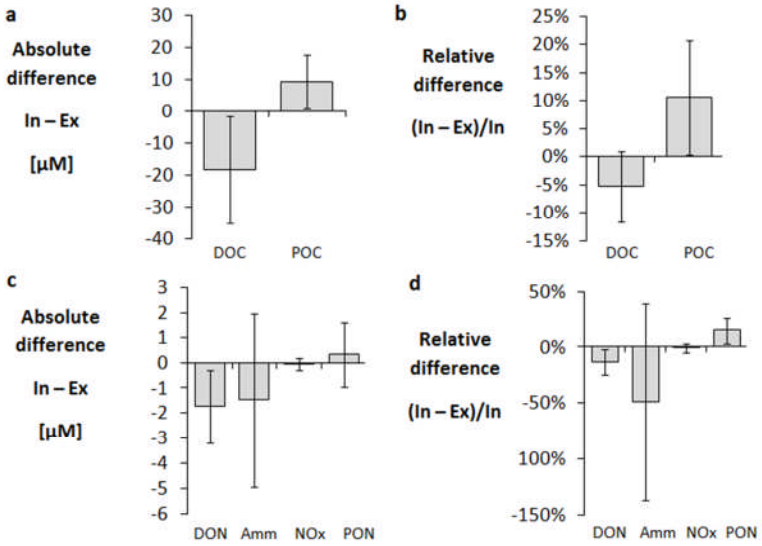

Figure 2: Absolute and relative differences between "In" and "Ex" concentrations (Average ± 95% CI) of Carbon (a, b) and Nitrogen (c,d) for Halichondria sp. (n=8).

Positive values suggest uptake and negative values suggest excretion. Error bars crossing the x-axis suggest non-significant difference from 0.

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