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Ammonium Excretion Rates of Giant California Sea Cucumbers (*Apostichopus californicus*)

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Abstract

Nitrogen is a key nutrient used to support the growth of primary producers. In the ocean, it can be supplied in various ways, including through decay, upwelling, nitrogen fixation, and via animal excretion. There is a possibility that ambient nitrogen availability in nutrient-rich cold waters could be enhanced by animal-mediated nitrogen production. Here, we used giant California sea cucumbers, Apostichopus californicus, to explore animal-mediated nitrogen contribution in temperate oceans by holothurians, asking specifically if (a) nitrogen levels decrease with increasing distance from sea cucumbers, and (b) larger sea cucumbers excrete ammonium at higher rates. We analyzed ammonium concentrations using fluorometry water samples collected internally, near (3-5 cm) and far (1 m) from sea cucumbers at Reed Point Marina, with average ammonium concentrations of 2.79 µmol (SD = 0.75 µmol) and 3.21 µmol (SD = 1.28 µmol) respectively. There was no significant difference in ammonium concentrations near and far from sea cucumbers. Ammonium concentrations in the two internal samples obtained were $\sim$ 7 times higher (22.96 $\pm$ 2.12 µmol) than the ambient samples, suggesting a strong dilution of sea cucumber nitrogen upon excretion. There was a strong positive correlation between excretion rate and sea cucumber mass ($R^2 = 0.79$). Based on our findings we are still unsure of the ecological impacts of sea cucumber fishing, which is becoming more prevalent in the northeast Pacific, as we can only see that giant California sea cucumbers at an individual scale do not contribute a significant amount of available nitrogen. Further research needs to be conducted as the role of a species in its environment is key to learning the potential ecological repercussions of removing it.

Keywords — Deposit feeders, Holothurians, Nutrient cycling, Nutrient availability, Producer productivity

1. Introduction

A debated dichotomy in ecology is whether primary producers are regulated mainly by top-down effects (i.e. by consumers), or bottom-up forces (i.e. resource availability) [1]. Primary production is affected by consumers, which are kept in check by predators (top-down effects) and are impacted by resource availability (bottom-up forces). However, debates on which mechanism has the greatest effect on primary producers (and the rest of the food web) have begun to shift towards

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hypotheses of how these mechanisms work together [1]. Nitrogen and phosphorus are common growth-limiting nutrients for photosynthetic organisms in marine, freshwater, and terrestrial environments [2]. Nitrogen and phosphorus are equally limiting in freshwater and terrestrial environments, but nitrogen is the most limiting nutrient in coastal marine environments [2]. Nitrogen is supplied to the ocean in various ways and is used to support primary producer nutrient requirements, primarily via nitrogen fixation, decay, upwelling, and animal excretion. Nitrogen can be provided to the ocean surface through both nitrogen fixation [3, 4], and ocean upwelling [5]. Ocean upwelling supplies nitrogen to the ocean surface created by the decay of both autotrophs (predominately phytoplankton) and heterotrophs [5], as well as from animal excretion [6].

The effects of animal-mediated nutrient contributions on primary producer productivity will differ across tropical and temperate regions due to differences in ambient light and nutrient availability [3]. Tropical waters of moderate depth cannot heat all the way through, which produces different water density layers. This reduces nutrient cycling in the tropics as mixing will only occur fully during the winter. This creates much lower nutrient levels in the upper layer of tropical waters, compared to cooler temperate waters which have the same density throughout the year and are able to mix constantly [7]. Due to this, nitrogen is more often limiting for autotrophs in the tropics than in temperate oceanic regions [7]. Animals will therefore likely play an important role in recycling nutrients back to autotrophs at low latitudes, since nitrogen levels are lower in the tropics [2, 7]. However, our understanding of how important excretion is for nutrient recycling in temperate waters is immensely limited. Given that temperate waters have higher nutrient levels than the tropics, the contribution of animal-mediated nitrogen should be relatively less important in temperate waters than in tropical waters.

The amount of nitrogen excreted by animals, and hence the importance of this source of available nutrients, will also depend on body size as metabolism and waste production scales proportionally with body size. Smaller species have a higher metabolism and thus a higher excretion rate per unit mass. Therefore, biomass consisting of smaller organisms will contribute more nutrients than an equal biomass of much larger animals [8, 9]. Also, within-species excretion rates scale allometrically with body mass (e.g., zooplankton, 11). However, the extent that mass alters excretion rates can vary in different species. For example, the per-gram excretion rates of smaller freshwater fish could be less than, equal to, or higher than those of larger individuals in the same species, demonstrating that both body size and taxonomy control excretion rates [8].

Sea cucumbers (class Holothuroidea) are model organisms for exploring the importance of animal-mediated nitrogen excretion in the ocean as multiple species of holothurians can be abundantly found in both temperate and tropical waters. Holothurians digest organic materials contained in sediments (i.e., deposit feeding) and then release waste as inorganic nitrogen in the form of ammonium, along with small amounts of phosphorus in the form of phosphate [10, 11]. Ammonium is mainly excreted from the respiratory trees through the anus and, to a lesser extent, through the body wall [10]. In the tropics, sea cucumber nitrogen excretion is of similar magnitude to the amount of nitrogen fixed by cyanobacteria. Holothurian excretion in the tropics enhances water nutrient levels, which positively impacts benthic microalgae productivity [10]. There is no comparable study of the possible impacts that sea cucumber-mediated nitrogen contributions have in richer temperate waters.

In this study, we used giant California sea cucumbers, Apostichopus californicus, to examine the importance of animal-mediated nitrogen excretion in temperate waters. Giant California sea cucumbers are the largest sea cucumbers found in northeast Pacific waters and are often locally abundant. Our specific objectives were twofold. First, we estimated the differences in nutrient levels within, near, and far from sea cucumbers. We predicted that nutrient levels would decrease with distance from sea cucumbers. Second, we investigated whether mass and excretion rates were correlated, anticipating a positive association. This pilot study is important because echinoderms, such as sea cucumbers, are an emerging fishery in the northeast Pacific [12]. Our findings will begin to address the potential ecological impacts of removing sea cucumbers, which have been so far overlooked.

2. Materials and Methods

We collected giant California sea cucumbers on September 7th, 2017 at Reed Point Marina, Burnaby, British Columbia, Canada (49.2913°N, 122.8823°W). Samples were collected near the boating docks, at the interface between the rocky reef area extending from shore and the sandy/muddy sediment, at approximately 3-4 m in depth. There was minimal water current near the sample site as the day was calm, there was minimal boat traffic that was located far enough away to cause negligible water disturbance, and the site was surrounded by docks that dampened water movement.

From the surrounding areas of seven randomly selected sea cucumbers, a SCUBA diver collected 14 water samples into individually numbered, 1 L Ziploc bags. Sea cucumbers were spaced at least 1 m apart to reduce any changes to the concentration of excreted ammonium surrounding them due to SCUBA diver turbulence (from fin kicks) while collecting samples from another sea cucumber. One 'near' sample was taken near each sea cucumber, approximately 3-5 cm from the animal, and a 'far' sample was collected roughly 1 m away. Each of the seven sea cucumbers were then brought up using a mesh bag, one at a time, and placed into holding troughs with flow-through seawater. Sea cucumber locations were roughly mapped to ensure that water samples were labelled with the same number as the relevant sea cucumber.

We first subsampled the 'near' and 'far' water samples. To do this, we cleansed a plastic syringe by withdrawing and expelling 20 mL of seawater from one of the bagged samples. We then withdrew a further 60 mL and pushed it through a 0.45 µm filter paper, of which the first 10 mL were expelled to cleanse the syringe tip (of any small organisms) and another 10 mL to cleanse a darkened 250 mL bottle. The darkened bottle was capped, shaken, and emptied. The remaining 40 mL of filtered seawater was then injected into the labelled bottle. We repeated this process for the other 13 samples. The filter paper was replaced when the syringe started to get blocked by filtered substances; it was changed approximately every two subsamples.

To obtain background (pre-incubation) levels of nitrogen, nine 8 L Ziploc bags were labelled and filled with seawater (of various volumes), which was filtered through a manifold with a 0.7 µm glass fibre filter. From an 8 L Ziploc bag, we took one 40 mL subsample of pre-excretion water and placed it into a 250 mL darkened bottle, repeating the above syringe and bottle cleansing procedures. This process was repeated for the other eight samples.

To obtain samples of sea cucumber excretion, we placed the seven sea cucumbers collected into the 8 L Ziploc bags filled with filtered seawater, as described above. We placed the sealed bags, seven of which contained the sea cucumbers and the other two had filtered seawater (controls, without a sea cucumber), into the flow-through holding troughs. After the incubation period of at least 30 minutes, we subsampled the seawater in all bags by the procedure described earlier. One 40 mL post-incubation subsample was collected from the seven bags containing sea cucumbers (which remained in the bag during sampling) and from the first control bag. We then took seven 40 mL subsamples from the second control bag to later be spiked with nitrogen to produce a standard curve for fluorometry.

Following post-incubation subsampling, each sea cucumber was removed from its holding bag and held vertically, out of the water, for at least one minute to encourage drainage of body cavity water. We collected this water and obtained two water samples from the two sea cucumbers that expelled a large amount of water. Sea cucumbers were then weighed on an electronic balance (to the nearest 2 g). There was probably weighing error as five of the seven sea cucumbers did not properly drain when held.

After the sea cucumbers were removed from the post-incubation 8 L Ziploc bags, the volume of seawater in the nine bags was measured in a large graduated cylinder. These volumes were used later in nitrogen concentration calculations.

In the laboratory darkroom, we estimated nitrogen levels with fluorometry, using the seven 40 mL seawater subsamples taken from the second control bag. To create a standard curve, six of the control bottles were spiked with 100, 300, 400, 500, 800, and 1200 µmol/L of NH_4^+, respectively. Post-incubation subsamples from the seven sea cucumbers and the two samples of sea cucumber internal water had nitrogen levels that were too high for the fluorometer to read. From these bottles, samples were taken and diluted using the pre-incubation first control bottle. Dilution was done in 1:4 ratios, using 500 µL of the high nitrogen level samples to 2000 µmol/L of the pre-incubation first control sample. Fluorescence values from diluted samples were multiplied by five to adjust for the dilution.

All sample bottles (near/far/internal and pre/post incubation samples) were shaken and then subsampled using an adjustable pipette and expelled into a mini cuvette to be used in a fluorometer. This occurred three times for each bottle and the mean fluorescence value of the three subsamples was calculated.

We accounted for the different volumes of each of the nine 8 L post-incubation bags by multiplying the concentration of nitrogen by the volume of water in the bag. This gave us the correct micromoles of nitrogen for each post-incubation sample. We also accounted for the differences in incubation times by expressing excretion rates of the seven sea cucumbers as µmol/h.

To allow conversion of sea cucumber fluorescence values to excretions rates, we created a standard curve by constructing a linear model relating the mean fluorescence values and respectively known nitrogen concentrations from the seven (second) control bottles. We used the regression equation of the standard curve to calculate ammonium concentrations of the sea cucumber samples.

Analysis of ammonium concentrations within, near, and far from the sea cucumbers was carried out in R (version 3.3.1). This was done using two linear models, one including distance while the other model was null (with no distance), which were compared with a log-likelihood test. Ammonium concentration was the response variable, distance (near and far) the fixed effect, and our sea cucumbers were used as a random variable. We obtained only two internal samples, which precluded formal analysis; however, we plotted their mean and 95% confidence interval to allow comparison with the near and far samples.

The association between excretion rate and mass was examined using a linear model with log excretion rate against log mass. Data points were plotted using a scatterplot with a best fit line.

3. Results

3.1. Effect of distance on nutrient levels

Distance from sea cucumbers did not alter ambient ammonium concentration (log-likelihood test, $p = 0.13$; Fig. 1).

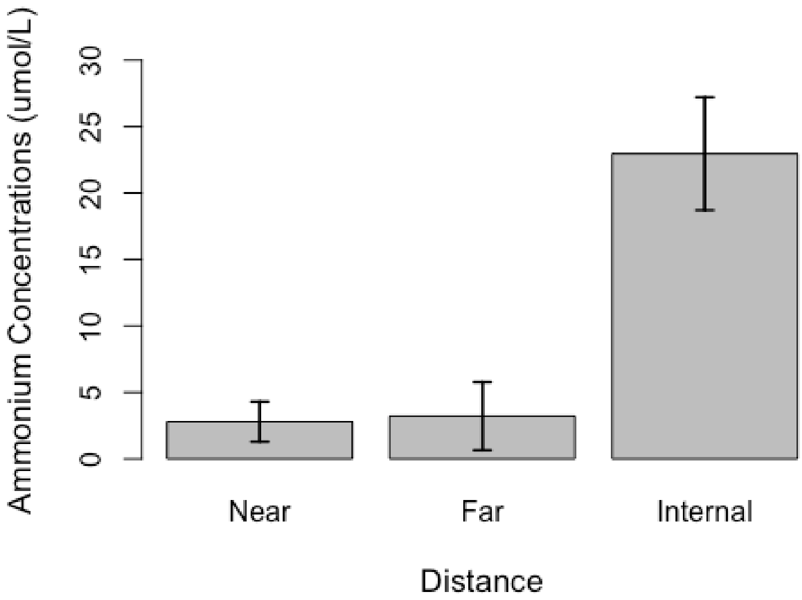

Figure 1: *Average ammonium excretion concentrations taken at three distances (internal, near (3-5 cm), and far (1 m)) from giant California sea cucumbers at Reed Point Marina. Bars are means with 95% confidence interval error bars. Near and far distances had a sample size of N = 7 sea cucumbers each, and internal was N = 2.*

3.2. Excretion rate in relation to sea cucumber mass

There was a strong positive correlation between ammonium excretion rate and sea cucumber mass; larger sea cucumbers had a greater ammonium excretion rate compared to sea cucumbers of lower mass ($p = 0.005$, $R^2 = 0.79$, $N = 7$; Fig. 2). Rounded, untransformed excretion rates (µmol/h) from smallest to largest sea cucumbers: 12, 7, 22, 30, 27, 41, 39.

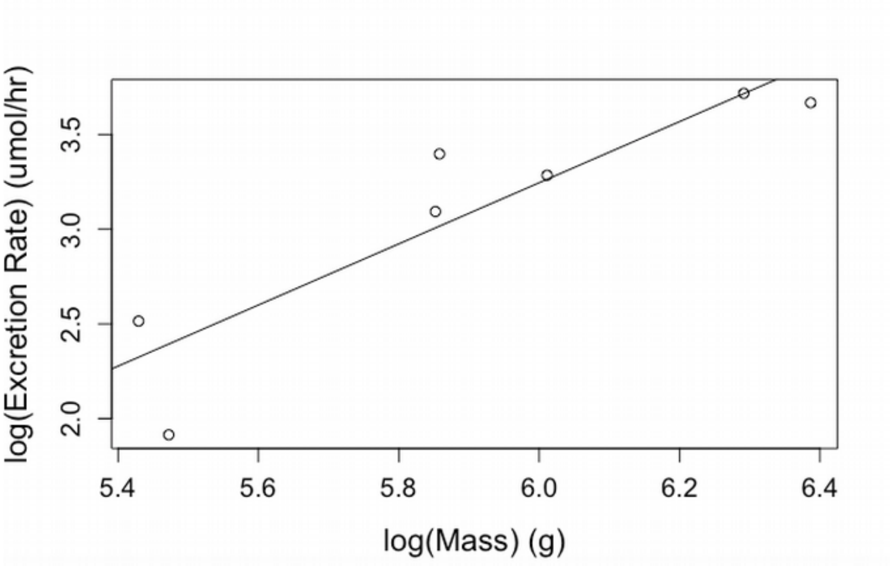

Figure 2: *Ammonium excretion rates of giant California sea cucumbers in relation to their mass at Reed Point Marina. Data points are single animal measurements ($N = 7$), represented on a log-log plot with a best fit line.* $Excretion = 0.492(Mass) + 2.310$.

4. Discussion

We used giant California sea cucumbers to further understand animal-mediated nitrogen contributions in temperate waters by holothurians. We observed no difference in ammonium levels near (3-5 cm) and far (1 m) from sea cucumbers at Reed Point Marina, but ammonium levels in internal samples were noticeably higher. This means that distance (outside of the sea cucumber) is not a factor in determining ammonium levels in our samples. We also found that larger sea cucumbers excrete more ammonium than smaller sea cucumbers. With further research, it would be interesting to determine if the extensive removal of holothurians by over-fishing could still have an impact on nitrogen recycling in temperate waters and thus cause a detectable change in primary producer productivity.

Distance is not a factor in determining ambient nitrogen levels as high nitrogen levels within sea cucumbers are immediately diluted once excreted. This was shown in our near and far samples as there was no significant change in ammonium concentration. We need to collect samples farther than 1 m away from sea cucumbers to ensure that ammonium concentrations do not, in fact, decrease past that point. However, our results contrast with the effect of distance as a factor altering ammonium concentrations in the tropics. In warm water, holothurian excretion increases water nitrogen levels directly behind the sea cucumbers [10]. Water expelled by one tropical sea cucumber can increase ammonium concentration for a short time over an area of approximately 0.2 m^2 per hour [10]. There is no comparable study of the impact that sea cucumber-mediated nitrogen contributions might have in richer temperate waters.

As predicted, larger sea cucumbers excrete more ammonium than smaller sea cucumbers. This conclusion is consistent with the results found for two other species located in the tropics, *Holothuria atra* and *Stichopus chloronotus* [10]. However, we did not investigate the intraspecific variability of per-gram excretion rates. For example, the per-gram excretion rates of smaller freshwater fish can be less than, equal to, or higher than those of larger individuals in the same species, demonstrating that both body size and taxonomy control excretion rates [8]. Whether the same variability exists within temperate sea cucumber species is unknown.

Mass is not the only factor that determines nitrogen excretion rates of sea cucumbers. Both the species of sea cucumber and temperature changes throughout the year contribute to differences in ammonium excretion rates. Ammonium excretion rates differed between two species of tropical sea cucumber, *H. atra* and *S. chloronotus*, meaning that different sea cucumber species can have different excretion rates [10]. In addition, metabolism is altered by temperature, which in turn affects ammonium excretion rates, meaning that excretion rates are higher in the summer than in the winter [10, 8].

It appears that temperate sea cucumbers do not excrete enough nitrogen to make a detectable increase in primary producer production. In general, the effects of animal-mediated nitrogen contributions on primary producer productivity differs between tropical and temperate regions due to differences in ambient light and nutrient availability [3]. In the tropics, holothurians released 0.52 to 5.35 mg m^{-2} day^{-1}, which is the same order of magnitude as the nitrogen fixation rate of cyanobacteria (0.5 to 5.6 mg N m^{-2} day^{-1}) [10]. This creates a short-term nutrient enhancement in the surrounding water and sediment, which then increases benthic microalgae productivity [10]. This shows that nitrogen released by holothurian excretion in tropical waters is a significant process for nutrient recycling. Given that temperate waters have higher nutrient levels than the tropics [2, 7], contributions of animal-mediated nitrogen should be relatively less important in the former than in the latter. Our results suggest that individual giant California sea cucumbers probably make a negligible contribution to the surrounding nitrogen levels, and thus might not affect nearby primary producer productivity to a great extent. However, holothurians are locally abundant in northeast Pacific waters, so the sum of all sea cucumber excretions might have an impact on the overall nitrogen levels in the ocean. More data is needed to test this idea.

There are many studies on the cascading effects of removing consumers, i.e. top-down effects [13]. However, research is more limited on the bottom-up effects, i.e. nutrient availability, on community organization [13]. Based on findings by Uthicke [10], we know that sea cucumber excretion in tropical waters is a significant process for nutrient recycling. Eliminating nitrogen-releasing species could create a detrimental effect on marine ecosystems, as nitrogen fixation and decay alone may not be able to supply enough available nitrogen to support primary producer survival and growth. However, the removal of numerous temperate sea cucumbers through over-fishing may only make a minor difference on primary producer survival as our results suggest that individual giant California sea cucumbers probably make a negligible contribution to the surrounding nutrient levels.

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