**Spatial dynamics of animal-mediated nutrients** **in temperate waters**

Em G Lim1, Claire M Attridge1, Jasmin M Schuster2,3, Kieran D Cox1, Kiara R Kattler1, Emily J Leedham1, Bridget Maher2, Andrew L Bickell2, Francis Juanes2, Isabelle M Côté1

1Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

2Department of Biology, University of Victoria, Victoria, British Columbia, Canada

3Hakai Institute, Campbell River, British Columbia, Canada

**Abstract**

Consumer-mediated nutrient cycling (CNC), which is the fertilization of primary producers by animals’ metabolic waste products, is known to drive variability in nutrient availability and thus primary productivity and community functioning in tropical waters. Yet, CNC may be an unappreciated contributor of variability in temperate systems. Therefore, we aimed to quantify and explain spatial variation in CNC by surveying fish and macroinvertebrates via Reef Life Survey methods and measuring their ammonium excretions at 27 rocky reefs (3 years) and 17 kelp forests of varying density (1 year) in Barkley Sound, British Columbia. Ammonium concentrations varied from 0.009 to 2.5 uM across rocky reefs, and the relationship between animal biomass and ammonium varied with tidal exchange–weakly positive at slack and ebb tides, but weakly negative at flood tide. Ammonium was significantly higher within than near kelp forests, a difference that increased with kelp forest biomass. Ammonium variation between kelp forests was described by complex interactions between animal biomass, kelp density, and tide exchange. We also explored fine-scale ammonium variability and found nutrient enrichment on a scale of meters was possible, but only under low flow conditions. Our results suggest CNC-driven variability acts on scales ranging from a few meters to entire reefs, contributing to finer-scale variability in nutrient availability than allochthonous nutrient sources such as upwelling. CNC may therefore play a larger role than assumed in structuring temperate marine ecosystems from the bottom up.

**Introduction**

1. Nutrients + variability in nutrient availability are super important for pp. (stay broad here, terrestrial, aquatic, and marine)
   1. Nutrient availability is a big bottom-up limiter of pp
   2. Productivity structures ecosystems from the bottom-up
   3. Variability in when + where nutrients are available has impacts on pp and ecosystems as a whole
   4. This happens on both large and small scales
   5. Therefore, understanding drivers of nutrient variability is important!
2. Sources of temporal and spatial nutrient variability in the ocean
   1. Availability of nutrients varies in space and time
   2. Availability of nutrients varies on large and small scales
   3. Abiotic/external nutrient source examples:
      1. upwelling, run-off, atmospheric deposition.
         1. All vary in space + time
   4. Biotic/internal/regenerative examples?
      1. Nitrogen fixation + regeneration by microbes
         1. Seasonal differences in rates?
      2. Animal excretion + egestion
         1. Timing (migrations), spatial (animals concentrate on some reefs and not others)
   5. Talk about abiotic/biotic and temporal/spatial and large/small?
3. Marine tropical vs temperate paragraph?
   1. Tropical areas receive little external input so small-scale variability of when and where animals are found (eg patch reefs) contribute to a lot of variability in nutrient availability
   2. External sources assumed to dominate variability in temperate regions (eg. upwelling, run-off)
      1. Also high wave action and currents thought to limit small scale variation?
   3. But maybe animals are contributing to variability in temperate places too?
      1. Could mention that ammonium is preferred over nitrates here?
      2. Cite Pfister mussel paper and the Aquilino paper
4. Set up what we’re doing?
   1. Intro Barkley Sound region?
   2. Intro into kelp forests and rocky reefs?
   3. Set up the experiment/hypothesis/questions

**Methods**

*RLS large scale*

To explore the large-scale variability of NH4+ on rocky reefs, we collected subtidal NH4+ samples paired with fish and invertebrate surveys using a globally standardized method (Reef Life Survey) at 27 subtidal sites in Barkley Sound, BC each year for three years (Table 1). A full explanation of the Reef Life Survey method are available online (http://www.reeflifesurvey.com/methods) and provided by Edgar and Stuart-Smith, (2009 and Edgar et al., (2020). At each rocky reef site, a pair of trained SCUBA divers assessed fish and invertebrate abundance and diversity along each side of a 50 m transect. First, fishes were counted and sized within 5 meters of the transect on either side, and then cryptic fishes (also sized) and large mobile invertebrates (> 2.5 cm) were counted within 2.5 meters on either side of the transect.

Immediately following the RLS survey, we collected three 60 mL subtidal seawater samples along the transect (at 0, 25, and 50 m) at consistent depths and stored the syringes in sealed plastic bags upon collection to prevent contamination. Seawater samples were filtered into amber bottles in the field and frozen for a maximum of two weeks (???) before NH4+ analysis. We confirmed that freezing samples for this duration did not affect NH4+ concentration. The first two years, we followed the fluorometric method using 40 mL seawater samples (Holmes et al., 1999), and in the third year we followed the fluorometric standard-additions protocol II (Taylor et al., 2007). For each survey, we took the average of the three NH4+ samples as the mean NH4+ concentration. Detailed ammonium analysis methodology is available in the supplement (???).

*Kelp pee medium-scale*

To explore within site variability, we explored NH4+ variability in and around kelp forests of varying density and composition at 17 sites in Barkley Sound, BC (Table 2). Our sites comprised giant kelp only, bull kelp only, and mixed forests of varying densities as well as a bare site as a no kelp control. First, to quantify the abundance and biodiversity of the kelp forest communities, trained SCUBA divers counted and identified fish and invertebrate along 50 m transects placed immediately outside kelp forests following standardized Reef Life Survey protocols as above. Next, we ran four 5 m long transects 5 m apart into the kelp forest to assess kelp density, canopy height, and biomass (Figure 2). We counted the number of canopy kelp individuals (bull or giant kelp) within 0.5 m of either side of the 5 m kelp transect to measure kelp density. To estimate canopy height, we measured the length of five random kelps per kelp transect; for bull kelp we measured the total length from holdfast to pneumatocyst *in situ*, but for giant kelp we collected five random individuals to measure from holdfast to apical meristem??? on dry land. To quantify biomass, we measured the sub-pneumatocyst circumference of the same five bull kelps per transect *in situ* in order to calculate individual biomass using X FORMULA. For giant kelp, we weighed the same five individuals per transect which were collected for total length measurements using X scale. We multiplied the mean biomass estimate for each kelp species by the species density in order to calculate a biomass/m2 estimate for each kelp transect, which we averaged over the four transects per forest to estimate overall mean forest biomass. We estimated total forest area by swimming around the perimeter of the forest on the surface with a Garmin GPS.

Finally, to compare NH₄⁺ concentration inside vs outside the kelp forest, we collected paired 60 mL syringes of seawater immediately outside the kelp forest within 0 – 2 meters from the substrate, and 5 m into the kelp forest (n = 3). These paired seawater samples were matched to the first three kelp density, biomass, and canopy height transects. We attempted to maintain a consistent depth for all three paired collections, and to fill a whirlpak of seawater from outside the kelp forest. Upon surfacing, we filtered 40 mL of each sample into amber bottles and also filled 8 amber bottles for use as standards with 40 mL of filtered seawater from the whirlpak. We stored all samples on ice for transportation back to the laboratory, at which point we measured NH4+ concentration in each sample bottle following the fluorometric standard-additions protocol II (Taylor et al., 2007). For each paired inside vs outside NH₄⁺ sample, we calculated ∆ NH₄⁺ = inside NH₄⁺ - outside NH₄⁺.

Biological and abiotic variable calculations for both large- and medium-scale experiments

For each Reef Life Survey, we calculated fish biomass from fish length following the formula (W = exp(log(a) + b\*log(L))) where W is fish weight, L is the fish length, a and b are species specific constants from FishBase (Froese et al., 2014). For invertebrates, only economically important species (abalone and scallops) and sunflower stars were sized, so we were only able to calculate weights for those species. For all others, we used published weight estimates from this region to estimate rough average weights for each taxon (Table 3). Animal abundance was calculated as simply the total number of fishes and invertebrates counted on the surveys. We calculated Shannon diversity, simpson diversity and species richness for each survey. We calculated the tide exchange by computing the rate of change of the tide height every minute, starting from the time each survey started and ending one hour later, and taking the average of those values. We downloaded tide height data from the website: <http://tbone.biol.sc.edu/tide/tideshow.cgi?site=Bamfield%2C+British+Columbia>. In order to define ebb, slack, and flood tide, we considered the rate of exchange over all six survey weeks (two weeks per year, three years) and <<< I did something with means and SD and centering and ended up with: ebb < - 0.1897325 < slack < 0.1897325 < flood. Come back to this >>>>.

*Cage experiments small-scale*

To assess the potential for animals to contribute to small-scale nutrient variability, we conducted two caging experiments *in situ* to measure animals’ effect on the NH4+ concentration in their immediate surroundings. Both experiments were conducted near Bamfield, BC; the first caging experiment took place at Scott’s Bay (48°50'05.2"N 125°08'49.3"W) using California sea cucumbers (*Apostichopus californicus*). We caged sea cucumbers in densities of 0, 1, or 2 individuals per X x X x X m mesh cage, which were spaced 3 m apart along two weighted lines (9 cages per line, n = 6, N = 18). We collected sea cucumbers from the site via SCUBA, measured contracted sea cucumber length and girth, and immediately placed them into the cages. Cage depth ranged from 3 – 5.8 meters, and sea cucumbers were left in the cages for 24 hours before we returned to collect water samples from each cage via SCUBA. We opened the mesh lids, which were secured with wire, just wide enough to collect a 60 mL syringe of seawater. Once at the surface, we filtered 40 mL of each sample into amber bottles and transported them on ice to the lab, where we measured NH4+ using the fluorometry (Holmes et al., 1999).

The second caging experiment took place in Bamfield inlet (48°49'53"N 125°08'11"W) using red rock crabs (*Cancer productus*). Our 12 cages contained either one large crab (carapace 15.9 – 15.0 cm), one medium size crab (14.4 – 11.6 cm), or a small control rock, scraped clean, to weight the cages similarly to the crabs (n = 4). We collected the crabs from the site using crab traps, and they were kept in flow through conditions in the lab for 2 – 10 days. Crabs were fed salmon every 2 - 4 days, and all crabs were fed the night before the experiment started. We constructed the cages from clear plastic X x X x X cm enclosures, with two X x X cm windows covered in a dual layer of X mm plastic mesh and X m mesh to allow for water flow. The cages were randomly distributed every 2 m along a lead line anchored with cement buckets 0.8 m below chart datum, which allowed samples to be collected by snorkel. We measured seawater ammonium concentration at the beginning, middle, and end of the nine-day experiment by drawing water samples using a 60 mL syringe and a narrow tube attached to the center of the cage. We swapped We filtered 40 mL of each sample into amber bottles which were stored on ice, before ammonium analysis via fluorometric standard-additions protocol II (Taylor et al., 2007). We ran the experiment from June 10 – 19, 2023 and replicated it a second time from June 19 – 28, 2023 following the same methodology. During both experiments, we replaced the crabs after 4 days with freshly fed, similar-sized crabs, at which point we re-randomized the order of the cages along the line. Mention that we weighed each crab? Mention that each cage had ulva??

*Statistical analyses*

All statistical analysis were conducted in R (v4.1.2, R Core Team, 2019) using RStudio (v1.3.1093, RStudio Team, 2016). We used tidyverse packages for data manipulation and visualization (Wickham et al., 2019), ‘vegan’ to calculate biodiversity indices (Oksanen et al., 2022), ‘glmmTMB’ for all modelling (Brooks et al., 2017), and DHARMa to check model fit (residuals?) (Hartig, 2022). All data and code are available at https://github.com/em-lim13/Ch2\_Spatial\_pee.

RLS large-scale

To determine whether there is significant variation in NH₄⁺ amoung sites, we constructed generalized linear mixed effect models with NH₄⁺ as the response variable, and animal abundance, tide exchange, an interaction between abundance:tide, Shannon diversity, and survey depth included as predictors, and a random effect of both site (1|site) and year (1|year). All predictors were centered around the mean using the scale function, with scale = FALSE. We used a gamma distribution (link = ‘log’). We constructed additional models with animal biomass instead of abundance, simpson’s diversity or species richness instead of Shannon diversity, but we determined abundance and Shannon diversity were the best metrics by comparing alternate models using AIC (report AIC values). We checked for collinearity of variables using car::vif, and all values were below 1.75. We visually inspected model residuals by plotting the output from DHARMa::simulateResiduals, and the model met all assumptions.

Kelp pee medium-scale

To determine whether NH₄⁺ concentration varies inside vs outside of kelp forests, we used a linear mixed effects model with ∆ NH₄⁺ (inside NH₄⁺ - outside NH₄⁺) as the response variable (n = 3 per site), and kelp species, mean forest kelp biomass, tide exchange, animal biomass, survey depth, Shannon diversity, and interactions between kelp biomass:tide exchange, kelp biomass:animal biomass, and animal biomass:tide exchange as fixed effects. All continuous predictors were centered around the mean using the scale function, with scale = FALSE. We included site as a random effect (1|site) to account for the fact that each site contributed three paired samples to the analysis and used a gaussian distribution. We inspected residuals and checked for collinearity as above, and the model met all assumptions. Should I mention models with alt variables?

Cage experiments small-scale

To quantify the effect of each caged animal on its surrounding NH₄⁺ concentration, we constructed separate linear models for each caging experiment. For the sea cucumber cage experiment, we regressed cage NH₄⁺ concentration against the treatment (0, 1, or 2 sea cucumbers) and cage depth (centered). Inspection of the residuals revealed no significant problems. For the red rock crab cage experiment, we constructed a generalized linear mixed-effects model with cage NH₄⁺ concentration as the response variable, treatment (no crab, medium crab, or large crab) as the predictor variable, and a random effect of sampling day. We used a gamma distribution (link = ‘log’) to ensure model residuals met all assumptions.

**Table 1.** List of rocky reef sites sampled using Reef Life Survey methods, and the years each site was surveyed.

|  |  |  |  |
| --- | --- | --- | --- |
| **Site code** | **Site name** | **Coordinates** | **Years sampled** |
| BMSC1 | Dodger Channel | 48.82894897, -125.1975708 | 2021, 2022, 2023 |
| BMSC2 | Kirby | 48.85039902, -125.1987686 | 2021, 2023 |
| BMSC3 | Ohiat | 48.85558319, -125.1837997 | 2021, 2022, 2023 |
| BMSC4 | Kii xin | 48.81511688, -125.1753311 | 2021, 2023 |
| BMSC5 | Taylor Rock | 48.82733154, -125.1966019 | 2021, 2022, 2023 |
| BMSC6 | Baeria Rocks South Island | 48.95023346, -125.1555481 | 2021, 2022, 2023 |
| BMSC7 | Baeria Rocks North Island Southside | 48.95464325, -125.1539917 | 2021 |
| BMSC8 | Baeria Rocks North Island Northside | 48.95508194, -125.1533737 | 2021, 2022, 2023 |
| BMSC9 | Eagle Bay | 48.83478928, -125.1470261 | 2021, 2022, 2023 |
| BMSC10 | Ross Islets Slug Island | 48.87051773, -125.160347 | 2021, 2022, 2023 |
| BMSC11 | Wizard Island South | 48.85746765, -125.1582336 | 2021, 2022, 2023 |
| BMSC12 | Wizard Island North | 48.858284, -125.1609192 | 2021, 2022, 2023 |
| BMSC13 | Effingham West | 48.8650322, -125.3137207 | 2021, 2022 |
| BMSC14 | Effingham Archipelago | 48.87908173, -125.2974014 | 2021, 2022 |
| BMSC15 | Raymond Kelp Rock | 48.88028336, -125.3128815 | 2021, 2022 |
| BMSC16 | Faber Islets | 48.89070129, -125.300499 | 2021, 2022 |
| BMSC17 | Wouwer Channel | 48.86548233, -125.3614807 | 2021, 2022 |
| BMSC18 | Eussen Rock | 48.91161728, -125.2670364 | 2021, 2022 |
| BMSC19 | Ed King SW Pyramid | 48.82860184, -125.2212982 | 2021, 2022, 2023 |
| BMSC20 | Ed King East | 48.83566666, -125.214798 | 2021, 2022, 2023 |
| BMSC21 | Dixon SW | 48.85205078, -125.1235657 | 2021, 2022, 2023 |
| BMSC22 | Dixon Inside | 48.85426712, -125.1170349 | 2021, 2022, 2023 |
| BMSC23 | Aguilar Point | 48.837589, -125.144145 | 2022, 2023 |
| BMSC24 | Swiss Boy | 48.916073, -125.131174 | 2023 |
| BMSC25 | Goby Town | 48.838595, -125.135015 | 2023 |
| BMSC26 | Hosie South | 48.9071, -125.037017 | 2023 |
| BMSC27 | San Jose North Island | 48.901183, -125.060433 | 2023 |

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