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TÍTULO PROYECTO: MARINE AMMONIFICATION: LINKING QUALITY TO QUANTITY IN NITROGEN REGENERATION
DISCIPLINA PRINCIPAL: OCEANOGRAFIA
GRUPO DE ESTUDIO: CS. DE LA TIERRA
INVESTIGADOR(A) RESPONSABLE: CAMILA DEL PILAR FERNANDEZ IBANEZ
DIRECCIÓN:
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FONDO NACIONAL DE DESARROLLO CIENTIFICO Y TECNOLOGICO (FONDECYT)
Moneda 1375, Santiago de Chile - casilla 297-V, Santiago 21
Telefono: 2435 4350 FAX 2365 4435
Email: informes.fondecyt@conicyt.cl
## MODIFICACIONES ACADÉMICAS

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### Otros Aspectos Importantes a Considerar

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PROJECT RESULTS:

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Within the nitrogen cycle, ammonium production has a pivotal role in fueling primary production and introducing nutrients in oceanic surface waters mainly via nitrogen regeneration. This project aimed at assessing ammonification as a process linking biogeochemical N cycling and microbial diversity and community structure. The general objective was to assess the abiotic and biological ammonification of dissolved organic matter in marine waters as a function of its origin and to evaluate its effect on the structure and activity of ammonium oxidizing communities.

We proposed 4 specific objectives that were tackled with complementary methodologies. The main results for each objective are listed below.

**Specific Objective 1.** - To evaluate the production rates of \( \text{NH}_4^+ \) via biotic and abiotic ammonification of dissolved organic matter from marine origin and representing different trophic stages of primary production (diatom vs diazotrophic dominance).

This objective was tackled using 3 complementary approaches: 1) We generated the first time series data set of incident PAR and UV radiation and nitrate regeneration fluxes off central Chile (36\(^\circ\)S) in order to perform controlled experiments using diatom cultures and natural DOM. This resulted in a published paper by a Master student \(^1\). 2) A cDOM study was conducted in variable productivity conditions and is the subject of a manuscript currently in preparation. 3) Cultures of phytoplankton were used to generate 15N-labelled DOM for future experiments. One MSc, a PhD thesis, two publications and one ms in preparation are reported.

Photoammonification experiments carried out with exudates of representative diatom species (*Chaetoceros muelleri* and *Thalassiosira minuscule*) allowed estimating the time scale of ammonium photoproduction, which can occur within 2 to 4 h of exposure \(^1\). The exudates of both strains showed ammonium production after exposure to PAR+UV as well as UVR in non-poisoned (abiotic + biotic ammonification) as well as poisoned samples (abiotic ammonification only). Final ammonium concentrations were always higher in non-poisoned compared to poisoned samples and resulted in higher rates of ammonium production probably by the combination of photo and biological ammonification. Rates in exposed samples were also higher than in dark controls evidencing the importance of photoammonification compared to dark DOM biodegradation. Abiotic ammonification in poisoned samples reached up to 1.5 \( \mu \text{mol L} \text{ h}^{-1} \) while biological ammonification is estimated at maximum values of 0.71 \( \mu \text{mol L} \text{ h}^{-1} \).

The production of ammonium from DOM coming from natural marine samples showed a coupling between bacterioplankton abundance and increasing \( \text{NO}_3^- \) and \( \text{NO}_2^- \) concentrations in sunlight-exposed treatments that was maintained during the dark recovery incubation. This indicates active ammonium oxidation in spite of light exposure. Consequently, low abiotic ammonification in our natural samples might be explained by, among other factors, active microbial \( \text{NH}_4^+ \) utilization (specifically nitrification) during the incubation that resisted solar radiation exposure\(^1\).

The response within the nitrate producing community to solar radiation and abiotic ammonium production was approached through a high frequency multidisciplinary approach the seasonal and single-diurnal variability of potentially active members of the prokaryote community was described at high frequency time scales in surface waters, with special attention to the relationship between nitrifiers and abiotic codtions for ammonification. Experiments combining 16S cDNA-based pyrosequencing, RT-qPCR of specific gene markers for nitrifiers (*amoA*, for general AOA, AOA-A, AOA-B, *Nitrosopumilus maritimus* and beta-AOB; and 16S rRNA gene for Nitrospina-like NOB), and solar irradiance measurements allowed describing the succession among archaea and bacteria ammonium oxidizers and nitrite oxidizers. Results showed a high variability in transcriptional levels during the dark which could be associated with sunlight intensity thresholds in winter although AOA and Nitrospina-like NOB transcript number were also potentially related to environmental substrate availability. Only *N. maritimus amoA* transcripts showed a significant negative correlation with solar irradiances in both periods suggesting sensitivity to sunlight.

Looking at contrasting seasons, the contribution of the number of *N. maritimus* (AOA) and Cenarchaeales (AEA) sequence reads to pyrolibraries in surface waters seemed to decrease with increasing solar radiation although slight differences between both groups were observed. AOA sequence reads showed a slight decrease in Austral winter compared to summer at high visible light intensities between 14:00 and 16:00 h (\( \geq 200 \text{ W m}^{-2} \)), whereas AEA sequence reads showed a clear decrease at the same time of day for both seasons. Samples collected at 5 m depth in summer
(February 2012) and winter conditions, were exposed for 4 h to artificial light (Visible and UVA+ visible). After exposure, the transcription of specific gene markers for nitrifiers (AOA-A, *N. maritimus*, beta-AOB, and *Nitrospina*) was normalized to the *in situ* transcription of the gene markers at the moment of sample collection, and then compared with transcript levels in the dark controls (Fig. 1). The ratios lower than 1 were considered as potential inhibition. A differential response to light spectra was observed between samples collected in summer versus winter. While in winter beta-AOB were inhibited by both spectra, AOA-A, *N. maritimus* and *Nitrospina* showed a potential stimulation (Fig. 1). In summer, beta-AOB, *N. maritimus*, and *Nitrospina* showed weak inhibitory effects of UVA while visible light alone did not have an evident effect. In addition, AOA-A was only slightly reduced compared with dark controls.

Figure 1: Effect of artificial light (PAR, open symbols; UVA-PAR, filled symbols) on the normalized transcription of specific gene markers for nitrifiers (*amoA* and 16S rRNA genes) collected at 5 m depth in representative months for upwelling-favorable and upwelling-unfavorable conditions. Gene transcription in the irradiated treatments and dark controls were normalized by the *in situ* gene transcription at the moment of sampling. Dashed line indicates a perfect linear relationship between normalized gene transcription from artificial light treatments and dark controls. For clarity, data coming from upwelling-favorable condition are highlighted by circles.

These findings support the idea that sunlight triggers a variable response among resident groups within the nitrifying community. An emerging hypothesis is that sunlight can determine the potential associations between specific archaeal and bacterial nitrifying ecotypes in shallow coastal waters. According to this, associations between *Nitrospina* and AOA-A could mostly occur in austral spring-summer while lower solar irradiances found in austral wintertime could enhance associations between *N. maritimus* and beta-AOB.

Within our approach to this objective, we developed isotopic enrichment techniques to phytoplankton cultures to obtain $^{15}$N-enriched DOM. This was done in collaboration with Dr Marcela Cornejo (Universidad Catolica de Valparaiso). A culture of *Nannochloris* sp. was held in f/2 culture media. An aliquot of 50 mL from the original culture was used to obtain images (Cytation 5) for its counting and identification. The phytoplankton assimilation of isotopic nitrogen was detected in the second day of the experiment, with enrichment values five orders of magnitude higher than initial values. These promising results show that the phytoplankton cultures assimilate the $^{15}$N-NO$_3^-$ offered in the culture at levels that allows generating a possible isotopic signal to be traced at higher trophic levels.
In order to better appreciate the role of photoammonification on biogeochemical cycles, we also assessed one of the most emblematic sources of DOM, chromophoric dissolved organic matter (cDOM). It is a biologically active component of the DOM, which we studied along a gradient of productivity, since cDOM can influence the optical properties of aquatic environments and therefore plays an important role in the photochemical and photobiological processes occurring in the euphotic layer. A comprehensive sampling was carried out at three locations of central and southern Chile (BioBío river, Puyuhuapi fjord and the Beagle Channel in southern Patagonia). Strong differences in the cDOM concentrations as well as among its optical parameters where found within the three zones. Salinity was identified as the main factor influencing cDOM changes and freshwater was the principal source in Biobío and Puyuhuapi Channel. However, this is not the case of Beagle Channel. Optical properties of dissolved organic matter allow identifying different cDOM pool suggesting that cDOM in Puyuhuapi and Beagle Channel is produced in situ. As DOC and cDOM variability appears to be decoupled at Puyuhuapi fjord and Beagle Channel, this new line of research can have implications for the local recycling of DOM.

**Specific Objective 2.** To explore the utilization of NH$_4^+$ by archaea and bacteria ammonium oxidizers as a function of the ammonification of different qualities of DOM by biotic or abiotic degradation.

This objective was approached by using biogeochemical/microbial techniques while studying natural (mesozooplankton, freshwater and terrestrial) of DOM in coastal marine environments. A PhD theses, two publications and one ms in revision are reported.

Two series of experiments were designed for evaluating the utilization of excreted nitrogen by ammonium oxidizing bacterioplankton. Organic and inorganic nitrogen excretion (as DON or ammonium) was measured, under different feeding diets (micro and nanoplankton; 150– 20 µm and < 20 µm, respectively). Special attention was put in combining a biogeochemical characterization of the excretion of dominant copepod species (*Paracalanus* cf. *indicus* and *Acartia tonsa*) with the description of the activity of the ammonium oxidizing microbial community in response to the addition of excreted products via the quantification of transcriptional gene *amoA* in archaea (*Candidatus Nitrosopomilus maritimus* and AOA-A) and bacteria (AOB). Copepods fed with the smaller fraction of food (<20 µm) and generated a greater accumulation of nitrogen compounds, mainly DON compared to the treatment fed with the 150-20 µm size fraction. In order to elucidate the response of nitrifying communities to newly excreted nitrogen by zooplankton, incubations of natural bacterioplankton were carried out with amendments coming from the excretion of copepods fed with different size fractions. During this step of the experimental design, the potential activity of AOB and AOA were detected by RT-qPCR *amoA* transcripts counts during the experiments. In general, AOA and AOB presented similar trends in their RT-qPCR *amoA* transcripts counts through time. In the treatment spiked with excretion products of copepods fed with 150-20 µm fraction, AOB and AOA showed a notorious activity increase at 4 h coinciding with a reduction of ammonium concentrations. The results (Fig 3) of the treatment spiked with excretion products of copepods fed with < 20µm fraction on the other hand, showed that only bacteria activity increased significantly at the initial time compared to the control, and both AOB and AOA contribution decreased during the rest of the incubation time.
The ongoing work will help answer the question on the direct utilization of DOM by bacterioplankton in the upper water column in diverse conditions already tackled in two previous publications. The experimental design for assessing the excretion of nutrients by mesozooplankton currently includes DOP as an excretion product. As part of an international effort (OUTPACE cruise; https://outpace.mio.univ-amu.fr/), we collaborated with colleagues from MIO (Marseille, France) in a series of experiments focused on the role of organic and inorganic nitrogen and phosphorus released by copepods on a gradient of oligotrophic conditions using three long duration stations located along an ultra to oligotrophy gradient. At each station, microcosm experiments were done on board and combined with active remineralization measurements (via microbial ammonification and nitrification). Molecular analyses showed the relevance of Alteromonadales and SAR11 and its association with the nutrients concentration at each experiment that was modified by copepod excretion therefore suggesting and active role of excretion in microbial community composition

![Figure 3: Excretion of N-based compounds over time off central Chile expressed as percentage of variation over time compared to the control treatment](image)

Specific Objective 3.- To evaluate the input of NH$_4^+$ coming from dissolution and degradation of food pellets and excretion by *Caligus rogercreseyii* in marine waters influenced by salmon farms.

The quantity and composition of uneaten food generated from fish farms depends on a number of factors including the type of feed (moist versus dry), number of fish per cage, the health of the fish (sick fish tend to have reduced appetites), frequency of feeding, type of feeding method (automatic versus hand feeding), and feed conversion ratios. Uneaten food pellets can produce NH$_4^+$ when in contact with the water column and are also susceptible to bacterial degradation as they are enriched in organic matter. We carried out several experiments in central Chile, which showed that pellets dissolution is fast (within 30 min) and can generate up to 10 times the observed ambient ammonium concentrations. Also, when added to a pre-filtered natural seawater sample, increased remineralization of ammonium was detected in natural bacterioplankton communities (Fernandez et al, ms in prep). Consequently, the combination of dissolution and ammonification can substantially impact the N reservoirs of near-cage environments and affect marine microbial communities.

The pesticides used against sea lice infections in salmon farming can also have potential effects on non-target microbiota and on the structure and also on the quality of organic matter. We recently published the first comprehensive study of the response of natural microbial communities to the addition of the anti-lice pesticides azamethiphos, deltamethrin and emamectin benzoate and their potential effect on photoautotrophic and chemoautotrophic carbon fixation in central-southern Chile (37°S to 42°S). The addition of pesticides on primary production samples was related to changes in carbon uptake, which were significant if a single pesticide was applied, mainly emamectin benzoate and azamethiphos. In surface waters of central Chile, emamectin benzoate produced a 60-90% decrease for both photo and chemoautotrophic carbon fixation. Enhanced rates were also observed for in situ primary production as a result of azamethiphos addition in
northern Patagonia. Such stimulation, although limited, was possibly related to the supply of nitrogen and phosphate for phytoplankton requirements by this organophosphate compound (Rain-Franco et al. 2018).

**Objective 4.** To determine the effect of NH$_4^+$ inputs by aquaculture (as food pellet dissolution and *Caligus* N excretion) in local microbial nitrifying communities (functional diversity, cDNA) and functional groups (bacteria and archaea ammonium oxidizers).

This objective tackled the impact of fish N release via labile DOM in salmon cages. An undergraduate student and a ms currently under review are reported. Salmon farming releases significant amounts of organic matter to the water column. A portion of this organic matter would correspond to salmon mucus, which is continuously released, acting as a protective barrier and remaining available in the water column for microbial communities. In order to understand this source of this organic matter the different excreted components must be analyzed and the mucus generated in the salmon cultures is an important part of this excretion. A study was carried out in the Puyuhuapi fjord, where two experiments of 24 hours were carried out at 2m and 100m depth. The objective was to understand the potential effect of mucus on natural bacterial assemblages inhabiting waters surrounding the salmon farming centers and its influence on NH$_4^+$ production and DOM degradation. The results indicate an effect of mucus addition in ammonium concentrations, bacterial abundance and DOC compared with the control experiments in surface waters. Moreover, active bacterial ecological indexes and composition changes were observed based on 16S rRNA sequencing. Evenness (Jaccard index 0.32–0.51) and diversity (Shannon H’ log index 1.67–2.09) changed as a response to mucus inoculum. Bacteroidetes, gammaproteobacteria and α-Proteobacteria also shifted due to the consumption generated by the available ammonium. Changes were however transient, thus salmon mucus could be a labile source of DOM that produce immediate and reversible alterations in microbial diversity at Puyuhuapi fjord (Aguire et al, in prep).

**References**

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OTRAS PUBLICACIONES / PRODUCTOS

Sin información ingresada.

CONGRESOS

Nº : 1
Autor (a)(es/as) : Rain-Franco, A., Fernandez, C.
Título (Idioma original) : First report of optical parameters of CDOM in Chilean Patagonian Fjords: Puyuhuapi channel (~44°S, 72°W)
Nombre del Congreso : XXXVII Congreso Ciencias del mar
País : CHILE
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Nombre y Apellidos del(de la) Alumno(a) : David Aguirre
Nombre y Apellidos del(de la) Tutor(a) : Camila Fernandez
Título Grado : Pregrado
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Título de Tesis : Toxicidad de compuestos antiparasitarios de uso actual en salmonicultura: efectos de benzoato de emamectina y deltametrina sobre la diversidad y viabilidad de comunidades microbianas marinas

Nombre y Apellidos del(de la) Alumno(a) : Claudia Rojas Perez
Nombre y Apellidos del(de la) Tutor(a) : Camila Fernandez
Título Grado : Magister
Institución : Universidad de Concepcion
País : CHILE
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Título de Tesis : The biogeochemical role of zooplankton for phosphorus and nitrogen in the ocean

Nombre y Apellidos del(de la) Alumno(a) : Valentina Valdes
Nombre y Apellidos del(de la) Tutor(a) : Camila Fernandez
Título Grado : Doctorado
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País : CHILE
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