Assessment of N_2 production in anoxic regions of Effingham Inlet, British Columbia.

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Nitrogen Gas Excess In Effingham Inlet

Non-technical summary

Fixed nitrogen is a vital nutrient which is limiting in most of the world's oceans. The dominant source and sink for fixed nitrogen globally are nitrogen fixation and denitrification respectively. In addition to denitrification, several newly discovered metabolic pathways have been discovered to reduce fixed nitrogen to N_2 gas. These processes are anaerobic, occurring only in oxygen free environments, and were determined to be present in the anoxic regions of Effingham Inlet, British Columbia. Within this fjord two sills deter circulation of oxygen rich water from Barkley Sound and subsequently from the Pacific Ocean. In these anoxic basins inorganic N_2 builds up as a result of denitrification. This is in excess of the equilibrium concentration predicted by the solubility characteristics of nitrogen. To quantify this excess, N_2/Ar ratios were measured. Biologically inert Argon gas serves to cancel out physical variables affecting the N_2 gas concentration. This leaves all variation between ratios due solely to metabolic reduction of fixed nitrogen to N_2. It was determined that the greatest concentrations of N_2 excess in Effingham Inlet corresponded to regions of anoxia. This was consistent with the finding that denitrification was a dominant process in these regions.

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Abstract

By assessing N_2 production in Effingham Inlet a general trend of increasing N_2 excess with increasingly anoxic water was discovered. This is due mainly to denitrification which reduces fixed forms of nitrogen to N_2 gas. Denitrification reduces organic nitrogen to molecular nitrogen in an anaerobic respiratory process under strictly anoxic conditions. This study entailed collection of samples and depth profiles from oxic as well as anoxic water columns in the three basins of Effingham Inlet. The primary method of calculating N_2 excess was the analysis of headspace gases equilibrated with the sample water to obtain N_2/Ar ratios. This method corrected for variations due to physical processes in the basins and bypassed assumptions about the N:P ratios of the source nitrogen that are required by other methods. Minimal ammonium concentrations of 0.14 to 0.16 μmol kg^{-1} are consistent with findings of low N_2 excess in the euphotic zone where N_2 was fixed and immediately consumed by organisms. Ammonium concentrations reached a maximum of 36.48 μmol kg^{-1} in the anoxic depths of the innermost basin corresponding to a maximum N_2 excess of 15.42 μmol kg^{-1}. These findings were corroborated by findings of the same order of magnitude in anoxic waters in the Arabian Sea and Black Sea.

Introduction

Nitrogen, in biologically available forms, is a major limiting nutrient in the world's oceans (Codispoti and Christensen 1985) and nitrogen gas is the main component of our atmosphere. Nitrogen dynamics in the biosphere consist of a complex set of biogeochemical processes which dominate the flux of nitrogen in the environment (Hinrichsen & Wulff 1998). Understanding these critical biogeochemical processes is fundamental to understanding any dynamic ecosystem. Nitrogen cycling is a complex system of oxidation reduction reactions which fuel biological productivity. The nitrogen cycle, nitrification specifically, is closely linked to ocean's ability to sequester carbon (McElroy 1983) due to the stoichiometry of nitrification. In much of the ocean oxygen is the preferred oxidant resulting in aerobic respiration; some regions however are oxygen deficient and can become completely anoxic. In these environments reactions must be able to proceed anaerobically and will not undertake these pathways if the energetically conservative oxygen pathway is available. In these distinct regions bacterial metabolic processes reduce fixed forms of nitrogen into inorganic N_2. The most significant N_2 sink is denitrification (Gruber and Saramiento1997, Deutsch 2001) but anaerobic ammonia oxidation (anammox) may also play a role (Thamdrup 2006, Kuypers 2005). N_2 export fuels all other parts of the nitrogen cycle and consequently impacts biological productivity as well as oceanic carbon sequestration.

This project assessed the extent of N_2 production in the anoxic depths of Effingham Inlet, British Columbia. This was accomplished by measuring N_2/Ar ratios and taking the difference between measured and background values to calculate N_2

excess, as done by Devol et al. (2006). There are no additional sources for N_2 in these anoxic regions leading to the assumption that all N_2 excess is a result of denitrification and potentially anammox or other metabolic pathways of nitrogen reduction. This is more accurate than calculating N* (Pers. Com. Bonnie X. Chang), an estimate of N_2 excess based on the Redfield Ratio. N* is unaffected by the biological pump (Deutsch et al. 2001) and makes the potentially flawed assumption that all source nitrogen has a Redfield N:P ratio.

In addition to measurements of N_2 production, the nitrogen gas evolved from the collected samples was analyzed for isotopic composition of molecular nitrogen. When denitrification takes place the lighter, ^{14}N, isotope of nitrogen is preferentially used, this leaves the surrounding water enriched in the heavier isotope. This process is called fractionation (Cline and Kaplan 1975). Mass spectrometer analysis of $^{15}N/^{14}N$ ratios determines of fractionation and can be an indicator of denitrification. Where denitrification is high, larger ratios are expected. Conversely, where denitrification is low, the ratio is expected to be lower. This study will use the more common version of this data, the delta N-15 value, denoted $\delta^{15}N$. The careful analysis of these ratios also provided a rough approximation of how much nitrate, NO_3, was reduced to N_2, and conversely, how much N_2 was produced.

The purpose of this study was to compare various methods of determining N_2 production and where in Effingham Inlet denitrification was most productive. It was determined that the only reasonable method of N_2 production measurements was by N_2/Ar ratios to determine N_2 excess. Interpretation of $\delta^{15}N$ data indicated that denitrification was present in both anoxic regions of Effingham Inlet and that it was not

present in the outermost basin where anoxic conditions are deterred by oxygenated ocean water flushing.

Methods

Study Site—On the western coast of Vancouver Island, Barkley Sound opens to the Pacific Ocean. In the northern reaches of the sound Effingham Inlet is a fjord which extends 17 km and is on average 1 km wide (Patterson et al. 2000). Complete circulation of the water in this fjord is deterred by two sills. Near the mouth of the Inlet the first sill is at a depth of 70 m and separating the middle basin from the inner basin is a sill at a depth of 40 m (Patterson et al. 2000). To characterize this unique fjord, water column profiles of salinity, temperature, oxygen and nutrients as well as water samples were collected at three stations, one in each of the basins, between 19 March 2010 and 22 March 2010. At Station Effingham 1 (Ef 1) samples were collected from 10, 50, 80, 100, and 110 meters. At Station Effingham 2 (Ef 2) samples were collected from 10, 50, 80, 150, 170, and 190 meters with duplicates at 170 meters. At Station Effingham 3

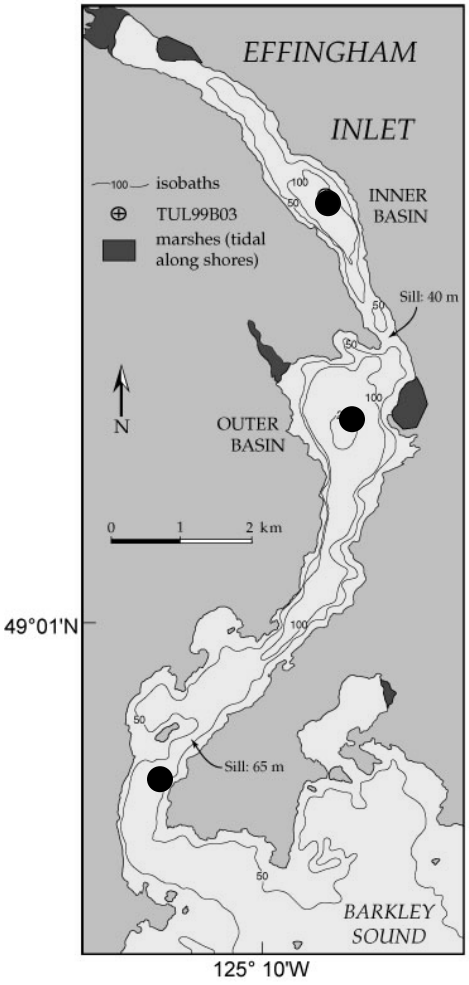

Fig. 1. Station locations indicated by black circles in Effingham Inlet. (Palaeontelogia Electronica 2004:1)

Station 1: 49° 04.27N, 125° 09.38W
Station 2: 49° 02.54N, 125° 09.13W
Station 3: 49° 00.67N, 125° 10.20W

(Ef 3) samples were collected from 10, 50, and 80 meters. Station Ef 1 was the innermost basin with completely anoxic bottom water. Station Ef 2 was the middle basin with anoxic bottom water and Station Ef 3 was the outermost basin with no anoxic conditions.

Nutrients and Hydrographic data—In addition to gas analysis samples, Carpenter-Winkler titrations were used to determine oxygen concentrations according to the method of Carpenter (1965). These concentrations, along with conductivity, temperature, and depth measurements were used to calculate % O_2 saturation of each sample. % O_2 saturation was also determined by isotopic analysis for consistency with nitrogen isotope analysis.

Sampling Method— Samples were collected from Niskin bottles cast by hand and CTD profiles were measured by a Seabird SBE-19 CTD with Beckman YSI O_2 sensor. 5-L Niskin bottles and the CTD were attached to the same handheld line and retrieved with the aid of a small winch. From each Niskin bottle samples for gas analysis were drawn into 185-ml evacuated glass flasks with 9 mm bore Louwers-Hapert single o-ring valves approximately halfway full. During the draw CO_2 was pumped into the neck of the flask to prevent any ambient air from entering the sample. Each bottle was poisoned with 100µL of saturated $HgCl_2$ prior to vacuum evacuation, the purpose of which is to any biological activity once the sample has been collected. The necks were then filled with CO_2 to prevent any contamination through the sealing O-rings. The samples were carefully packed and the necks refilled with CO_2 upon return to the lab to await analysis.

Laboratory Methods—Ratios of N_2/Ar and $\delta^{15}N$-N_2 measurements were made following the method of Emerson et al. (1999). Samples were weighed and then rotated in a constant temperature water bath for 24 hours. Headspace gases reached equilibrium

with the dissolved gases in the water samples during this time. Immediately following equilibration the water phase was vacuum pumped out (>95%), leaving a small cap of water to prevent any leakage of the headspace gas. On a cryogenic vacuum line a known amount of ^{36}Ar spike was added to each sample and the headspace gas was purified with liquid nitrogen traps. The temperature of liquid nitrogen captures $CO_{2(g)}$ and water vapor but leaves the remaining elements in the gas phase. The sample gas was then frozen into an aluminum finger at liquid helium temperature. Once closed to the atmosphere, the frozen samples were allowed four hours to return to a gas phase and were then analyzed on a Finnigan Delta XL isotope ratio mass spectrometer (IRMS). Each sample run on the mass spectrometer was referenced to the in-house standard. Each standard is referenced to air. The ionization efficiency of the IRMS is heavily dependant on O_2 concentration, for this reason the standards were chosen to most closely match the % O_2 saturation calculated for each sample to maintain the lowest slope correction. Each sample was measured for mass ratios 28:40, 29:28, 36:40, and 32:40 (if the oxygenated standard was used). These ratios are of the molecular weights of N_2:^{40}Ar, ^{15}N-N:N_2, ^{36}Ar:^{40}Ar, and O_2:^{40}Ar respectively.

Analytical Methods—Mass spectrometer measurements of 29:28, 28:40 and 36:40 were converted to 28:36 ratios and a background value chosen. The background value for this study site was chosen to be the N_2/Ar ratio from 50 meters depth in Effingham 2 due to its oxic character and therefore lack of denitrification. [N_2] excess was calculated for each sample by:

$$[N_2]_{excess} = (N_2/Ar_{sample} - N_2/Ar_{bkg}) \times [N_2]_{equilibrium}$$

From the mass spectrometer isotope analysis the $\delta^{15}N$ of the samples was calculated. $\delta^{15}N$ is a measure of the isotopic ratio of the sample compared to the isotopic ratio of air, referenced through the specific standard chosen. The mathematical analysis is as follows:

$$\delta^{15}N = [(R_{Sample}/R_{STD}) - 1] \times 1000$$

R_{Sample} and R_{STD} are the Ratios of N^{15}/N^{14} in the sample and standard respectively. This calculation must be done twice, once to reference the sample to the standard and again to reference this new value to air.

For the final mathematical analysis a simple mass balance was set up to calculate predicted average $\delta^{15}N\text{-}NO_3$ in the region.

$$(\mu gATMS\ N_2) \times (\delta^{15}N\text{-}N_2) + (\mu gATMS\ NO_3)(\delta^{15}N\text{-}N_3) = (\mu gATMS\ N_{pool}) \times (\delta^{15}N_{pool})$$

In this calculation, N_{pool} refers to the measured mass and measured $\delta^{15}N$ of the sample which includes all species of nitrogen. Also, µgATMS refers to µg of monatomic N which must be used in place of µg of the diatomic molecule N_2 for even stoichiometric agreement between the various species of nitrogen.

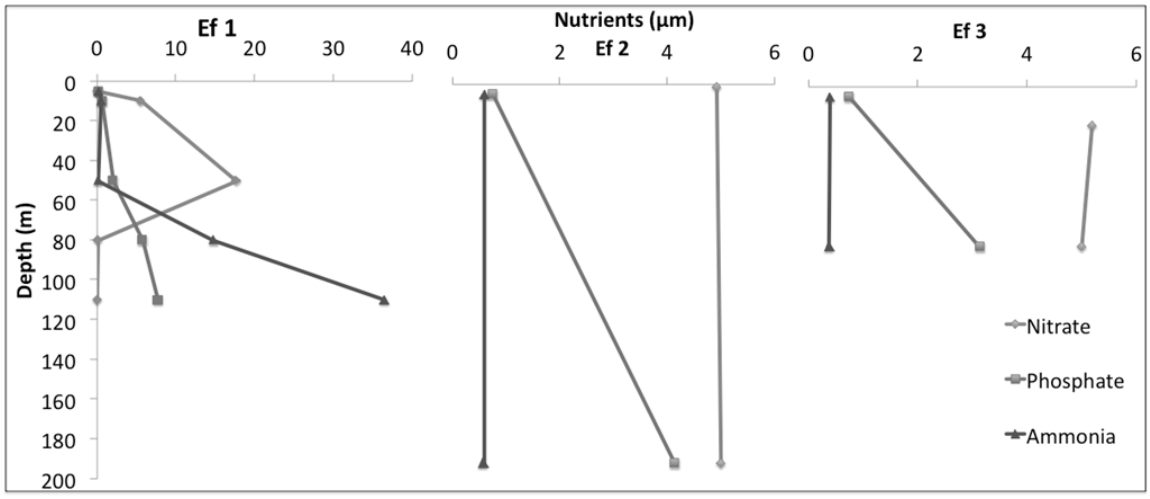

Fig. 2. Nutrient concentration in µmol kg^{-1}. In the anoxic Ef 1 basin the increase of phosphate and ammonium and decrease of nitrate near 50 m indicate nutrient utilization (dominant metabolism) changed at this depth.

Nutrient and hydrographic data—At the surface of Station Effingham 1 phosphate, nitrate, and ammonium were essentially 0 μM (Figure 2). With increasing depth, phosphate increased steadily, ammonium remained close to 0 μM then increased rapidly between 50 and 110 meters. Nitrate increased to a maximum of 17 μM at 50 meters then dropped to zero by 80 meters and remained constant. In Effingham 2 phosphate and ammonium were below 1 μM at the surface and nitrate was near 5 μM at the surface and 190 meters. Ammonium was 0 μM and phosphate increased to 4.14 μM at 190 meters. Effingham 3 was similar to Effingham 2 in nutrient magnitudes and trends.

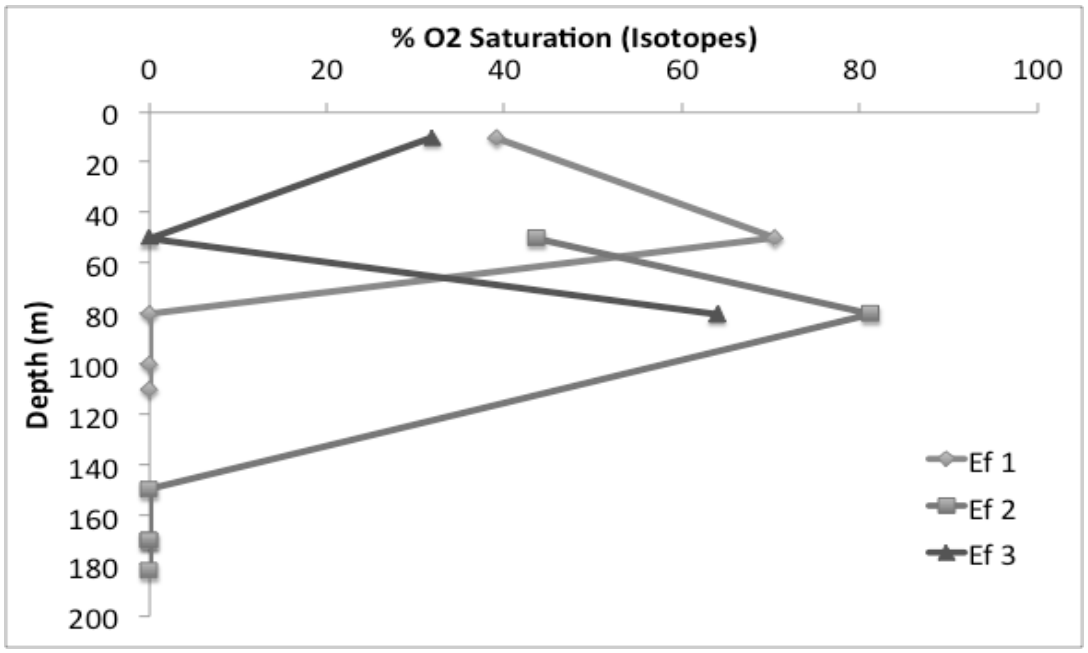

Fig. 3. % Oxygen saturation determined from the oxygen concentration determined by mass spectrometry.

Oxygen saturation (Figure 3) was determined by analysis of mass spectrometer data. Solubility characteristics of oxygen determined the predicted concentration at each depth for each sample's given temperature and salinity. Measured concentrations were divided by the predicted then the ratio multiplied by 100 to give a percent O_2 saturation. Ef 1 and 2, the two basins with anoxic bottom layers, followed trends similar to one

another. At the surface in Ef 1 oxygen was 40% saturation. At 50 meters it increased to an O_2 max of 70.4% and then decreased to 0% from 80 meters to the bottom at 110 m. In Ef 2 an O_2 max was reached at 81.23% at 80 meters then decreased to 0 at 150 and remained 0 to the bottom. At Station Ef 3, surface O_2 was 31%. It reached a minimum near 0% at 50 meters then increased to 63% saturation at depth.

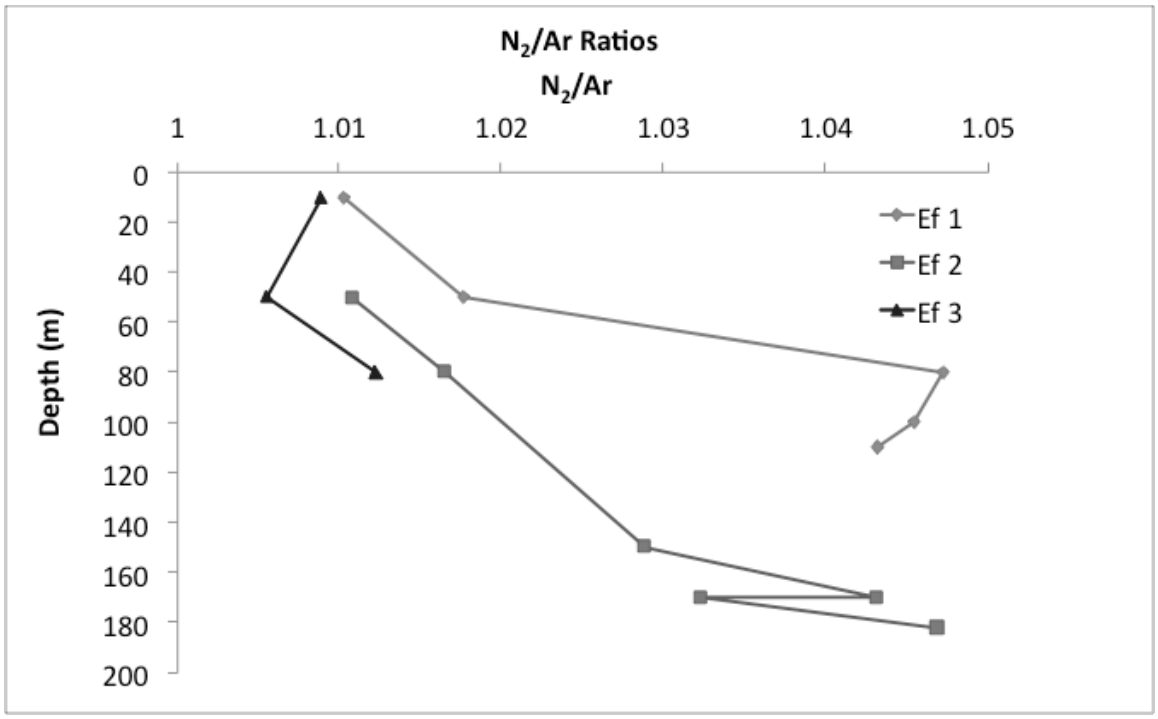

Fig. 4. N_2/Ar ratio with depth at each of the three stations sampled.

N_2/Ar ratios— During sample purification and freezing into aluminum fingers, a known amount of ^{36}Ar spike was added. The concentration of naturally abundant ^{40}Ar is too small to measure directly, IRMS however is used to determine the ratio of 36:40 and absolute concentrations of $N_{2(g)}$ and $Ar_{(g)}$ in the sample can be calculated with solubility coefficients from Hamme and Emerson (2004). Argon is biologically inert but shares solubility characteristics with oxygen making it an ideal gas to measure with N_2 to remove variations in N_2 due to physical temperature changes and bubble processes. Absolute N_2/Ar (Figure 4) varied greatly between depths and ranged from 1.010 to 1.050

in Effingham 1. In Effingham 2, the ratio ranged from 1.011 to 1.053. Effingham 3 samples were limited but the two samples measured ranged from 1.005 to 1.016.

N_2 excess—As calculated by the N_2/Ar method N_2 excess measurements fell within the range of 16.46 to -2.72 μmol kg^{-1}. (Figure 5). In Effingham 1 surface N_2 excess was 2.17 μmol kg^{-1}, meaning the concentration of N_2 was higher than expected under equilibrium conditions. With depth, N_2 increased to 16.2 μmol kg^{-1} then decreased slightly over the remained 30 to the bottom. In Effingham 2 N_2 excess was low at the surface and increased with depth to an average of 14 μmol kg^{-1} between 160 and 190 meters. In Effingham 3 an excess of 6.99 μmol kg^{-1} was present at the surface, N_2 was depleted to -2.8 μmol kg^{-1} at 50 meters and was near 0 μmol kg^{-1} at 80 meters.

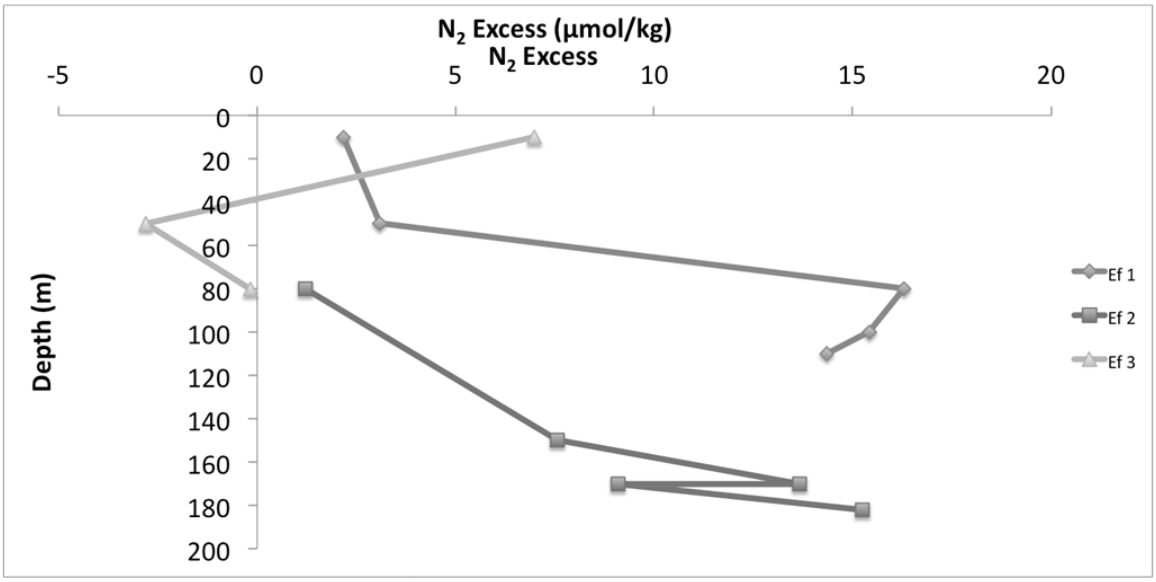

Fig. 5. [N_2] excess (in μmol kg^{-1}) at each of the three stations in Effingham Inlet

$\delta^{15}N$-N_2 of gross and excess N_2—From the IRMS, ratios of 29:28 were measured. The difference between this ratio and a 15:14 ratio is negligible when comparing measurements of δ^{15}N-N_2 thus all profiles are in terms of the nitrogen isotopes 15 and 14.

$\delta^{15}N$'s in Effingham Inlet varied greatly between depths (Figure 6). At Effingham 1 a surface $\delta^{15}N$ of 0.554 Mil rose to 1.121 at 50 m and 1.275 at 80 m. It then rose to 1.337 near the bottom at 110 m. Effingham 2 was more widely varied, from 0.492 at 50 m which rose to a max of 1.3 at 80 m then decreased at 170 m to 1.7 and again rose to 1.28 at the bottom. Effingham 3 surface del was 0.264 Mil rose to 1.6 at 50 m and dropped to 1.8 at 80 m.

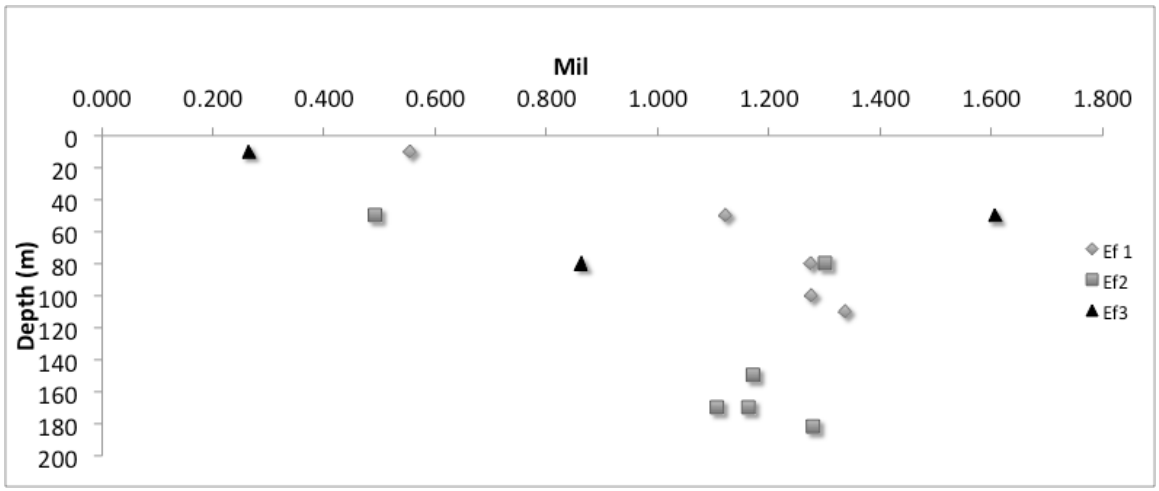

Fig. 6. $\delta^{15}N$-N_2 [Mil] measurements in Effingham 1, 2, and 3.

Mass Balance—The mass balance of $\delta^{15}N$ measurement taken at 10 meters in Effingham 1 resulted in an average $\delta^{15}N$-NO_3 of 52.67 Mil. Additional mass balances for the remainder of the samples fell within the same order of magnitude. Values at all stations at all depths exceeded N_2 excess calculations by a factor of 3 or more.

Discussion

Each stage of the nitrogen cycle, save denitrification and nitrogen fixation, converts one species of fixed nitrogen to another through reduction and oxidation reactions which transition nitrogen between oxidation states (Figure 6). These

transformations take place during the assimilation and utilization of fixed nitrogen by plankton and higher order trophic levels. When these organisms die the various forms of organic nitrogen they posses are degraded and the ammonia released undergoes nitrification into nitrite. Nitrite, nitrate and ammonia are the integral nitrogen species associated with denitrification and anammox and can be used to infer whether these metabolisms are present. In Ef 1, nitrate decreased to 0 μmol kg^{-1} and ammonia concentration rose, beginning around 50 meters depth. This corresponded to the O_2 low saturation which decreased from 70% to 0% over the same depth. Low oxygen and low nitrate concentrations indicate that denitrification was a dominant process in the bottom waters of Station Ef 1. Station Ef 2 was also determined to have low O_2 at depth, though nitrate concentrations did not decrease at depth, denitrification may still have been a factor. Station Ef 3 was in the outermost basin of Effingham Inlet where freshly oxygenated water was not deterred from flushing this basin by a sill. At this station O_2 saturation was high at depth signifying that aerobic respiration was the most likely dominant process.

Quantification of the extent of denitrification can be accomplished by studying the export of NO_3 deficit out of the zone of denitrification (Naqvi, 1987; Gruber and Sarmiento, 1997; Devol et al., 2006) or by N_2 excess (Codispoti et al., 2001; Devol et al., 2006). The method used to calculate N* relies on the stoichiometric values of the Redfield ratio. According to Deutsch et al. (2001):

$$N^*=N-16P+2.9 \mu mol\ kg^{-1}$$

By this calculation the first term on the right hand side of the equation is the sink of N* from direct loss of fixed N. The second term accounts for the loss of N from the organic

nitrogen pool, the final term is a constant to set the global mean to 0 μmol kg^{-1}. N* estimates of nitrate deficit are dependant on the assumption that all of the nitrogen that went into N_2 production was organic matter with a Redfield N:P ratio. While this tracer is a useful tool in the euphotic zone it was designed for general water column N deficit calculations and is not specific to anoxic regions. Additionally, estimations of N deficit by the N* method can be complicated by reactions of phosphate with oxides at the interface between suboxic and anoxic zones. The estimates obtained by this calculation indicated that the N deficit was upwards of 50 μmol kg^{-1} in the anoxic depths of Station Ef 1 where the N_2 excess method calculated an excess N_2 of 15μmol kg^{-1}. This discrepancy was most likely due to an inability of the N* calculation to account for non-Redfield reservoirs of nitrogen in the production of N_2 and other variables.

Denitrification and other anaerobic respiration metabolisms are slightly less energetically favorable than aerobic respiration and therefore are only found to occur in anoxic conditions. These metabolisms include anammox, sulfur reduction by thiotrophic organisms (Tuttle and Jannasch 1973), and other less studies metabolisms. In nearby Saanich Inlet, British Columbia ammonia oxidizing crenarchaea were the dominant species in the anoxic regions of the fjord (Zaikova 2010). Ammonium concentrations in Effingham 1 increase in the anoxic basin providing a fuel for anammox, $NH_3 + NO_2 \rightarrow N_2$. Due to the similarity between season and location between the Zaikova study and the 2010 Ocean 444 cruise in Effingham Inlet, as well as the presence of ammonium to source anammox, similar species were most likely present in the anoxic regions of Effingham Inlet during this study.

N_2 excess increased with increasing depth in the anoxic basins. The low and negative values in the euphotic zone are indicators that nitrogen fixation, $N_2 \rightarrow NH_3$ may have converted all available N_2 into ammonia. This ammonia was most likely being immediately taken up by other organisms which is why the nutrient profile indicated near complete depletion of ammonia. Outside of the euphotic zone dead organisms degrade rapidly which frees the ammonia from this organic detritus. For this reason the profile of ammonia increases. In the deepest, most anoxic regions of Effingham Inlet, N_2 excess values shoot up to 17 or 18 μmol kg^{-1}. These values correspond with denitrification, $NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$, or the other pathways which reduce nitrogen. In these regions high N_2 excess corresponded to near complete exhaustion of the nitrate pool, which was expected. These results were corroborated by studies in similar anoxic conditions in other regions of the world (Table 2).

Location	$\Delta N_2/Ar$ (%)	N_2 excess (μmol kg^{-1})	Reference
Arabian Sea	2.5-3.5	8-12	Devol et al. (2006)
Black Sea	1.5-3.0	7.5-20	Fuchsman et al. (2008)
Effingham Inlet	1.005-1.052	-2.7-18	Current Study

Table 1. Comparison studies carried out in the Arabian Sea, Black Sea, and Effingham Inlet.

The transition between molecular nitrogen and fixed nitrogen is a specialized metabolic process and is performed only by diazotrophs (Deutsch et al. 2001). In areas where N_2 excess is negative, meaning nitrogen gas concentration is below equilibrium concentrations, the most likely explanation is nitrogen fixation. If diazotrophs remove

gaseous nitrogen from the N_2 reservoir and fix it faster than physical processes replenish the pool, then N_2 excess would be negative. A study of diazotroph abundance would be integral in further analysis of the nitrogen dynamics of this fjord.

Conclusions

Nitrogen to argon ratios used to calculate N_2 excess above equilibrium concentrations can quantify the amount of biologically mediated N removal in the anoxic basins of Effingham Inlet. These calculations do not rely on the metabolic pathways assumed for previous studies, nor do they require assumptions to be made about N:P ratios of the degraded organic matter. In Effingham inlet between 19 March 2010 and 22 March 2010 significant N_2 excesses corresponded with oxygen depleted water and low nitrate concentrations. All of these are indicators that denitrification and perhaps other pathways are reducing nitrogen and that this region plays a role as a N_2 source. The N_2 produced in the depths replenishes the pool of inorganic nitrogen available to diazotrophs in the euphotic zone. These organisms then provide nutrients for all manner of biological production to begin again. The profiles and measurements taken in Effingham were consistent with data published for similar anoxic regions but more extensive sampling is required to determine which metabolic pathways are most dominant in this region.

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