A simple and precise method for measuring ammonium in marine and freshwater ecosystems

Robert M. Holmes, Alain Aminot, Roger Kérouel, Bethanie A. Hooker, and Bruce J. Peterson

Abstract: The accurate measurement of ammonium concentrations is fundamental to understanding nitrogen biogeochemistry in aquatic ecosystems. Unfortunately, the commonly used indophenol blue method often yields inconsistent results, particularly when ammonium concentrations are low. Here, we present a fluorometric method that gives precise measurements of ammonium over a wide range of concentrations and salinities emphasizing submicromolar levels. The procedure not only solves analytical problems but also substantially simplifies sample collection and preservation. It uses a single working reagent (consisting of orthophthaldialdehyde, sodium sulfite, and sodium borate) that is stable for months when stored in the dark. The working reagent and sample can be mixed immediately after sample collection and the reaction proceeds to completion within 3 h at room temperature. Matrix effects and background fluorescence can be corrected without introducing substantial error. This simple method produces highly reproducible results even at very low ammonium concentrations.

Résumé : La mesure exacte des concentrations d'ammonium s'avère essentielle à la connaissance de la biochimie de l'azote dans les écosystèmes aquatiques. Malheureusement, la méthode au bleu d'indophénol généralement utilisée donne souvent des résultats incohérents, notamment lorsque les concentrations d'ammonium sont faibles. Nous présentons ici une méthode fluorométrique qui permet de déterminer avec exactitude une large gamme de concentrations d'ammonium et de salinités notamment au niveau sub-micromolaire. En plus de résoudre des problèmes analytiques, cette procédure simplifie de façon appréciable la collecte et la conservation des échantillons. Elle fait appel à un seul réactif (constitué d'orthophtaldiadéhyde « OPA », de sulfite de sodium et de borate de sodium) qui demeure stable pendant des mois lorsque conservé à l'obscurité. Le réactif et l'échantillon peuvent être mélangés immédiatement après le prélèvement et la réaction s'effectue en trois heures à la température ambiante. Les écarts dus aux effets de matrice et à la fluorescence de fond peuvent être corrigés sans introduire une erreur appréciable. Cette méthode simple donne des résultats très faciles à reproduire même à de très faibles concentrations d'ammonium.

[Traduit par la Rédaction]

Introduction

Ammonium determination is one of the most common yet troublesome measurements made by limnologists and oceanographers. A recent intercomparison study involving more than 100 laboratories worldwide demonstrated that the marine community as a whole cannot measure ammonium concentrations accurately (Aminot et al. 1997), particularly at submicromolar concentrations, which are the norm in many systems (Fig. 1). It is unlikely that the freshwater community can do any better. At a minimum, uncertainty in ammonium measurements hampers our ability to discern the details of nitrogen cycling in aquatic ecosystems; at worst, it leads to incorrect conclusions about ecosystem processes or invalid comparisons across sites. For example, errors in measurement of ammonium concentration are propagated throughout complex models of nitrogen cycling, making invalid subsequent inferences.

A number of methods exist for measuring ammonium (Garside et al. 1978; Goyal et al. 1988; Genfa and Dasgupta 1989; Gibb et al. 1995; Kérouel and Aminot 1997), with the indophenol blue technique being by far the most widely used (Solorzano 1969; Brzezinski 1987; Catalano 1987). The continued publication of methods attests to the general dissatisfaction with existing techniques. All of these methods have some merit, but most suffer from lack of generality or overcomplexity. Specific criticisms of the indophenol method include the presence of high and variable blanks and difficulties with low concentration samples (Patton and Crouch 1977; Catalano 1987). In addition, the reagents are toxic and must be disposed of as hazardous waste.

The method that we present here is an adaptation of a recently published continuous-flow fluorometric technique (Kérouel and Aminot 1997). Our modified method optimizes analysis of samples with low ammonium concentrations ($<0.5\ \mu mol \cdot L^{-1}$), but it is also applicable to samples with high concentrations. It is a manual method requiring no specialized equipment other than a fluorometer, in contrast with other fluorometric methods requiring high-performance liquid chromatography separations and continuous-flow instru-

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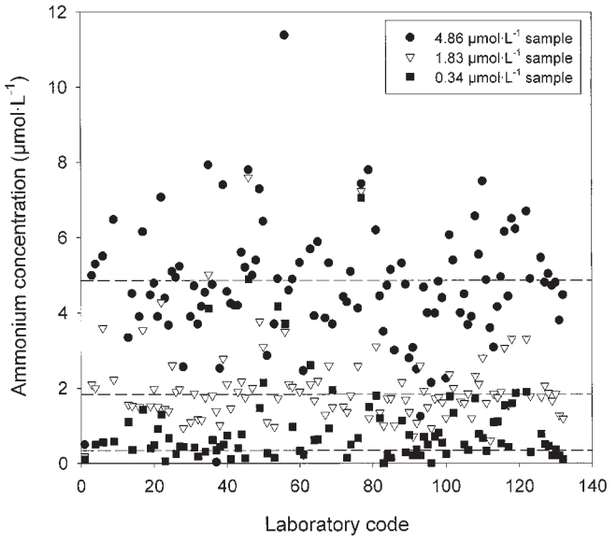
Fig. 1. Results of International Council for the Exploration of the Sea intercomparison study for ammonium in seawater. The horizontal broken lines show the "true" concentrations of the three samples that were sent to each of 132 participating laboratories. Modified from Aminot et al. (1997).



mentation (Gardner and St. John 1991; Kérouel and Aminot 1997).

We begin by discussing general considerations about measuring ammonium, next describe the reagents and equipment needed for the manual fluorometric method, and then present the step-by-step procedure. We then show how the method was validated using laboratory standards and consider how to deal with background fluorescence (sample autofluorescence) and matrix effects. Finally, we apply the method to various marine and freshwater samples. We conclude that the method is broadly applicable, relatively simple, and accurate even at very low ammonium concentrations.

Materials and methods

We present two variations of the method. The first (Protocol A) is optimized for samples with submicromolar ammonium concentrations. It uses large-volume samples and a high sample to reagent ratio. The second variant (Protocol B) is well suited for samples with higher ammonium concentrations. It uses a smaller sample volume and a lower sample to reagent ratio, which minimizes matrix effects but decreases sensitivity. We emphasize Protocol A, but we show how Protocol B may be preferable when ammonium concentrations generally exceed ~0.5 µmol·L^{-1}.

General considerations for measuring ammonium

A successful method for measuring ammonium in natural waters should address (*i*) sample collection in the field, (*ii*) sample preservation between collection and analysis, and (*iii*) analysis of the sample in the laboratory ("laboratory" is used generically and includes shipboard or even streamside). Too often, insufficient attention is given to sample collection and preservation; perfect measurements in the laboratory are meaningless if the sample's ammonium concentration does not reflect precisely the ammonium concentration at the field site when it was collected.

Because sources of contamination during sample collection and preservation are numerous and varied, great care must be taken if contamination is to be avoided using this or any other method. Part of the problem is that sample collection and preservation procedures vary greatly among investigators. Samples may be filtered during collection, upon returning to the laboratory, or not at all. Furthermore, samples may be analyzed immediately upon returning to the laboratory or "preserved" until later analysis by refrigerating, freezing, acidifying, or adding toxic compounds such as mercuric chloride or chloroform. These various techniques may impact the ammonium concentration of the sample differently and make comparison across sites tenuous.

Some steps that we use to minimize contamination and insure that the sample's concentration remains unchanged between collection and analysis are to (*i*) use large-volume samples (typically 80 mL for Protocol A but only 2.5 mL for Protocol B), (*ii*) use sample bottles with solid plastic caps (no liners), (*iii*) add the reagent when the sample is collected, and (*iv*) not open the sample bottle until just prior to reading on the fluorometer. We prefer relatively large-volume samples because contamination effects may be relatively smaller. In addition, larger sample bottles with wide mouths (we use 125-mL Pyrex media bottles for low-ammonium samples) are easier to clean, as are caps without liners. The reagent is added in the field immediately after sample collection, eliminating the need for sample preservation. Samples are always kept capped and are not transferred from their original bottles until just prior to fluorometric analysis, thus eliminating contamination from atmospheric ammonia. Finally, because the process of filtration can be problematic (Eaton and Grant 1979; Schierup and Riemann 1979) and since fluorescence is relatively unaffected by particulates, samples are generally not filtered unless the water is highly turbid.

In some cases, it may be impractical to begin incubations immediately after sample collection. In these cases, samples should be preserved as well as possible and returned to the laboratory for later reagent addition and analysis, recognizing that adequate preservation may be impossible for samples with very low ammonium concentrations. If sample preservation is unavoidable, we suggest filtration in the field, ideally using an in-line filtration system and glassfiber filter (such as Whatman GF/F) so the sample is filtered before entering the sample bottle, followed by freezing until analysis. Note that glass bottles may break upon freezing, even if adequate space is left in the bottle for expansion. If freshwater samples are filtered, be sure to first flush filters with sufficient sample water, since glassfiber filters initially adsorb ammonium from freshwater samples (Eaton and Grant 1979). If samples cannot be filtered in the field, it may be better to omit filtration and freeze as soon as possible instead of exposing the sample to contamination in the laboratory during filtration prior to freezing.

Background fluorescence and matrix effects

Two additional issues that we will address are background fluorescence (BF) and matrix effects (ME). BF results from substances in the sample that autofluoresce; this fluorescence must be subtracted from observed fluorescence in order to determine fluorescence resulting from ammonium in the sample. ME are caused by substances in the sample that alter the intensity of the fluorescence caused by orthophthaldialdehyde (OPA) reacting with ammonium in the sample. Although ME are often small (particularly when using Protocol B), they can be substantial in some samples and must be considered. We present methods for dealing with BF and ME that allow accurate correction of results.

Reagents

The method uses a stable working reagent (WR) that is common to Protocols A and B. The active ingredient in the WR is OPA. Although OPA is also used to measure amino acids, when combined

with sodium sulfite (instead of mercaptoethanol), it loses sensitivity to amino acids and thus is essentially specific to ammonium (Kérouel and Aminot 1997). The other two reagents in the WR are a borate buffer and sodium sulfite. The borate buffer, without OPA or sodium sulfite, is used to evaluate sample BF (see Background fluorescence and matrix effects section). Only freshly drawn ultrapure deionized (DI) water should be used to prepare standards and solutions. Since relatively large quantities of WR are used (typically 10–20 mL·sample^{-1}), we make it in large batches; the recipe below makes ~2 L.

Sodium sulfite solution

Add 1 g of sodium sulfite (we use Sigma S-4672) to 125 mL of DI water. The resulting solution is stable for ~1 month when stored at room temperature in a glass bottle.

Borate buffer solution

Add 80 g of sodium tetraborate (we use Sigma S-9640) to 2 L of DI water. Stir or shake thoroughly to dissolve.

OPA solution

Add 4 g of OPA (we use Sigma P-1378) to 100 mL of ethanol (use a high-grade ethanol because impurities in ethanol can autofluoresce; we have used both Carlo Erba and Fisher brands with good results). OPA is light sensitive, so it should be protected from light while dissolving in ethanol and stored in the dark.

WR

In a large (>2 L) brown polyethylene bottle, mix 2 L of borate buffer solution, 10 mL of sodium sulfite solution, and 100 mL of OPA solution. Ideally, allow the WR to "age" for 1 day or more prior to use because its blank will decrease over time. The resulting WR is stable for at least 3 months when stored in the dark at room temperature. To summarize, the final WR should contain the chemicals at the following concentrations: borate buffer (40 g·L^{-1}, 21 mM in the WR), sodium sulfite (40 mg·L^{-1}, 0.063 mM in the WR), and OPA in ethanol (50 mL·L^{-1} in the WR).

Analytical equipment

We use a Turner Designs 10-AU field fluorometer equiped with optical kit No. 10-303. The optical kit consists of a near ultraviolet mercury vapor lamp, a 350-nm interference excitation filter with a 25-nm bandpass, a 410- to 600-nm combination emission filter, and a 1:75 attenuator plate to reduce signal strength. Samples are manually introduced into the fluorometer in borosilicate test tubes, which are used once only. There is no need to preclean test tubes. The sample is read immediately after entering the test tube; thus, any ammonium contamination that might be in the test tube does not have time to react before being read. Alternatively, a single test tube can be reused by rinsing thoroughly with DI water between samples.

Standard preparation

Preparation of standards is nontrivial. Our recommended method for making standards is out of the ordinary and therefore requires explanation. Since we favor adding the WR immediately after the sample is collected in the field, standard reactions should also be started in the field unless the period between sample collection and returning to the laboratory is very short.

Protocol A standards

Take a set of sample bottles to the field filled with precisely 80 mL of DI water. Also take some ammonium stock solution (we typically use 50 μmol·L^{-1}, but more or less concentrated solutions may be preferable in some cases) and an adjustable pipette (we use a 100- to 1000-μL Eppendorf). During the period when samples are collected, standards are made by pipetting stock solution into the sample bottles filled with DI water, and then the WR is added. When calculating standard concentration, one must account for the volume of the stock solution added to the DI water.

We obtain better results using the above protocol than when we prepare standards in volumetric flasks and then transfer to sample bottles, perhaps for the following reasons. First, every time the sample is transferred between glassware, contamination can occur. Second, in freshwater standards and samples, ammonium may bind to the surface of glass; thus, the solution coming out of the volumetric flask may be less than expected (Kérouel and Aminot 1997). By preparing the standard in the bottle when the reagents are added, any ammonium from the water sample that has bound to the bottle will participate in the reaction.

Protocol B standards

Standards are prepared similarly to Protocol A, except that 2.5 mL of DI water and a quantity of ammonium stock solution are added to the sample containers that are already filled with 10 mL of WR. For example, addition of 2.5 mL of DI water and 100 μL of 50 μmol ammonium·L^{-1} stock solution to the WR-filled sample bottles results in a 1.923 μmol ammonium·L^{-1} standard.

Basic procedure — protocol A

Supplies and preparation

1. Acid-washed sample bottles, ~125 mL volume. Extra bottles are needed for determination of the BF and ME of selected samples.
2. WR, enough for 20 mL for each sample and standard.
3. Small graduated cylinder to measure 20 mL of WR.
4. Several sample bottles filled with 80 mL of DI water. These bottles will be used to make standards.
5. Ammonium stock solution.
6. Adjustable pipette and pipette tips to add ammonium stock solution to standards and ME samples (see Background fluorescence and matrix effects section).

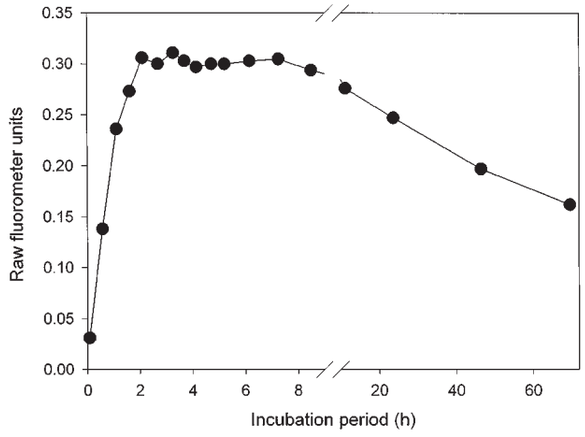
Field procedures

1. Rinse bottle once with sample water and then fill to the 80-mL mark.
2. Add 20 mL of WR and shake and then store in the dark at ambient temperature.
3. Prepare standards (see Protocol A standards section) by adding ammonium stock solution and WR. Standards should be made during the period when samples are collected. If sample collection takes >4 h, more than one set of standards may be needed in order that samples and standards will be at a similar stage of reaction.
4. If the ME is unknown, collect additional samples from selected locations (try to span the range of sample types to be collected; for example, encompass the full salinity range). Amend these samples with the same amount of ammonium stock solution as was used for an intermediate concentration standard. The response of the amended samples will be compared with the standards in order to determine the ME (see Background fluorescence and matrix effects section).
5. If background fluorescence is unknown or variable, collect samples from selected locations for BF determination. The borate buffer solution can be added upon return to the laboratory, since no incubation period is necessary.

Laboratory procedures

1. After incubating for at least 2–3 h, pour samples and standards into test tubes and immediately read on the fluorometer. More than one sample per minute can typically be read.
2. Calculate sample ammonium concentration. The procedure for calculating a sample's concentration involves four steps:

Fig. 2. Time course of the reaction at 22°C. Sample ammonium concentrations were 0.2 μmol·L^{-1}.



(*i*) measuring the raw fluorescence of the sample ($F_{\text{sample}_{\text{obs}}}$), (*ii*) subtracting BF ($F_{\text{sample}_{\text{BF}}}$), (*iii*) correcting for any ME, and (*iv*) calculating sample concentration using the standard regression (see Background fluorescence and matrix effects section for details of calculations).

Basic procedure — protocol B

Supplies and preparation

1. Sample containers filled with 10 mL of WR. We generally use Wheaton 20-mL HDPE scintillation vials as sample "bottles." Instead of washing the vials, we find it easier to incubate them with WR for at least 1 day and then discard the WR and refill with precisely 10 mL of WR. The incubation process removes any ammonium that may be present in the sample container. Take additional sample containers filled with WR for standards and ME samples.
2. Pipette to add 2.5 mL of sample or DI water into sample bottles.
3. Ammonium stock solution and an adjustable pipette for adding stock to standards.
4. Empty bottles for collecting samples for BF analysis.

Field procedures

1. Using a pipette or other accurate measuring device, add 2.5 mL of sample to a sample container that has been filled with 10 mL of WR. Shake and store in the dark.
2. Prepare standards (see Protocol B standards section) during the period when samples are being collected. As noted previously, if sample collection takes several hours, more than one set of standards may be needed in order that samples and standards will be at a similar stage of reaction.

Laboratory procedures

Same as for Protocol A.

Results and discussion

Characterization of the reaction

The kinetics of the reaction were investigated to determine the required incubation period, and spectral characteristics of the fluorescent compound were evaluated to assess optimal excitation and emission wavelengths. The tests were made using Protocol A but are equally applicable to Protocol B.

Time course of reaction

To investigate the time course of the reaction, 18 standards (0.2 μmol ammonium·L^{-1}) were prepared simultaneously and analyzed at intervals over a 3-day period until all standards had been analyzed (Fig. 2). The reaction reached maximum fluorescence after ~2 h, remained relatively stable for several more hours, and then gradually declined. Ideally samples should be read during the period of plateau, ~2–8 h after mixing sample and WR. If for logistical reasons this is not feasible, it is still possible to get good results even after fluorescence has begun to decline, since samples and standards degrade at the same rate.

We also tested the effect of reducing or elevating incubation temperature on the time course of the reaction. It was possible to substantially speed the reaction by incubating in a 40°C water bath or slow it by placing the reacting samples on ice. In general, however, we favor reaction at ambient temperature (generally 18–25°C in our case) unless it is not feasible to wait 2–3 h for the fluorescence to plateau. Note that since the speed of the reaction is temperature dependent, samples and standards should begin at approximately the same temperature. This is particularly true for Protocol A, since sample volumes are large and there is little dilution with WR. In the case where samples and standards begin at very different temperatures, it is best to allow additional incubation time (>4 h) so that both are fully reacted before reading their fluorescence.

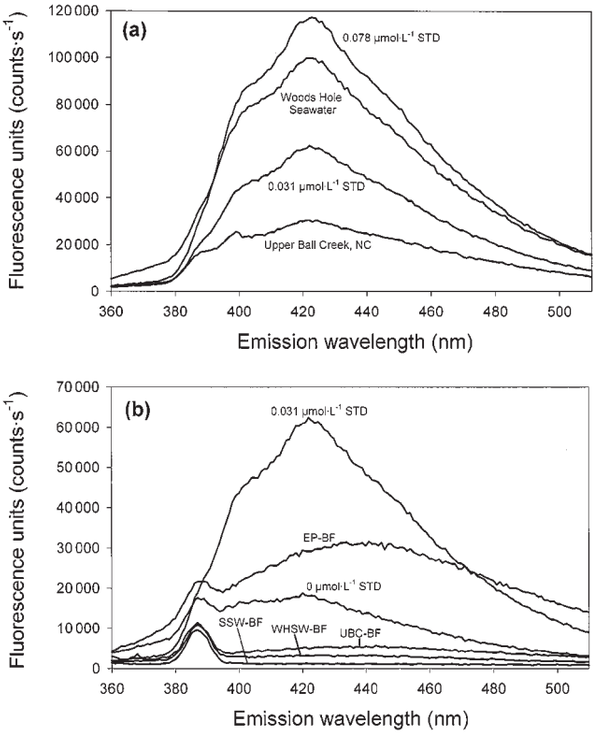
Excitation and emission spectra

In order to choose optimal excitation and emission wavelengths for the method, we measured spectral characteristics of samples and standards using a Photon Technology International scanning fluorometer. We measured the excitation spectra for a 12.5 μmol ammonium·L^{-1} standard and found that maximum emission occurred when the excitation wavelength was ~360 nm. For subsequent scans, we used an excitation bandwidth of 340–360 nm.

For both samples and standards, maximum emission was at ~420 nm, but the peaks were rather broad, ranging from ~380 to >500 nm (Fig. 3*a*). We concluded that excitation below 380 nm and quantifying emissions above 400 nm was appropriate. We find that our filter set (350-nm peak, 25-nm bandpass excitation, 410–600 nm emission) works well, but other combinations within the prescribed range should also be adequate.

We had hoped to be able to minimize interference from BF (sample autofluorescence) by selecting a narrow-bandwidth emission filter that separated OPA-ammonium fluorescence from BF. To quantify BF, we add borate buffer and measure fluorescence. This is done to account for dilution by the WR and because fluorescence is pH dependent. Unfortunately, overlap between OPA-ammonium fluorescence and BF is relatively large (Fig. 3*b*), so complete separation with a narrow-bandwidth emission filter is not possible. However, by focusing around the peak OPA-ammonium fluorescence of ~420 nm, the signal to noise ratio could be increased. Note that in most cases tested, BF is rather small, generally less than the signal of the 0 μmol·L^{-1} standard, which itself fluoresces because the OPA in the WR has a small amount of fluorescence. The peak observed at

Fig. 3. (*a*) Emission spectra for standards and samples after incubation with WR for 4 h. Peak fluorescence for both samples and standards was 421–423 nm. (*b*) Emission spectra for standards (with WR) and samples (without WR) to determine if appropriate filter selection might allow OPA-ammonium fluorescence to be separated from sample BF. EP, Eel Pond in Woods Hole, which was brown in color when these samples were taken due to storm runoff; SSW, Sargasso seawater; WHSW, Woods Hole seawater; UBC, Upper Ball Creek, a small stream in North Carolina.



~385 nm (Fig. 3*b*) is due to the Raman signal of water and is avoided by selecting an emission filter with a wavelength >400 nm.

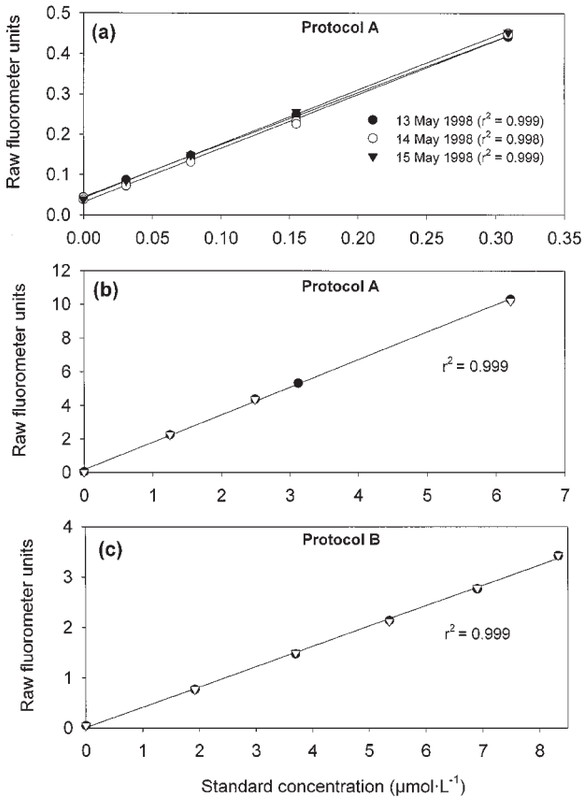
Standards

Using Protocol A, we made 0, 0.031, 0.078, 0.155, and 0.309 µmol ammonium·L^{-1} standards on 3 consecutive days. Duplicate standards of each concentration were run on the first 2 days, and a single replicate of each concentration was done on the third day. In all cases, the resulting linear regressions were good, with little variability among days (Fig. 4*a*). There were no outliers. The signal strength of the lowest standard (0.031 µmol·L^{-1}) was unambiguous, approximately double the blank fluorescence. The reagent blank (or possibly ammonium contamination in our DI water) was equivalent to ~0.03 µmol·L^{-1}.

We also ran standards up to 6.2 µmol·L^{-1} using Protocol A and fluorescence remained linear (Fig. 4*b*). In other experiments, we ran samples up to 25 µmol·L^{-1} and could have adjusted the instrument to read even higher concentrations.

Protocol B also yielded good results (Fig. 4*c*). We are able to analyze ammonium in samples up to ~50 µmol·L^{-1} without dilution or adjusting the sensitivity of the fluorometer

Fig. 4. Standard curves. (*a*) Low-concentration ammonium standards run on 3 consecutive days using Protocol A. On the first two dates, duplicates were done for each concentration standard, whereas on the third date, there was only one standard per concentration. (*b*) Higher concentration ammonium standards analyzed using Protocol A. Duplicates of each concentration were analyzed. (*c*) Standards analyzed using Protocol B. Duplicates of each concentration were analyzed.



using Protocol B. Below ~0.5 µmol·L^{-1}, however, variability increased, so we recommend Protocol A for samples with less than ~0.5 µmol·L^{-1}.

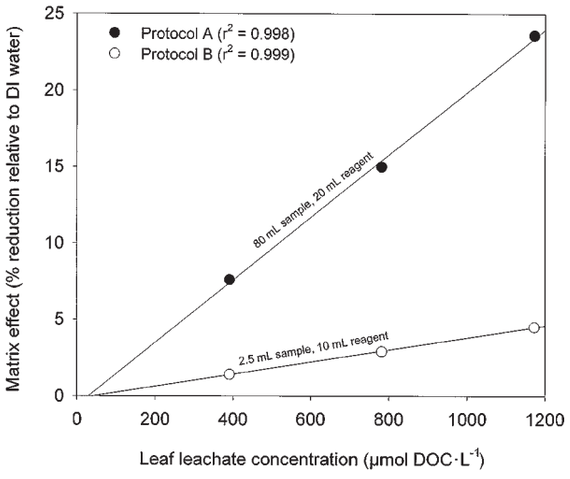
Background fluorescence and matrix effects

A sample's BF is determined by mixing the sample with borate buffer instead of WR. No incubation period is necessary. The fluorescence of the sample when combined with borate buffer ($F_{\text{sample}_{\text{BF}}}$) is subtracted from its fluorescence when incubated with WR ($F_{\text{sample}_{\text{obs}}}$) to determine the sample's ammonium fluorescence ($F_{\text{sample}_{\text{NH}_4}}$):

$$F_{\text{sample}_{\text{NH}_4}} = F_{\text{sample}_{\text{obs}}} - F_{\text{sample}_{\text{BF}}}. \quad (1)$$

ME are caused by substances in the sample such as sea salt or dissolved organic matter that make the sample behave differently than standards. ME are an issue for most methods, including the common indophenol blue technique for measuring ammonium (Loder and Glibert 1977; Stewart and Elliott 1996). To determine the magnitude of matrix effects, we amended samples and standards with the same amount of ammonium and compared the response:

Fig. 5. Quantification of ME with different amendments of leaf litter leachate. ME is plotted as percent reduction of signal in amended samples relative to DI water standards.



$$(2)\qquad \mathrm{ME} = \left\{\frac{[(F_{\mathrm{std}_{\mathrm{spike}}} - F_{\mathrm{std}_{\mathrm{zero})}} - (F_{\mathrm{sample}_{\mathrm{spike}}} - F_{\mathrm{sample}_{\mathrm{obs}}})]}{(F_{\mathrm{std}_{\mathrm{spike}}} - F_{\mathrm{std}_{\mathrm{zero}}})}\right\} \times 100\%.$$

If there were no ME, samples and standards would respond identically. More typically, the response is somewhat attenuated in samples relative to DI water standards, which must be considered when calculating sample concentration.

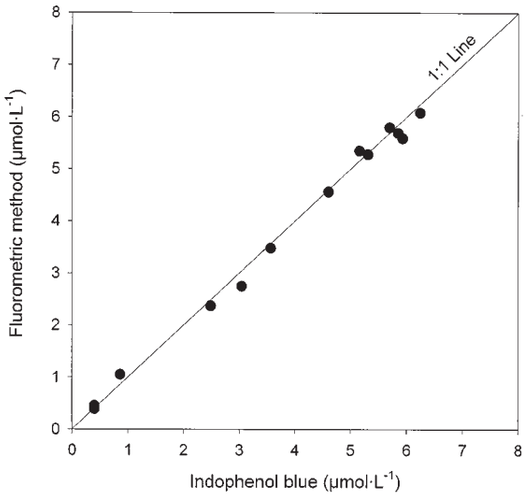
When analyzing seawater samples using standards made in DI water, the ME is ~14–17% when using Protocol A but is reduced to ~3–5% for Protocol B. That is, a 1 µmol ammonium·L^{-1} amendment added to a seawater sample using Protocol A fluoresces only ~85% as much as a 1 µmol·L^{-1} amendment of DI water. To investigate the ME associated with dissolved organic matter, we amended DI water with leaf litter leachate (to dissolved organic carbon concentrations of 391, 782, and 1172 µmol·L^{-1}) and quantified the ME (Fig. 5). At the highest dissolved organic carbon concentration (1172 µmol·L^{-1}), the ME was ~24% using Protocol A but only 4.5% for Protocol B. Thus, Protocol B has the advantage of requiring a smaller ME correction but is less well suited for samples with very low ammonium concentrations because of reduced sensitivity. BF and ME estimates are combined to determine corrected sample fluorescence ($F_{\mathrm{sample}_{\mathrm{cor}}}$) (eq. 3). The value of $F_{\mathrm{sample}_{\mathrm{cor}}}$ is entered into the standard regression to compute the sample's ammonium concentration:

$$(3)\qquad F_{\mathrm{sample}_{\mathrm{cor}}} = F_{\mathrm{sample}_{\mathrm{NH_4}}} + \left[F_{\mathrm{sample}_{\mathrm{NH_4}}}\left(\frac{\mathrm{ME}}{100}\right)\right].$$

Comparison with indophenol blue method

We compared the fluorometric method with the indophenol blue method using marine and freshwater samples, some amended with ammonium. Results obtained from the two methods were comparable (Fig. 6). Protocol B was used for the fluorometric method, and the indophenol method followed Solorzano (1969) using 3-mL samples.

Fig. 6. Comparison of fluorometric and indophenol blue methods. Marine and freshwater samples were measured simultaneously with both methods. The similarity of results demonstrates the comparability of the two methods.



Field samples

The fluorometric method (Protocols A and B) was used on a variety of samples, both marine and freshwater (Table 1). BF ($F_{\mathrm{sample}_{\mathrm{BF}}}$) ranged from 1.6 to ~30% of observed sample fluorescence ($F_{\mathrm{sample}_{\mathrm{obs}}}$), and ME ranged from 4 to 18%. ME for Protocol B did not exceed 6%. The relatively high BF of the Parker River sample is not surprising, since the sample was highly colored with dissolved organic matter. Moreover, while the BF for some samples seems rather high, this is largely an artifact of the low ammonium concentrations of the samples. When converted to ammonium concentration equivalents, the observed BF ranged from only ~0.007 to 0.13 µmol·L^{-1}. Ammonium concentrations for all samples were <0.5 µmol·L^{-1}, and replication was good in all cases. Samples of Woods Hole seawater and Parker River water were analyzed using both protocols, and results differed by 0.03–0.05 µmol·L^{-1}. Although replication was good using both protocols, in general, we are more confident in analyzing samples with less than ~0.5 µmol·L^{-1} using Protocol A. Since the Sargasso seawater sample contained no measurable ammonium, standards could be made in the Sargasso seawater, which would greatly reduce the ME when analyzing seawater samples.

We also tested the method on a series of samples from the Kuparuk River and Toolik Lake, at the site of the Arctic LTER project in northern Alaska. The stream samples were taken along a 4.3-km reach of the river that has been subject to a long-term phosphorus fertilization experiment (Peterson et al. 1993, 1997). Primary production is stimulated in the phosphorus-fertilized reach, and consequently, we might expect a downstream decline in ammonium concentration. Ammonium concentrations are always low, and prior to summer 1998 when we began using the fluorometric ammonium method, any such pattern that might have been present was obscured by analytical noise. However, ammonium analy-

Table 1. Analysis of ammonium concentrations in a variety of marine and freshwater samples using both variations of the method (Protocols A and B).

Sample	Protocol	Observed fluorescence ($F_{sample_{obs}}$) (raw units ± SE)	BF ($F_{sample_{BF}}$) (raw units ± SE)	Sample NH_4 fluorescence ($F_{sample_{NH_4}}$) (raw units ± SE)	ME (relative to DI water) (%)	Corrected sample fluorescence ($F_{sample_{cor}}$) (raw units ± SE)	Sample concentration ($\mu mol \cdot L^{-1}$ ± SE)[a]
Woods Hole seawater	A	0.611±0.0013	0.010±0.0000	0.601±0.0013	15	0.692±0.0015	0.43±0.001
Woods Hole seawater	B	0.179±0.0007	0.005±0.0003	0.174±0.0007	4	0.182±0.0003	0.40±0.001
Parker River, Mass.	A	0.679±0.0038	0.178±0.0003	0.501±0.0038	18	0.591±0.0045	0.36±0.003
Parker River, Mass.	B	0.202±0.0012	0.058±0.0009	0.144±0.0012	6	0.153±0.0011	0.31±0.003
Sargasso seawater	A	0.027±0.0022	0.004±0.0000	0.023±0.0022	17	0.027±0.00260	0.00±0.002
Upper Ball Creek, N.C.	A	0.066±0.0010	0.020±0.0005	0.046±0.0010	5	0.049±0.0013	0.01±0.001

Note: Error terms represent SE of three replicates.
[a]For Protocol A standards, the slope of the regression equation was 1.556, the *y*-intercept (blank) was 0.029, and the r^2 was 0.9998; for Protocol B standards, the slope of the regression equation was 0.3489, the *y*-intercept (blank) was 0.043, and the r^2 was 0.9995.

Fig. 7. Ammonium concentrations measured in samples collected along the Kuparuk River, Alaska, the site of a long-term phosphorus addition experiment (phosphate added at 0.3 km). Samples were collected 20 July 1998. As expected, ammonium concentrations declined downstream of the phosphorus addition site because primary production (and nutrient uptake) was stimulated by the phosphorus addition.

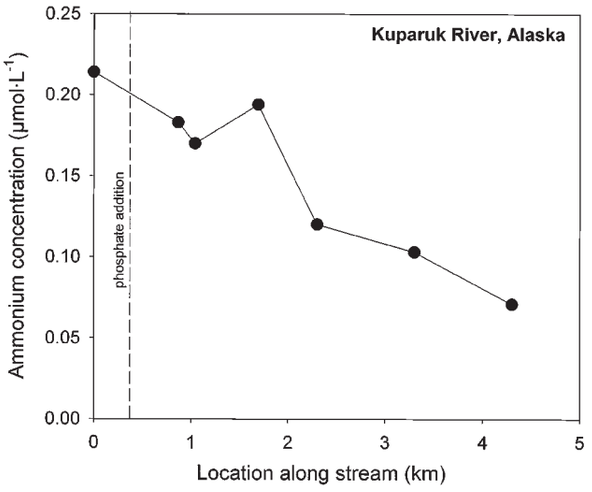
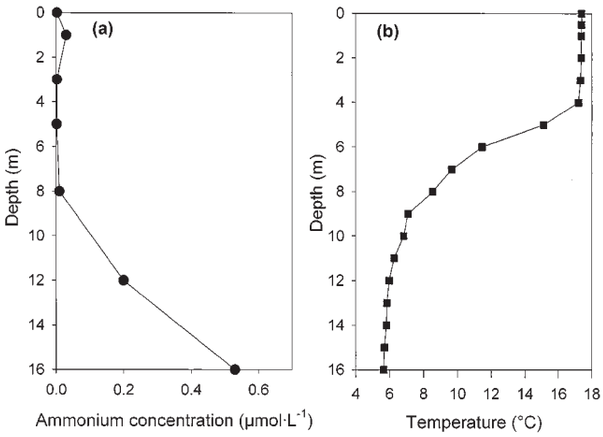


Fig. 8. Toolik Lake, Alaska, (*a*) ammonium and (*b*) temperature profiles from 22 July 1998. Ammonium concentrations were extremely low in the epilimnion and metalimnion but rapidly increased in the hypolimnion.



sis using the fluorometric method demonstrated the expected pattern; ammonium concentration declined from ~0.2 $\mu mol \cdot L^{-1}$ at the upstream end of the reach to 0.07 $\mu mol \cdot L^{-1}$ ~4 km downstream (Fig. 7).

The ability to measure extremely low ammonium concentrations using the fluorometric method also increased our understanding of nitrogen cycling in the oligotrophic Toolik Lake (Fig. 8). In the epilimnion, ammonium concentrations were generally <0.02 $\mu mol \cdot L^{-1}$ but increased to almost 0.6 $\mu mol \cdot L^{-1}$ in the hypolimnion. As was the case in the Kuparuk River, this pattern was previously much less apparent because concentrations were typically too low for us to measure using the indophenol method.

Conclusions

We have used the method on a variety of samples from diverse sites and in all cases have been pleased with its performance. We presented two variations of the method (Protocols A and B) and should note that other sample volumes and sample to reagent ratios can be used; for example, 10 mL sample and 10 mL reagent may be optimal for some applications. In our first field season using the fluorometric method at the Arctic LTER site (summer 1998), the ability to measure ammonium at low concentrations with good accuracy provided insight into the functioning of the Kuparuk River and Toolik Lake ecosystems that previously was obscured by analytical noise. The fluorometric ammonium method has also allowed us to improve our measurements of the isotopic composition of ammonium that we conduct at the Arctic LTER site and other sites (Holmes et al. 1998, 1999; Mulholland et al. 2000), as accurate ammonium concentration measurements are critical for these analyses. We conclude that the method is broadly applicable, relatively simple, and will advance our understanding of ecosystem processes by providing more reliable ammonium concentration measurements.

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