EuroGO-SHIP
Enhancing ocean observations

Advancing Ocean Best Practices

A EuroGO-SHIP Exploitable Result

Co-funded by the European Union

UK Research and Innovation

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Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

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Advancing Best Practices

The Preservation of Nutrient Samples

Presented by: Malcolm Woodward (Plymouth Marine Laboratory, UK)

13th October 2025

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Team, Scope and Objectives

Analytical Teams:
- Malcolm Woodward - Plymouth Marine Laboratory (PML), United Kingdom
- Stefano Cozzi - Consiglio Nazionale delle Ricerche (National Research Council - CNR), Trieste, Italy

Scope:
- Nutrient sample storage: compare 2 of the most widely used methods of long-term nutrient sample storage (freezing and pasteurisation) and recommend on best practice for sample storage in the future;
- The pilot activities involved two partners from the EuroGO-SHIP consortium (UK and Italy) and took advantage of planned cruise programmes in the English Channel, Eastern North Atlantic, and the Mediterranean.

Specific objectives and goals:
- To describe the rationale, methodology, and data sources for the nutrient sample storage experiments.
- To present a summary of the results of the nutrient preservation activity.
- To identify and discuss the lessons learned and best practices from the experiments.
- To provide recommendations and suggestions for the future.

Methodology

Background and methodological history to Nutrient Sample Storage

There are a very **broad range of methods** that have been reported in the literature over past decades:

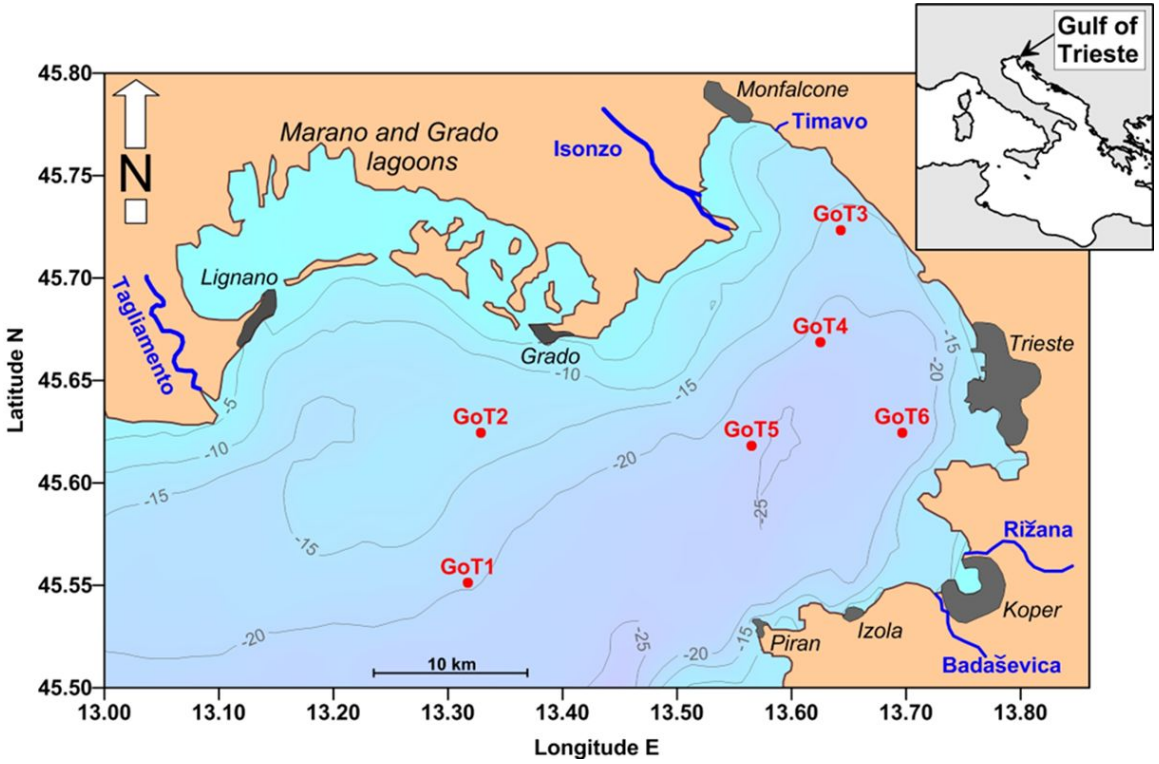
- **Freezing** at -20°C: most common (simple and non-invasive to the samples), but sample difficulties in shipping from distant oceans.
- **Pasteurisation**: heating sample to 80°C for two hours, cooling and storing in dark. Samples can be then shipped. This requires a good accurate oven.
- **Poisoning** with addition of Mercuric Chloride: good method but problems with user handling / poison / marine pollutant / waste handling / reduced efficiency of analytical Nitrate reduction, loss of column.
- **Chloroform** and other compounds: toxic and hazardous to human health.
- **Acidification** with HCl: samples must be neutralised before analysis by sodium hydroxide. Addition of material to sample twice risks contamination and acid can also cause hydrolysis of organic phosphate compounds.
- **Alkalinisation** with sodium hydroxide: samples to be neutralised with HCl before analysis, and again the addition of material can contaminate the sample.

Freezing and Pasteurisation were chosen for the nutrient pilot study as they are the most used by community

Sampling Methodology

Sampling activity in the Gulf of Trieste

- Nutrient samples collected in May 2023 in six stations in the Gulf of Trieste (GoT), using small boats for coastal water monitoring programs.
- Samples collected at the surface and bottom using 5-liter horizontal Niskin bottles and stored in 5-liter high density polyethylene (HDPE) tanks.
- Tanks were kept refrigerated and in the dark until the processing of all sub-samples in the laboratory of CNR ISMAR.
- Sampling stations in different sites of the gulf, variably affected by atmospheric forcings, circulation of water masses and runoff.



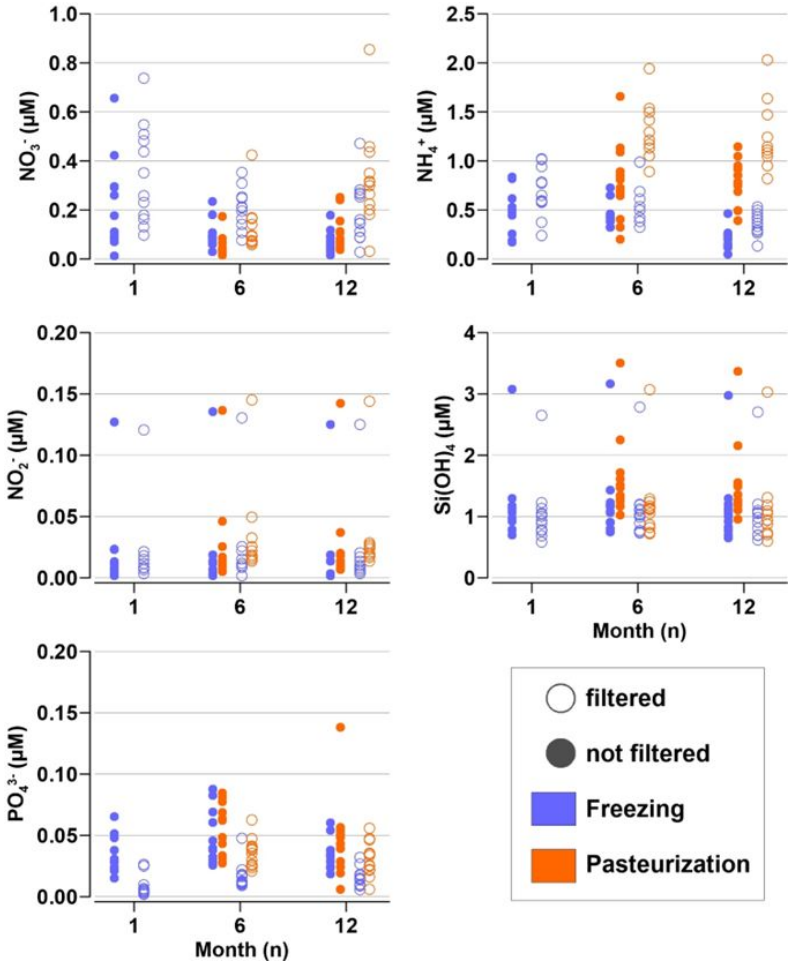
Sampling stations in the Gulf of Trieste where meteorological conditions, CTD data and nutrient samples were collected.

Key Findings

Data and results summary CNR

Larger sample taken at sea and processed on land: analysis at 1, 6 and 12 months.

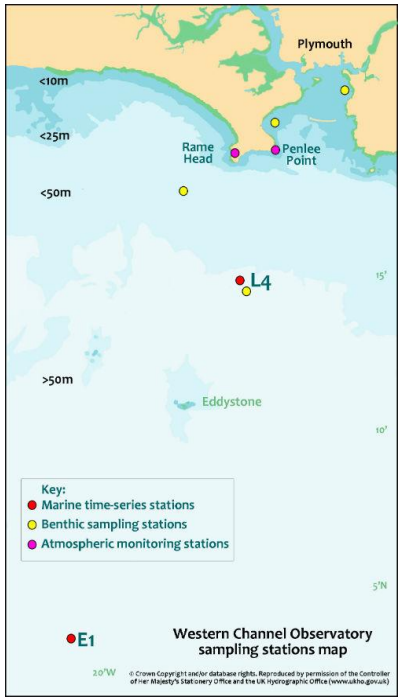
- Nutrient concentrations do not stay constant over one year in frozen and pasteurised samples: microbial activity is not completely stopped.
- Syringe filtration of samples with MCE membrane filters (0.22 µm pore size) can cause NO_3^- and NH_4^+ release due to plankton cells breakage.
- Possible overestimation of PO_4^{3-} concentration in non-filtered samples due to dissolution of natural particulate phosphorus (P).
- In non-filtered samples, pasteurisation can increase concentrations of $PO_4^{3-}$and $Si(OH)_4$, because of remineralisation of particulate and dissolved organic P and biogenic silicon.
- In filtered samples, pasteurisation increases concentration of PO_4^{3-} with respect to frozen samples, due to possible remineralisation of dissolved organic P.

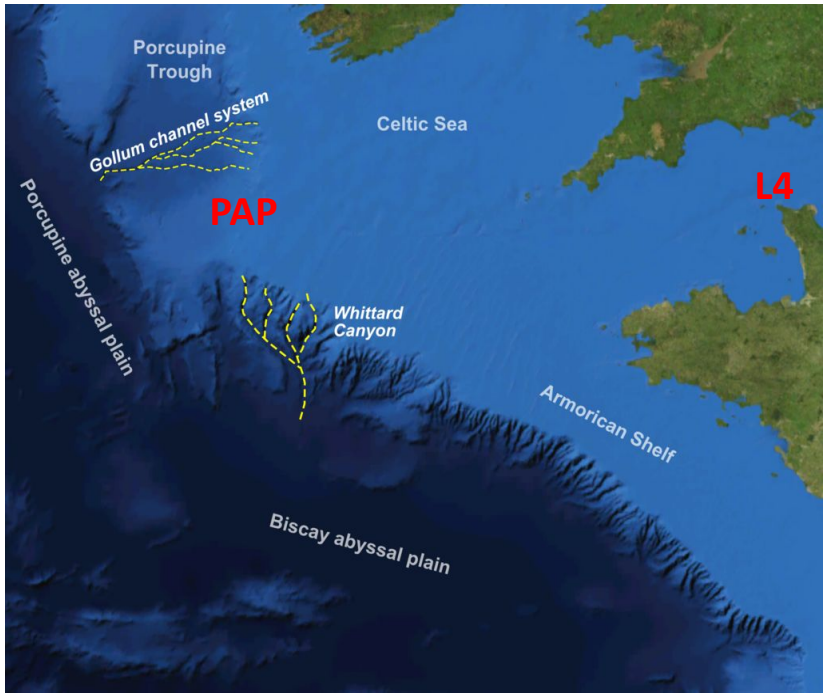


Methodology_PML

Sampling programme in Western Channel and Eastern North Atlantic

Sampling sites in the Western Approaches and Eastern North Atlantic





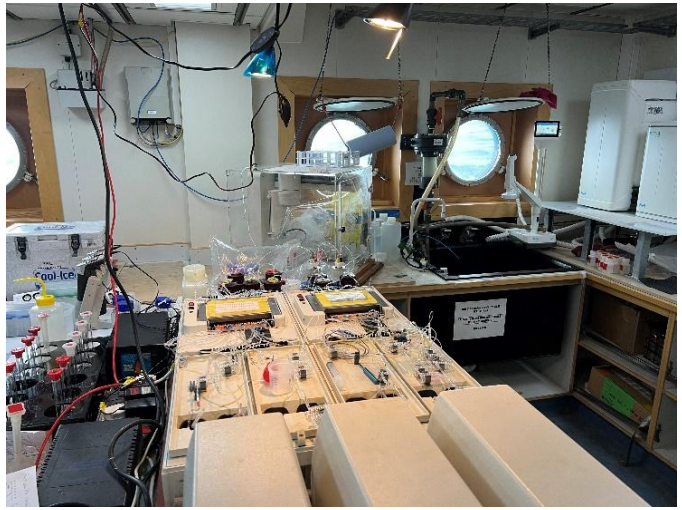
Coastal station at L4 and three stations at PAP (Porcupine Abyssal Plain) with varying depths and nutrients, cruise on board the RRS James Cook.

Methodology
Sampling and Analysis

EuroGO-SHIP

Nutrients:

- First sampling station at L4 in the Western Channel taken at 50 metres water depth: 24 Niskin Bottle CTD/Rosette. Full water column analysis, 3 CTD bottles reserved for the EuroGO-SHIP sampling. At sea analysis carried out with a 5 channel SEAL analytical AAIII, a colorimetric, segmented flow autoanalyser (NO_3^-, NO_2^-, NH_4^+, $Si(OH)_4$, PO_4^{3-}).
- Samples also taken during CTD bottles sampling for analysis to check on homogeneity of the 20 litre bottles.
- Clean sampling handling techniques employed: acid washed bottles, 'nutrient free vinyl gloves'. Sampling according to the GO-SHIP nutrient manual.

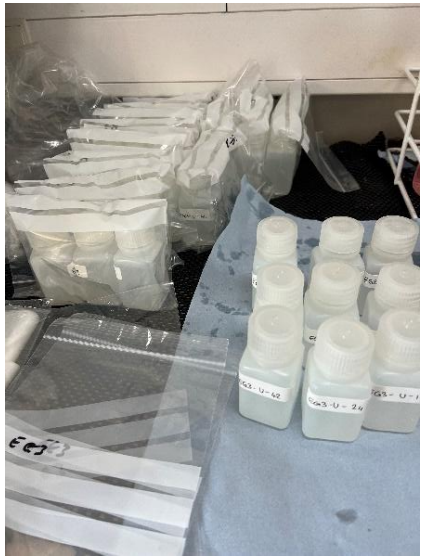


Methodology
Pasteurisation

Nutrients:

- Triplicate samples taken for every sample and randomly packaged.
- Acid cleaned 60 ml HDPE bottles used for sample freezing and pasteurisation of NO_3^-, NO_2^-, NH_4^+, $Si(OH)_4$, PO_4^{3-}. 100 ml Glass Schott bottles used for pasteurisation of NH_4^+ only.
- Sufficient samples taken for up to 15 months of future analysis.
- 45 HDPE bottles sampled for freezing and pasteurisation + 45 Glass bottles for pasteurisation (at every station).

Pasteurization method as described in Daniel et al 2012*: 80°C for two hours.



Oven heating profile was tested

* Marine Chemistry, 2012. Anne Daniel, Roger Kérouel, Alain Aminot. Pasteurization: A reliable method for preservation of nutrient in seawater samples for inter-laboratory and field applications.

Methodology
Homogeneity

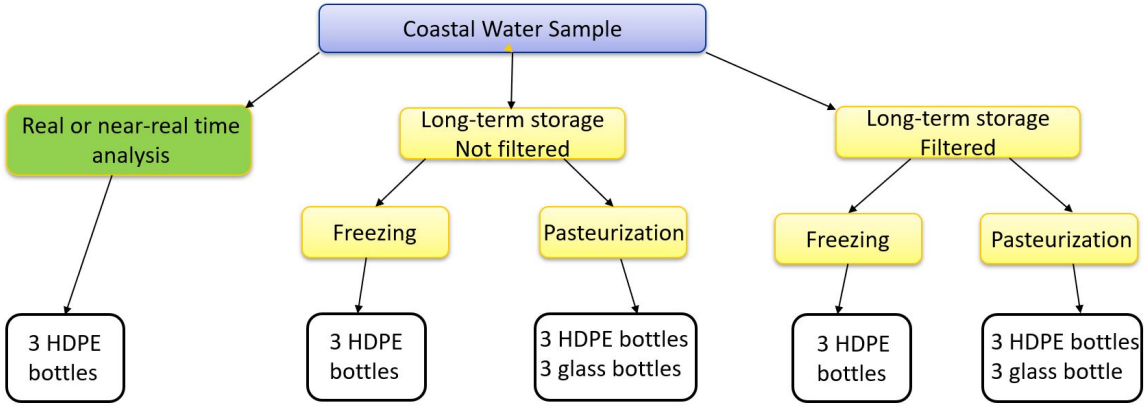
Nutrients:

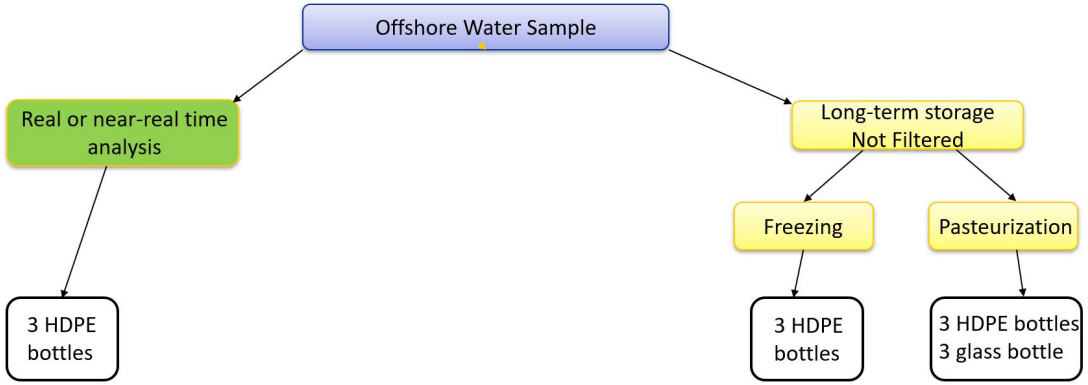
- Samples were also taken for analysis to check on homogeneity of the 20 litre bottles.

Homgeneity subsampling of the CTD bottles					
	Nitrite	Nitrate+Nit	Ammonium	Silicate	Phosphate
	µM	µM	µM	µM	µM
Subsample bottle A	0.03	0.09	0.54	0.52	0.14
Subsample bottle B	0.04	0.09		0.52	0.15
Subsample bottle C	0.03	0.09	0.59	0.48	0.14
Subsample bottle D	0.03	0.09	0.60	0.49	0.14
Subsample bottle E	0.03	0.09	0.60	0.50	0.15
Subsample bottle F	0.04	0.10	0.66	0.54	0.16
Subsample bottle G	0.04	0.09	0.63	0.57	0.15
Subsample bottle H	0.04	0.09	0.62	0.53	0.15
Subsample bottle I	0.04	0.10	0.63	0.50	0.15
Average Subsample	**0.03**	**0.09**	**0.61**	**0.52**	**0.15**
Standard deviation	**0.00**	**0.00**	**0.04**	**0.03**	**0.01**

Methodology
Experimental procedures

Experimental outline for coastal and offshore samples:





Methodology
Water samples

Nutrients:

- Aim: sample different water masses with contrasting nutrient concentration ratios.
- Nutrients at L4 were depleted throughout the water column as this was post spring bloom at this coastal site.
- PAP samples were from 3 deeper depths and correspondingly varied nutrient concentrations.
- Sample set 2 was from 200 m at the PAP site, set 3 from 4824 m, and set 4 from 500 m.

	NO_3^-	NO_2^-	NH_4^+	$Si(OH)_4$	PO_4^{3-}
EG 1 (50 m)	0.25	0.04	0.62	0.59	0.16
EG 2 (200 m)	8.07	0.03	0.03	3.13	0.46
EG 3 (4824 m)	22.73	0.03	<0.03	46.87	1.53
EG 4 (500 m)	13.29	0.02	<0.03	5.58	0.78

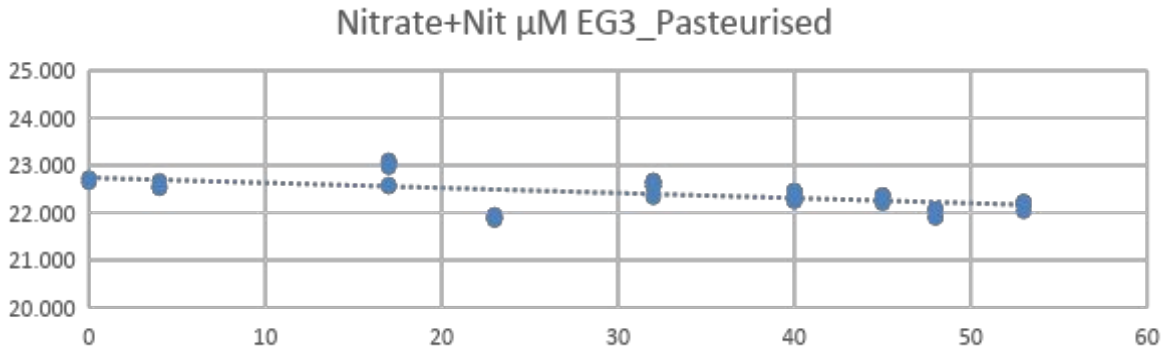
Before the analysis frozen samples were defrosted according to the GO-SHIP nutrient manual: 45 min at 50°C and then 45 min at room temperature (Becker *et al*., 2020)

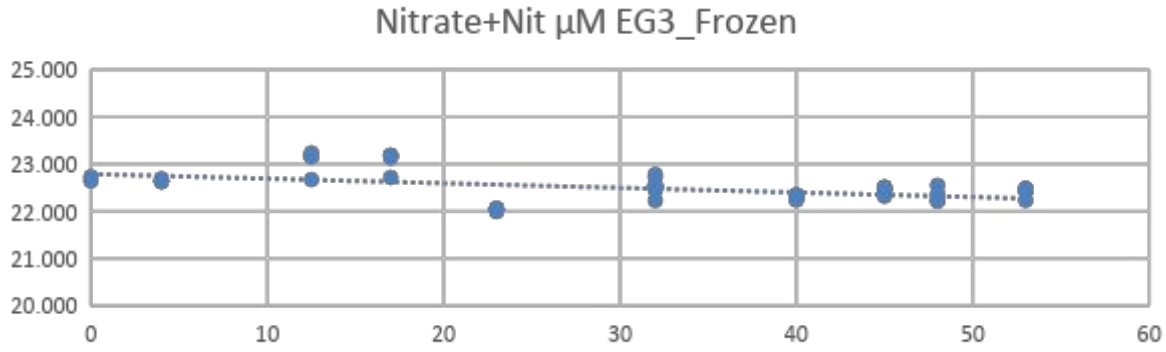
Atlantic Water CRMs were analysed on every run to ensure analytical and data quality and comparisons. (KANSO, Japan).

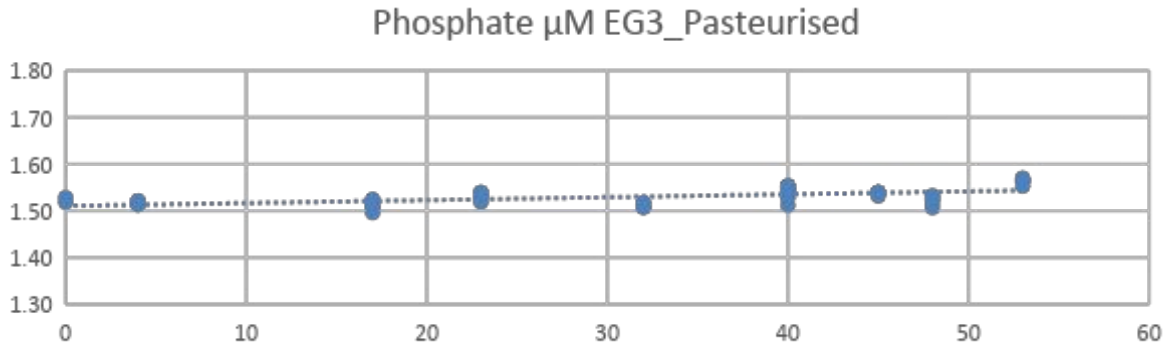
Key Findings
Results

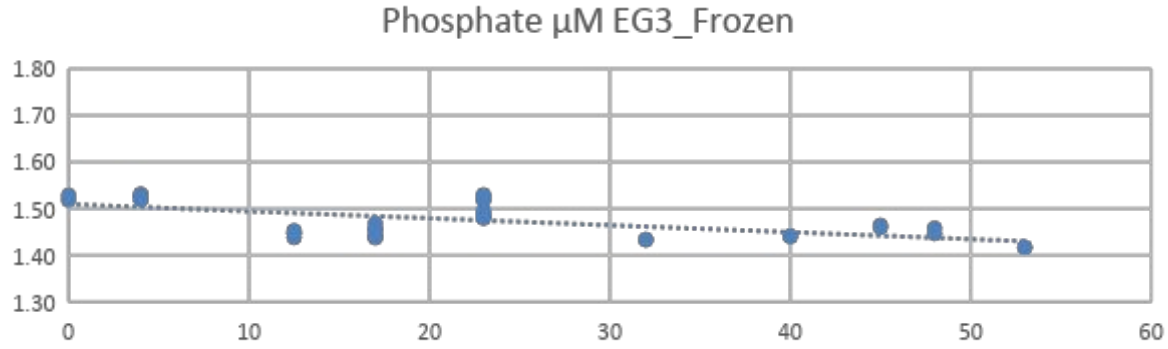
EuroGO-SHIP

Nutrients: EG3 (4824 m)









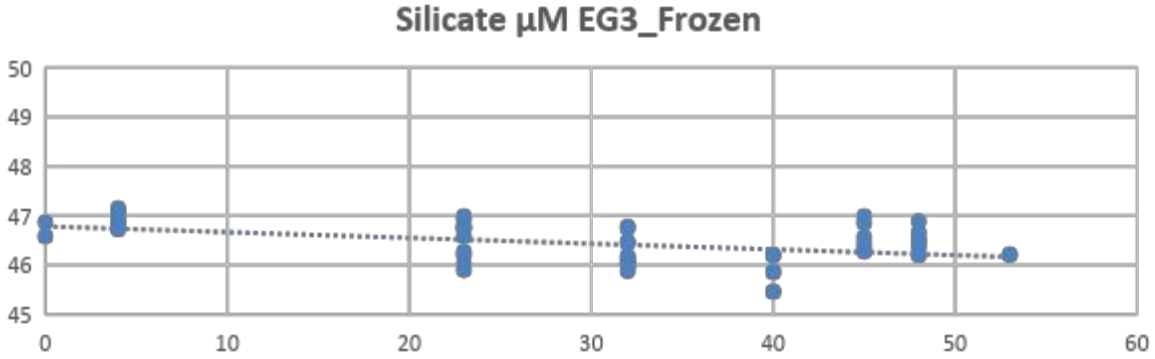
High initial conc, <2% loss for pasteurisation and <1% frozen.

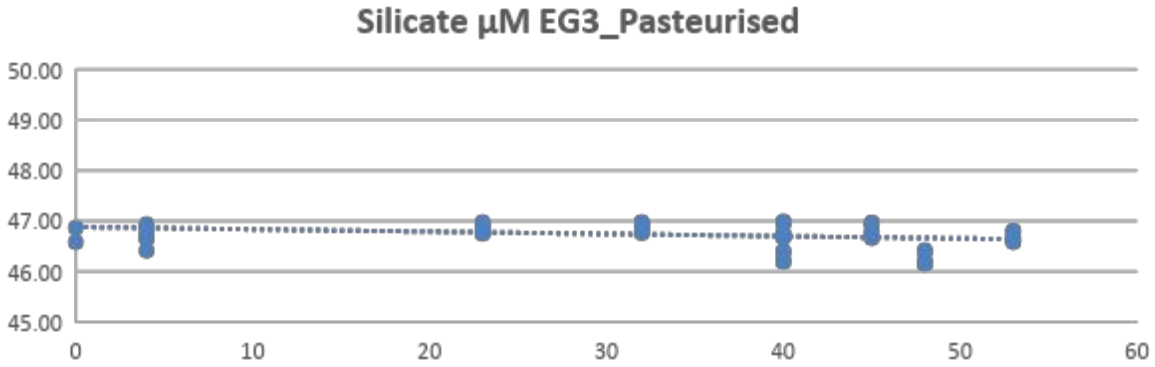
<1% Increase for pasteurisation and ~7% loss frozen.

Key Findings
Results

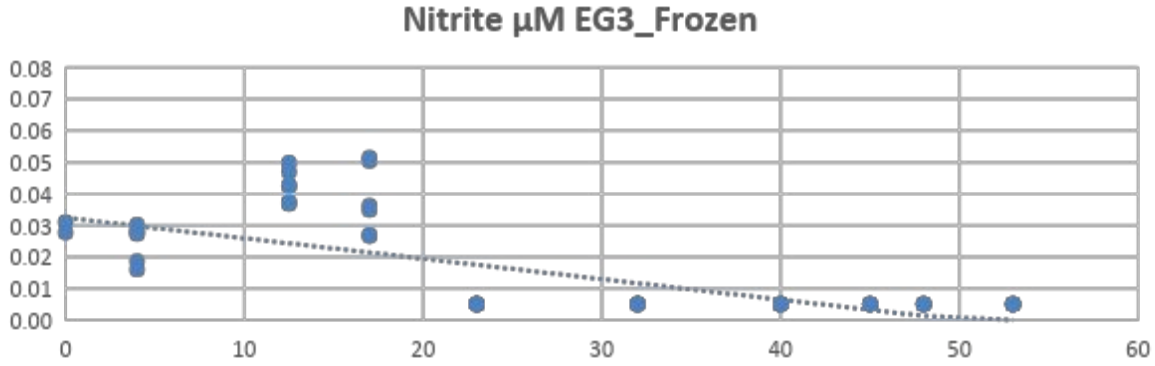
EuroGO-SHIP

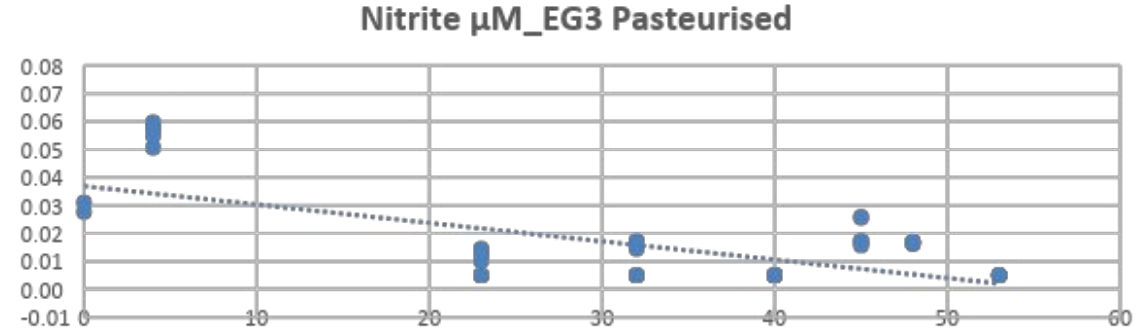
Nutrients: EG3 (4824 m)





Less than 1% loss for both preservation methods.





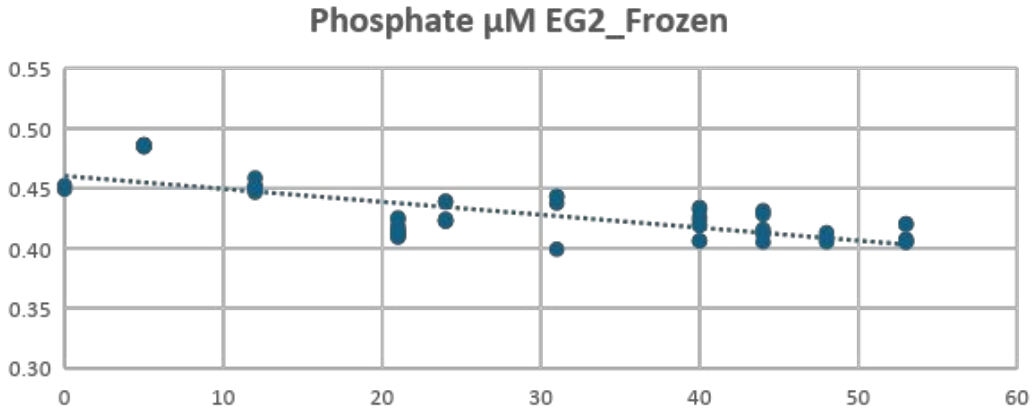
Both losses, large variations in sample bottle duplicates for both, but low concentrations close to the detection limits.

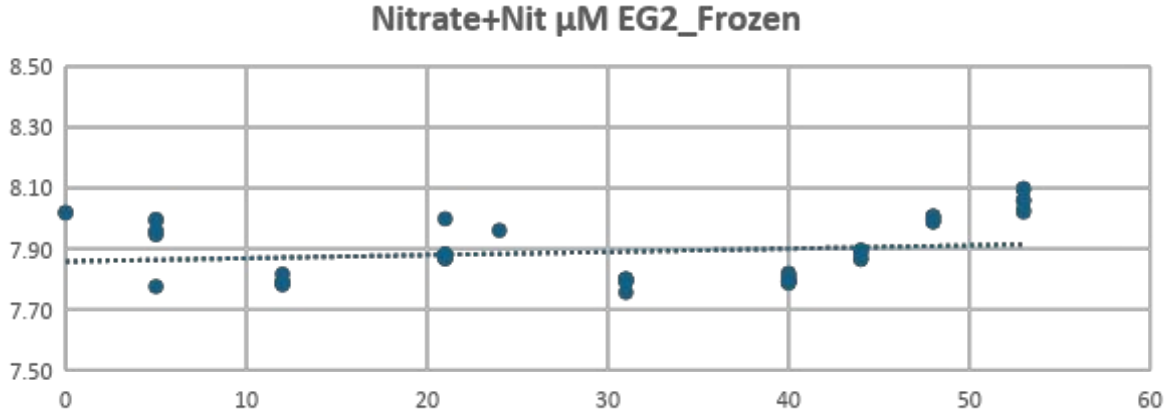
Variations within duplicate samples.

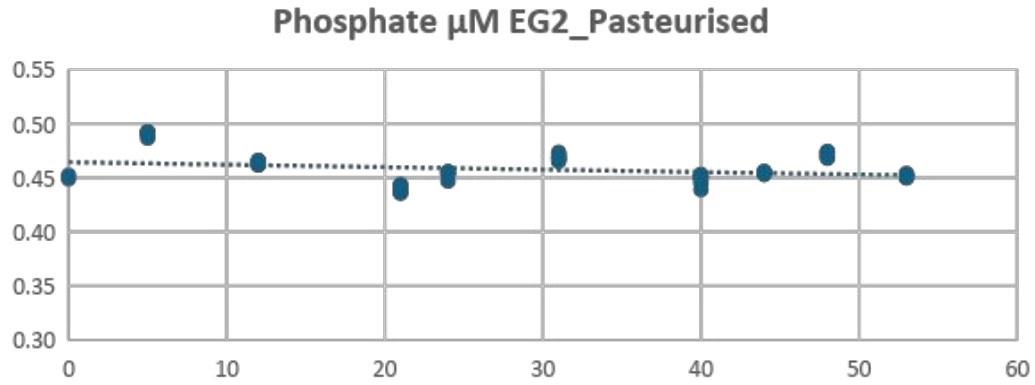
Key Findings
Results

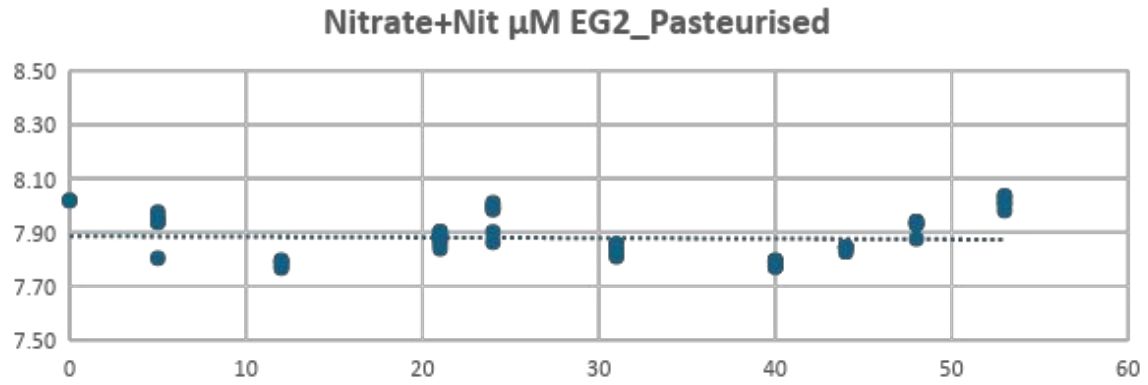
EuroGO-SHIP

Nutrients: EG2 (200 m)









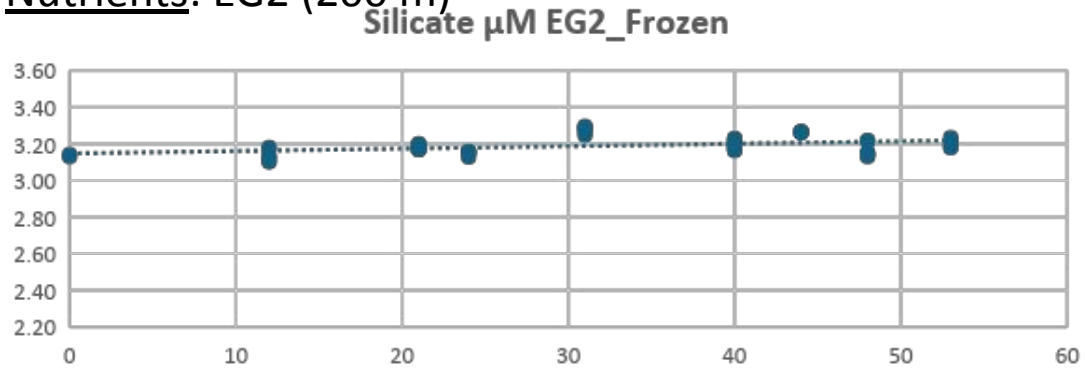
Small overall loss for both, but less for pasteurised.

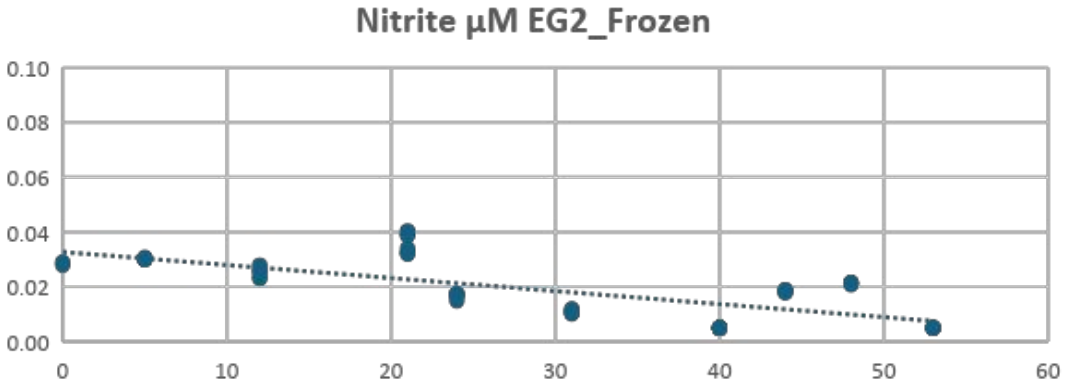
Small increase for frozen for both, less for pasteurised.

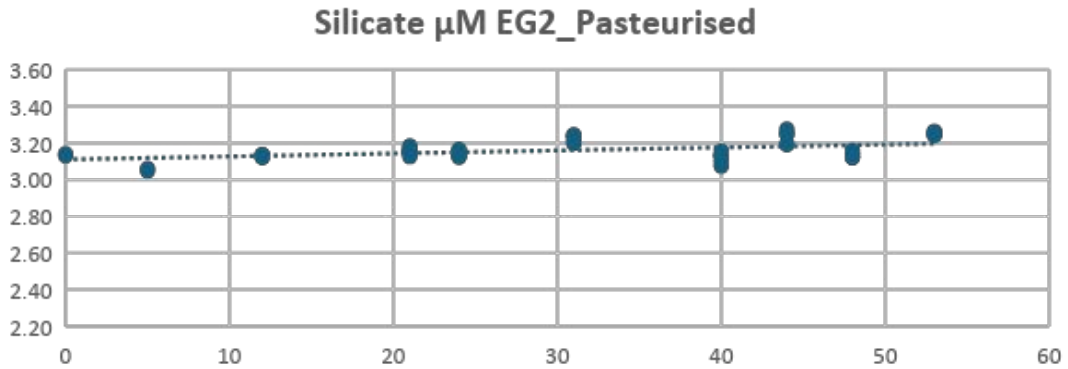
No significant differences, within analytical uncertainty? Variations within duplicate samples.

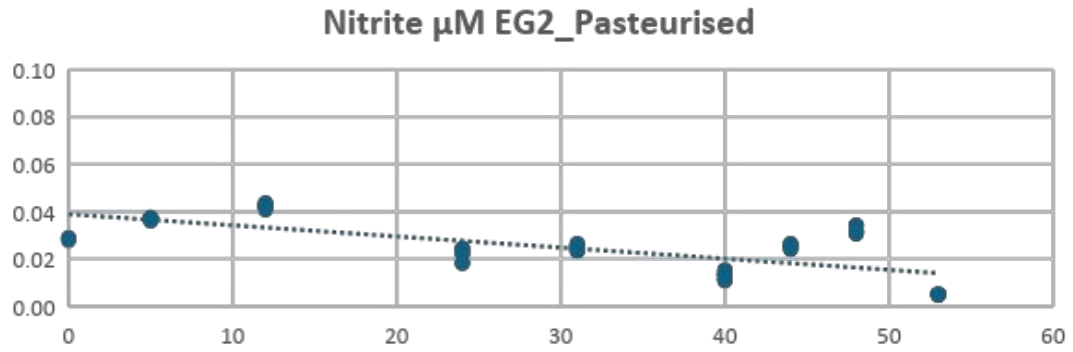
Key Findings
Results

Nutrients: EG2 (200 m)









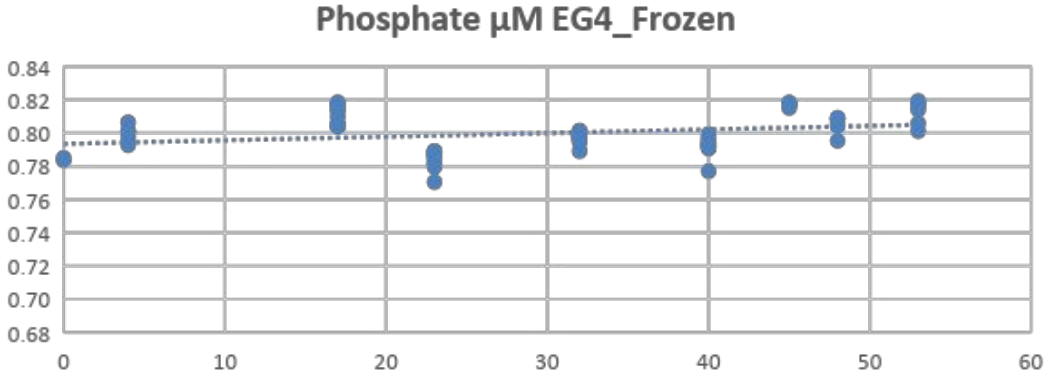
Small overall increases for both.

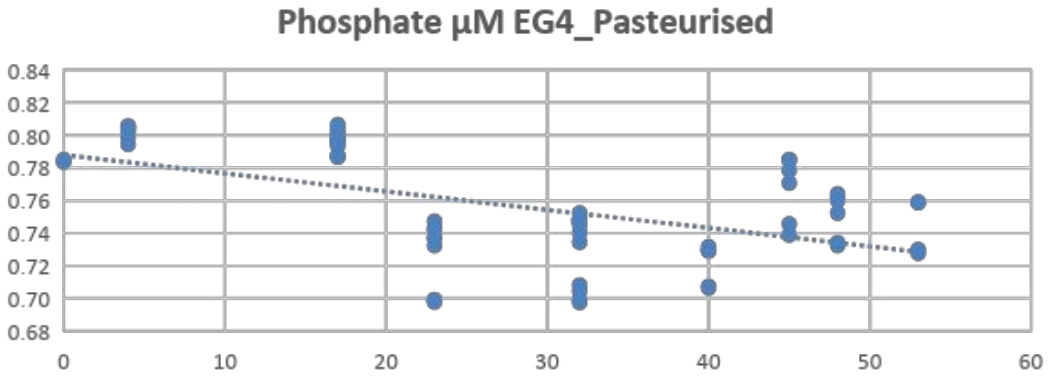
Small losses for both, very low values again.

No significant differences for silicate, within uncertainty? Variations within duplicate samples.

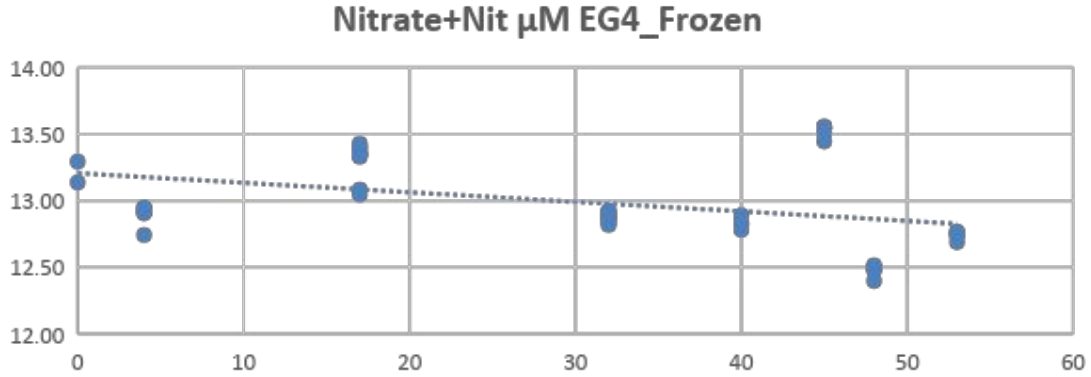
Key Findings
Results

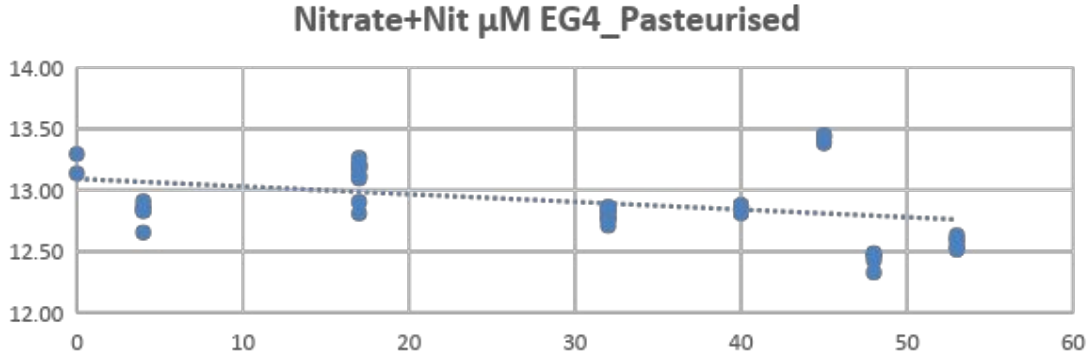
Nutrients: EG4 (500 m)





Small increase for frozen but within a 2-3% variation. Losses for pasteurisation, very poor inter sample repeatability for some.

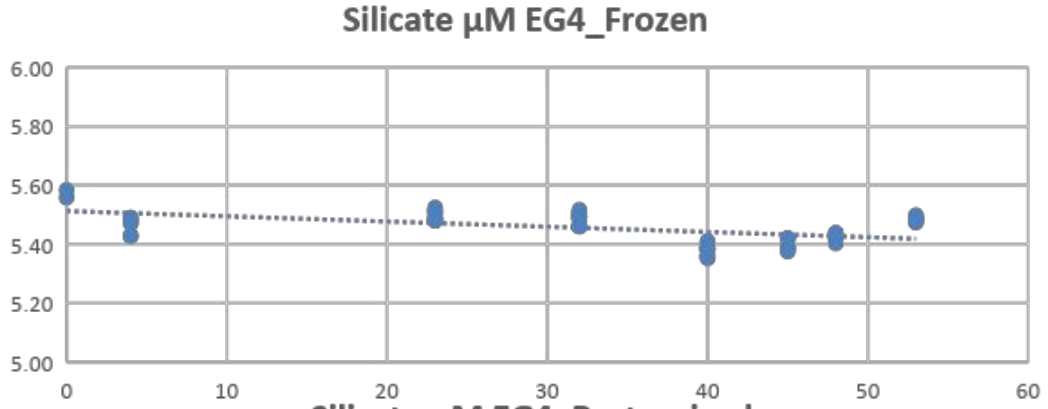


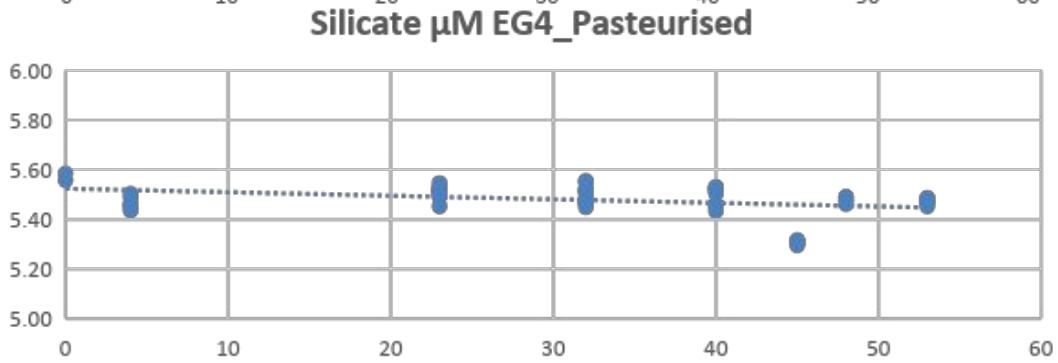


Nitrate: Small overall losses for both, variations with duplicates.

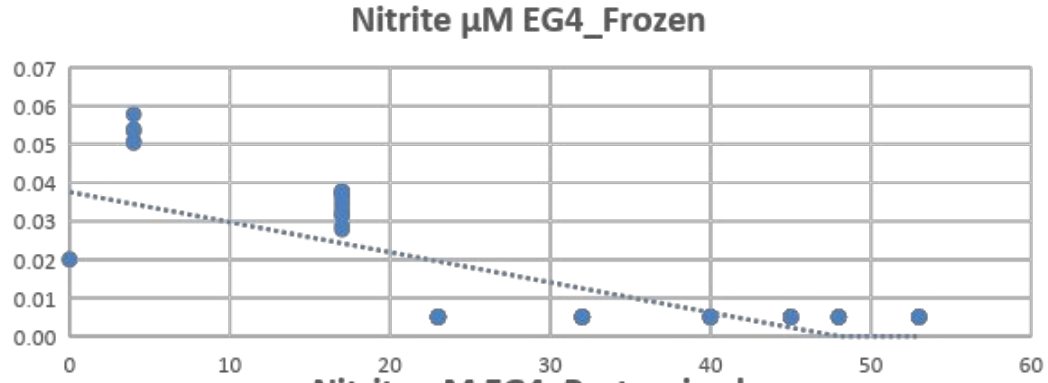
Key Findings
Results

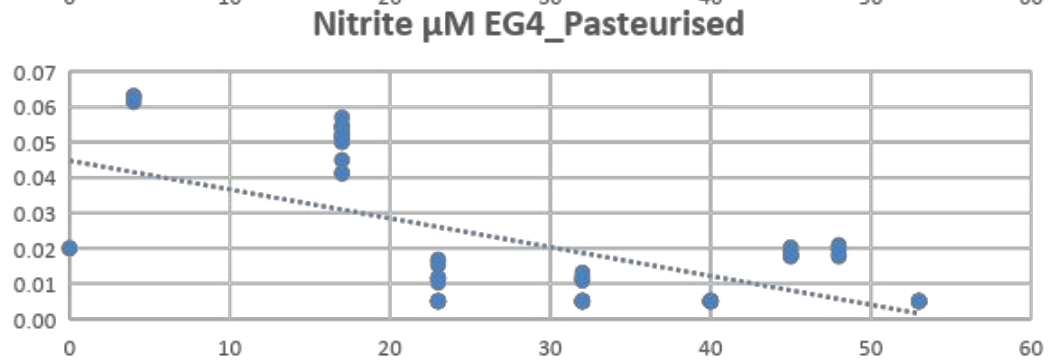
Nutrients: EG4 (500 m)





Small overall decreases for both, within uncertainty.





Losses overall for both, but very low values again.

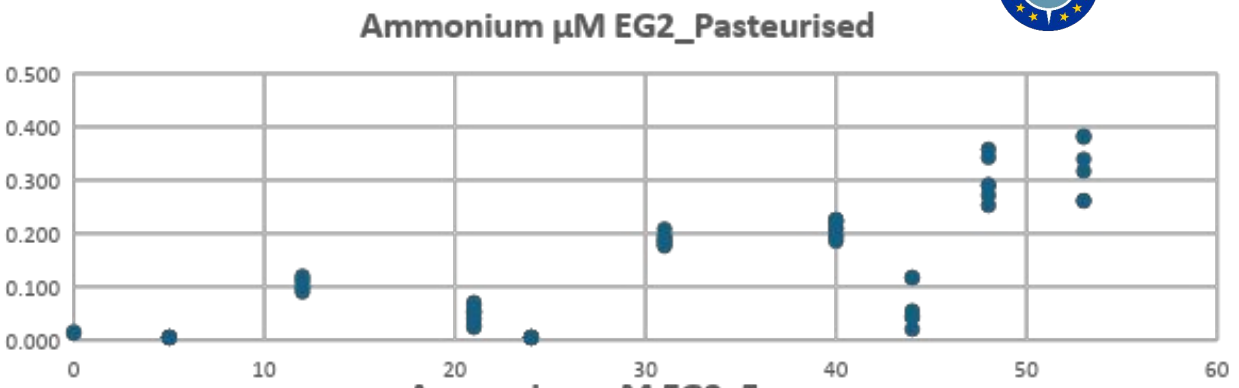
Variations within duplicate samples.

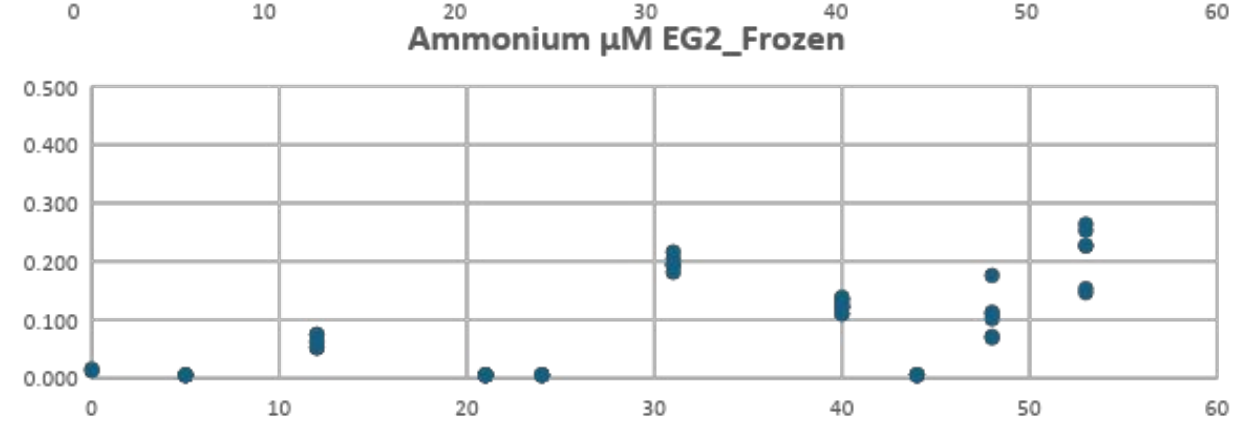
Key Findings

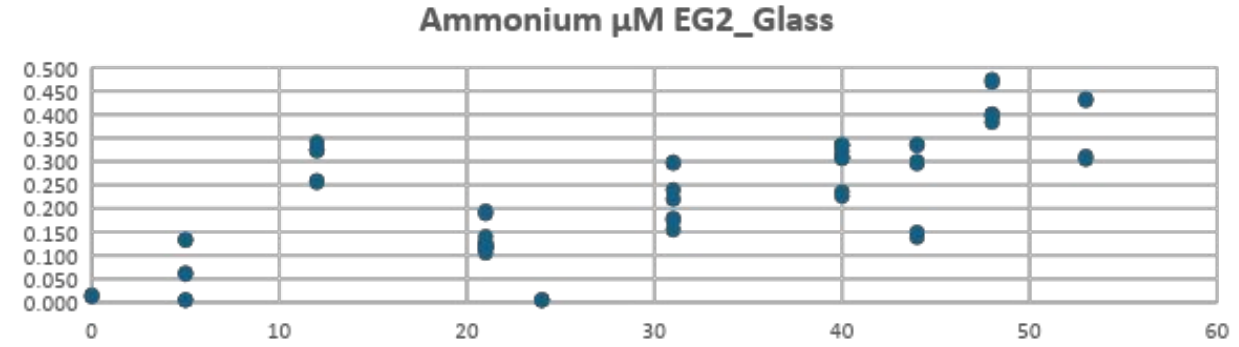
Results

Nutrients: Ammonium: EG2

Large variations in concentrations, overall increasing concentration trend for all. Glass appears to be the worst.





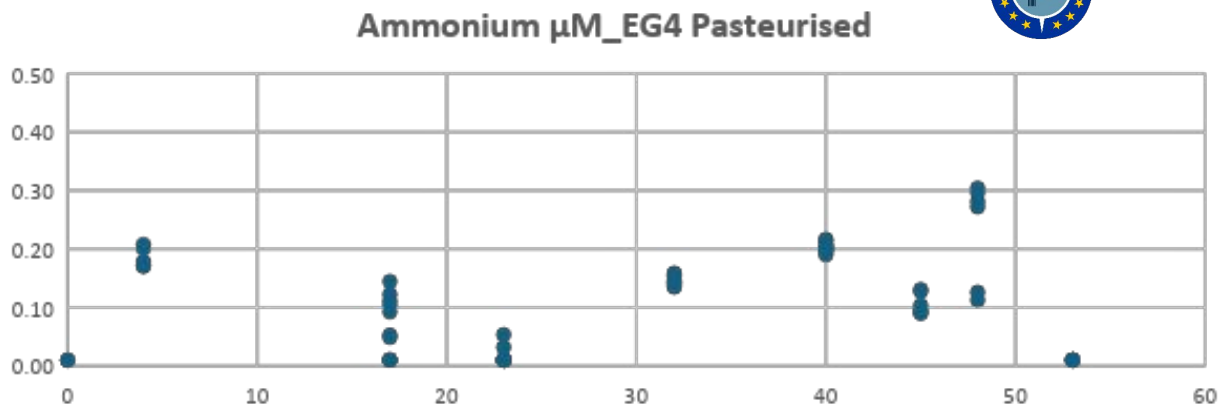


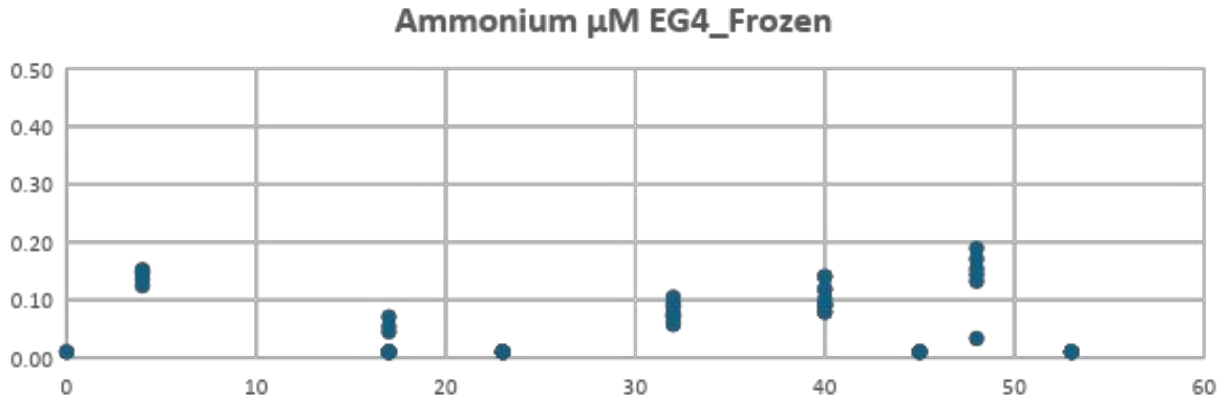
Key Findings

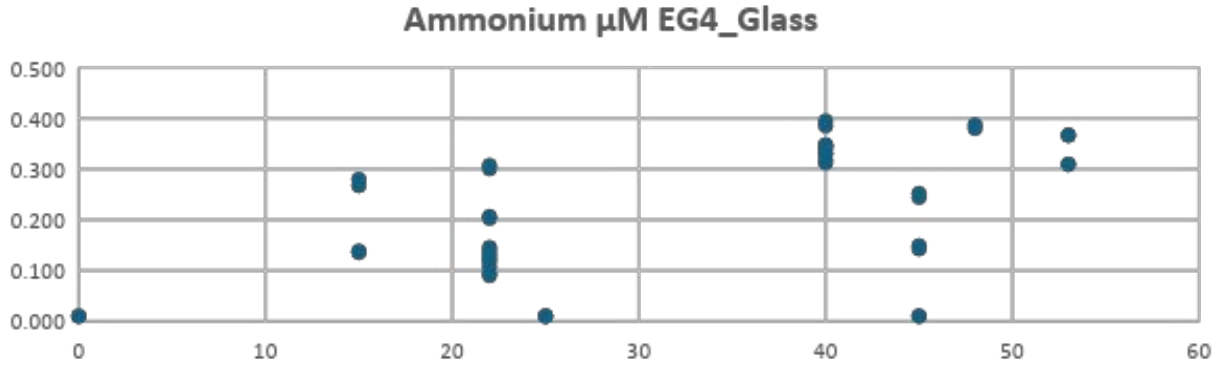
Results

Nutrients: Ammonium: EG4

Large variations in concentrations, overall increasing concentration trend for all. Glass appears to be the worst.







Conclusion

Key Points for Best Practices

- Generally, losses for PO_4^{3-} and NO_3^- for non-filtered open ocean samples are within a 3% error which may be considered acceptable.
- The lower the initial concentrations, the greater the overall percentage change and error.
- Silicate is preserved well by both techniques, loss/gain within 2-3%.
- Nitrite a loss trend for both with ‘big’ variations with duplicates, but the initial concentrations were close to detection limits.
- Ammonium storage for all situations shows an increasing concentration trend for all, poor duplicates, but most initial concentrations are at or below detection limit. Glass vials preferred to HDPE bottles using pasteurisation.
- Presence of particulates/biological material showed the preservation efficiency varies among different nutrients.
- Syringe filtration (0.22 µm) can cause breakage of plankton cells and release nutrients, must use lower pressure.

Conclusion

Key Points for Best Practices

- Enhanced PO_4^{3-} in non-filtered samples suggests dissolution of natural P particulates increase the concentration, maybe due to the high temp and low pH in the analytical analysis method.
- Filtration (low pressure) should always be carried out in sampling of coastal waters. However, pasteurisation increases the PO_4^{3-} where the organic P is high in respect to inorganic P, so in this case, this technique should be avoided.
- In non-filtered: some evidence that pasteurisation increases silicate and PO_4^{3-} concentrations because of dissolution and remineralisation of biogenic silica and/or dissolved organic P. Pasteurisation is ideally carried out on filtered samples.
- Reproducibility of methods of determination varies inversely with the concentrations, but the breakage of plankton cells during filtration and dissolution of suspended/organic matter decrease the analytical precision.

Conclusion

Recommendations for Best Practices

- The current best practice documentation for nutrient analysis and sampling and analytical methodology considerations is found in the GO-SHIP nutrient manual (Becker *et al*., 2020).
- This however only discusses nutrient sample preservation, referring to various published methods (e.g., freezing, pasteurisation, poisoning, acidification, alkalinisation), but does not make recommendation for best practices.
- The two most commonly used methods were therefore used for this EuroGO-SHIP nutrient preservation experiment so as to be able to better advise the European nutrient community as to best practice recommendations for the future.

Conclusion

Recommendations for Best Practices

- When deciding between the two techniques, the availability of a freezer or a reliable large volume oven for sample heating should obviously be considered. Probably, because most labs have readily available freezers, freezing is favoured as the simplest technique involving very little sample manipulation and why generally the favoured method to preserve samples, when it is not possible to analyse at sea.
- A pasteurisation oven must be of suitable size, must be fan operated so as to ensure uniform heating of all the sample bottles to the required 80°C, and it should be initially tested for the continuity of the temperature within all parts of the oven using a temperature probe.
- The considerations of sample shipping need to be considered. If long distances are involved, it may be advantageous to sample quality to use pasteurisation as those samples are easier to ship, rather than keeping frozen samples frozen across time zones for a number of days in transit.

Conclusion

Recommendations for Best Practices

- Wherever possible, analysis of dissolved nutrients should be carried out at sea, or in the field, using fresh samples analysed as soon after collection as possible.
- This is especially true where the nutrient concentrations are depleted in the water and are approaching detection limits of the analytical procedures.
- The long-term results for using freezing and pasteurisation are variable and not one technique of preservation is seen as being superior to the other.
- Generally, decisions about the method chosen will therefore be an individual laboratories choice, depending on the availability of a freezer and/or an oven suitable for the pasteurisation method.
- It will also depend on the reliability of returning frozen samples back from the sampling location, which may well be from a Research Ship docking in a foreign port thousands of miles form home. Pasteurised samples can conveniently be returned along with the rest of the scientific equipment once they have been through the process and are preserved.

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EuroGO-SHIP focused on enabling the European community conducting hydrographic observations at sea to provide higher quality and more sustainable data flows to a broad range of end users, more effectively.
The project worked toward strengthening European capabilities to deliver world-class Oceanographic science, which is key to informing policies and to meet the goals of the EU Mission “Restore our Ocean and Waters by 2030” andthe wider objectives of the EU Green Deal.

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