RC2

1) Summary

The study demonstrates that all tested prokaryoplankton species import and reduce INT. Still, the O_2 consumed and INT reduced ratio is species-dependent, making it impossible to assume a single universal conversion factor.

The authors quantify species-specific toxicity and adjust incubation times (5–20 min) to avoid bias, also showing good agreement between O_2 measured by optodes and by Winkler titration.

In copiotrophs (*Halomonas, Ruegeria, Agrococcus*) the INTR–O2C relationship has a strong linear fit; in oligotrophs and cyanobacteria (SAR11, *Synechococcus, Prochlorococcus*), the fit is significantly weaker, but the linear model lies within the model for natural plankton communities (Fig. 4).

The conclusion is that in situ studies must derive the O2C/INTR relationship locally and check for toxicity in the studied community.

2) Scientific questions

The study addresses the quantification of prokaryotic plankton respiration and the validity of the in vivo INT method.

Novelty

- Comparative multi-taxa dataset under controlled conditions.
- Derivation and comparison of slopes/intercepts per species,
- An operational framework to define toxicity and optimal incubation times.

Conclusions

Conclusions are well supported: no single O2C/INTR factor exists, and studies must derive species-specific relationships (especially in eutrophic systems dominated by copiotrophs).

Methods and assumptions

- Robust, O_2 measured by optodes and Winkler titration, avoiding hypoxia.
- INTR with INT 0.2 mM, killed and media controls, propanol extraction, and calibration curve.
- Quantitative toxicity criterion and choice of incubation.
- Per-cell rates calculated from FC/CFU counts.

Support for results

- Toxicity curves (Appendix A), per-cell rates (Appendix B with Winkler and optodes), and species-specific relationships are presented.
- Table 1 with toxicity, abundance, and O2C/INTR values from replicates per species.

Traceability and reproducibility

Solutions, equipment, calibrations, and regression models.

The authors declare the availability of the data in the public BODC repository and provide a DOI. However, at the time of this review, the dataset could not be accessed through the provided link. Authors are requested to verify the DOI and ensure the data are fully accessible to the public before final publication.

We have double checked that the link works. We also provide another link that links to the webpage were the DOI is also stated:
https://www.bodc.ac.uk/data/published_data_library/catalogue/10.5285/2be8f599-592c-5de2-e063-7086abc02acd/

Credit and originality

Context and limitations of the method (classic ETS, constant O2C/INTR assumption) are well referenced and contrasted; the authors' contribution (species test and comparative analysis) is clearly stated.

Title

Clear. Respiration rates of marine prokaryotes and implications for the in vivo INT method.

Abstract

Complete. States the problem, approach, organisms, main finding (O2C/INTR variability), and methodological implications.

Structure and clarity

Introduction–Methods–Results–Discussion–Conclusions–Appendices. Figures and tables are well integrated with clear cross-referencing.

Language

Technical English is fluent and precise.

Mathematical formulation and symbols

Units are consistent, equations and parameters are defined.

References

Appropriate in number and quality, covering the state of the art.

Supplementary material

Pertinent:

Appendix A (toxicity curves) and Appendix B (per-cell O_2 rates).

3) Major comments

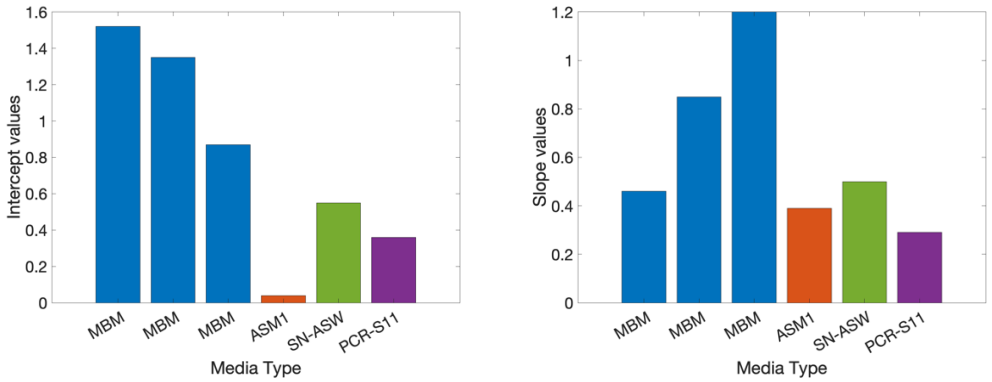
Ecological generalization and growth media

Although the discussion notes possible effects of growth medium and respiratory chain diversity, extrapolation to natural communities could be strengthened with an analysis showing how much slopes/intercepts vary across media.

We have modified the text in the discussion Section 4.1 (line 355) "An analysis of the O_2C/INT_R relationship for each type of media (Appendix C) shows that the highest intercepts correspond to the MBM, middle values to SN-ASW and PCR-SII, and the lowest to ASM1, which is coincident with eutrophic, mesotrophic and oligotrophic environments where the copiotrophs, cyanobacteria and SAR11 respectively are found. From the four types of media, only the MBM was repeatedly used with three species (*Halomonas v.*, *Ruegeria p.* and *Agrococcus l.*), which could partially explain the differences between these copiotrophic species from the mesotrophs and oligotrophs. However, if the media were the main driver of the O_2C/INT_R relationship, then more similarity would be expected

between the copiotrophs and more dissimilarity between the oligotrophs which were grown on three different media. This is the opposite to what was found here."

Added a plot showing how the intercepts and slopes vary across media (Appendix C)



Underlying physiological mechanisms

The discussion proposes several hypotheses (cell wall, AOX, etc.) to explain the observed variability. To unify these ideas, the creation of a conceptual diagram is recommended. This figure would serve as a visual summary, linking the proposed mechanisms with their theoretical effects on the O_2C and INTR.

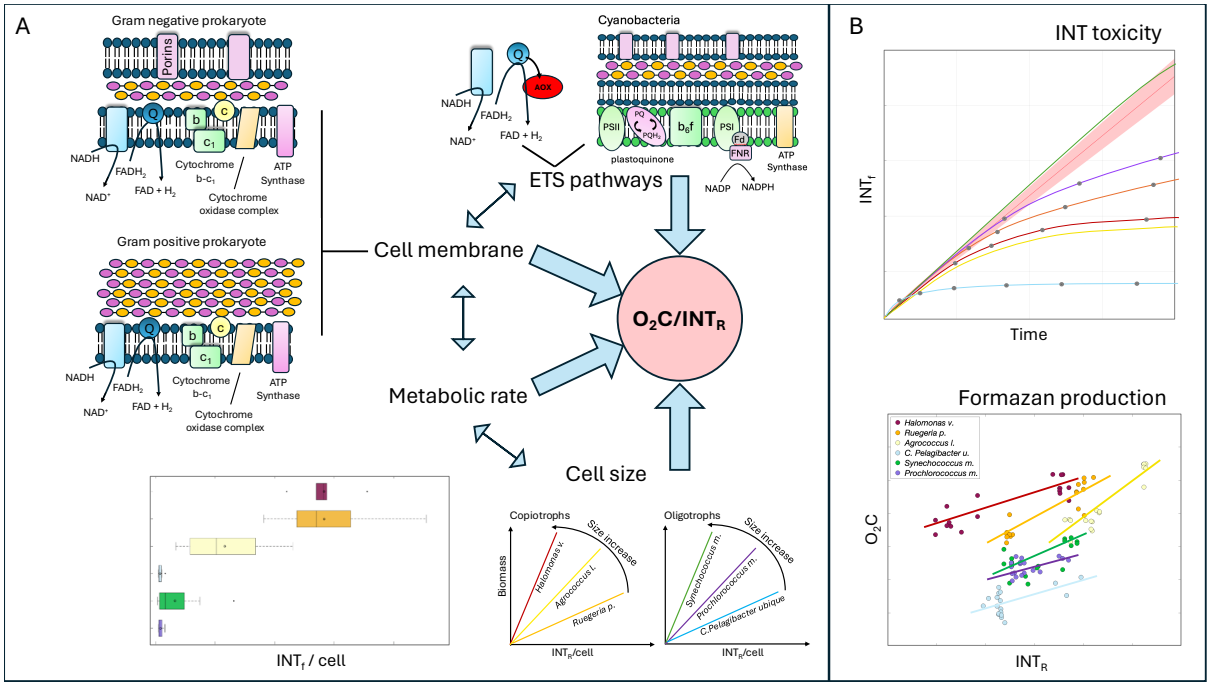


Figure X. A) Main hypothesis of that can explain the O_2C/INT_R relationship. ETS pathways (AOX, respiratory chain of cyanobacteria), cell membrane (Gram negative and Gram positive), metabolic rate, and cell size. B) Representation of the results from this study showing

variability of INT toxicity, and formazan production versus oxygen consumption. Big blue arrows represent the elements that affect O_2C/INT_R relationship. Double arrows represent the interaction between the elements that affect the O_2C/INT_R relationship. Species are represented with colours: *Halomonas venusta* (dark red), *Ruegeria pomeroyi* (orange), *Agrococcus lahaulensis* (clear yellow)*, C. Pelagibacter ubique* (cyan), *Synechococcus marinus* (green) and *Prochlorococcus marinus* (violet).

4) Editorial recommendation

- Accept with minor revisions. The manuscript provides new and relevant evidence for the *in vivo* INT method, and the conclusions are well-supported and do not require new experiments.