

{rokbox title=|Vertical profiles of prokaryotic abundance and heterotrophic prokaryotic activity in the Southern Ocean :: Image: Authors|
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Chie Amano, **Eva Sintes**, Noémie Lebon, Julia Steiger, Danilo Prijovic, Thomas Reinthaler, Ingrid Obernosterer, Kristin Bergauer, Gerhard J Herndl, 2026.

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[-cell heterotrophic activity in deep-ocean prokaryotic communities quantified by BONCAT and microautoradiography](#)

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Abstract: Prokaryotes play a central role in marine biogeochemical cycles, yet quantifying their activity requires sensitive methods, particularly in the deep ocean where their biomass and metabolic rates are low. One widely used method to determine single-cell activity of prokaryotes is bioorthogonal non-canonical amino acid tagging (BONCAT), which offers a non-radioactive approach to measure protein synthesis. However, direct comparisons between BONCAT and radioisotope-based techniques across ocean depth gradients remain limited, particularly for low-activity prokaryotic communities. To address this knowledge gap, we applied BONCAT to quantify single-cell heterotrophic activity in prokaryotic communities from surface to bathypelagic depths (1000–4000 m) in the Southern Ocean near the Kerguelen Islands. Employing picolyl azide-based copper-catalysed click chemistry, we compared BONCAT (L-homopropargylglycine [HPG] incorporation) with microautoradiography (3H-methionine uptake). BONCAT consistently detected active cells throughout the water column, with HPG-derived total fluorescence intensity closely correlating with both microautoradiography ($R^2 = 0.91$, $P < 0.001$) and bulk methionine incorporation ($R^2 = 0.94$, $P < 0.001$). This strong relationship between BONCAT and microautoradiography was maintained into the upper bathypelagic depths, where detecting single-cell activity becomes challenging. Our results demonstrate that BONCAT provides estimates of single-cell heterotrophic activity consistent with microautoradiography in deep-ocean samples, supporting its application as a non-radioactive alternative in low-activity environments.

Keywords: marine biogeochemical cycles, BONCAT, prokaryotic communities, single-cell heterotrophic, microautoradiography.