

Supplementary Material

Cellular uptake and biotransformation of arsenate by freshwater phytoplankton under salinity gradient revealed by single-cell ICP-MS and CT-HG-AAS

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Table S1. Chemical composition of modified C medium and PIV metal used in this study.

Reagent	Concentration
UPW + 35‰ artificial sea water	1 L
Tris(hydroxymethyl)aminomethane	500 mg L ⁻¹
Ca(NO ₃) ₂ · 4H ₂ O	150 mg L ⁻¹
KNO ₃	100 mg L ⁻¹
KH ₂ PO ₄	30.7 mg L ⁻¹ (225.6 µmol L ⁻¹) 136.1 µg L ⁻¹ (1.0 µmol L ⁻¹)
MgSO ₄ · 7H ₂ O	40 mg L ⁻¹
Vitamin B12 stock solution	1 µg L ⁻¹
Thiamine-HCl stock solution	1 µg L ⁻¹
Biotin stock solution	1 µg L ⁻¹
PIV metal	3 mL L ⁻¹
Chemical composition of PIV metal	
Na ₂ EDTA · 2H ₂ O	1 g L ⁻¹
FeCl ₃ · 6H ₂ O	196 mg L ⁻¹
MnCl ₂ · 4H ₂ O	36 mg L ⁻¹
ZnSO ₄ · 7H ₂ O	22 mg L ⁻¹
CoCl ₂ · 6H ₂ O	4 mg L ⁻¹
Na ₂ Mo ₄ · 2H ₂ O	2.5 mg L ⁻¹

Table S2. Chemical composition of artificial seawater used in this study.

Reagent	Composition
NaF	2.9 mg L ⁻¹
H ₃ BO ₃	25 mg L ⁻¹
SrCl ₂ · 6H ₂ O	12.2 mg L ⁻¹
KBr	100 mg L ⁻¹
NaHCO ₃	196 mg L ⁻¹
KCl	683 mg L ⁻¹
CaCl ₂ · 2H ₂ O	1.4346 g L ⁻¹
NaCl	23.954 g L ⁻¹
Na ₂ SO ₄	4.004 g L ⁻¹
MgCl ₂ · 6H ₂ O	10.787 g L ⁻¹

Table S3. Device conditions and optimization requirements for SC-ICP-MS.

Experimental condition		Performance check	
Nebulizer gas flow rate	0.45 L min ⁻¹	Be	>4000
Makeup gas flow rate	0.7 L min ⁻¹	In	>30000
Dwell time	50 μ s	U	>2000
Stabilization time	0 μ s	Ce ^{2+/} Ce	$\leq$ 0.03
Measurement mode	Oxygen DRC	CeO	$\leq$ 0.025
Target molecule	AsO		
Oxygen gas flow rate	0.5 L min ⁻¹		
AFT	250 V		

Table S4. Cell status of *S. paradoxum* and *P. duplex* under salinity stress (*S. paradoxum*, 1‰; *P. duplex*, 2‰).

<i>S. paradoxum</i> (1‰)			<i>P. duplex</i> (2‰)		
State	Condition	Fragmentation (%)	State	Condition	Fragmentation (%)
A	Normal	0.0	A	Normal	11.0
B	Shortening of a cell protrusion	18.3	B	Farmless/ Unstructured	37.6
C	Swelling	29.3	C	Aggregation	
D	Karyorrhexis	43.6	D	Initial stage of swelling	27.8
E	Pyknosis		E	Color phase change	
F	Karyolysis, Karyorrhexis	8.7	F	Late stage of swelling	24.2

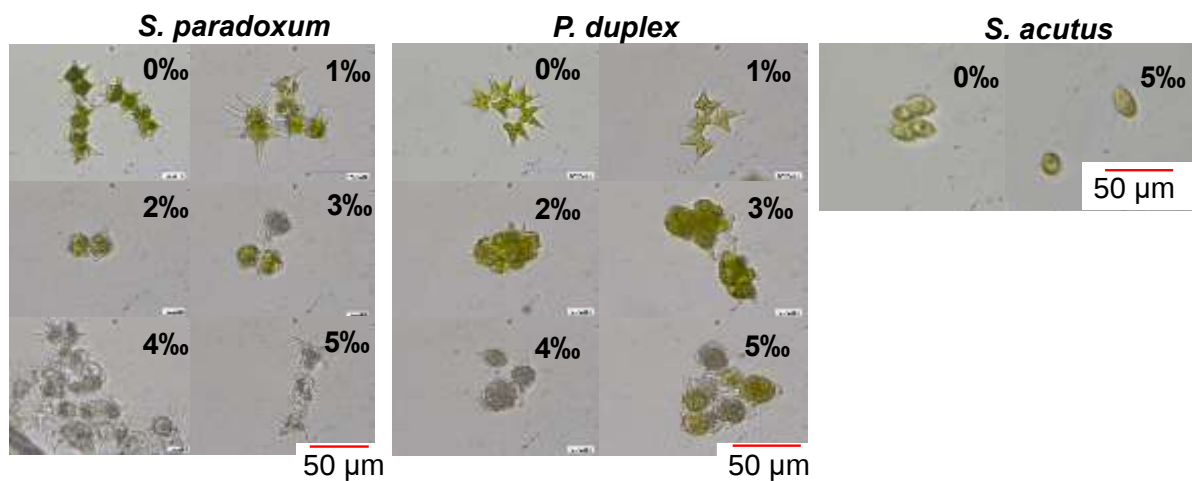


Fig. S1. Microscopic images of three phytoplankton species, (a) *S. paradoxum*, (b) *P. duplex*, and (c) *S. acutus*, at varying salinity gradients on 14th day of culture. Culture conditions: As^{V} , $0.1 \mu\text{mol L}^{-1}$ and PO_4^{3-} , $1.0 \mu\text{mol L}^{-1}$.

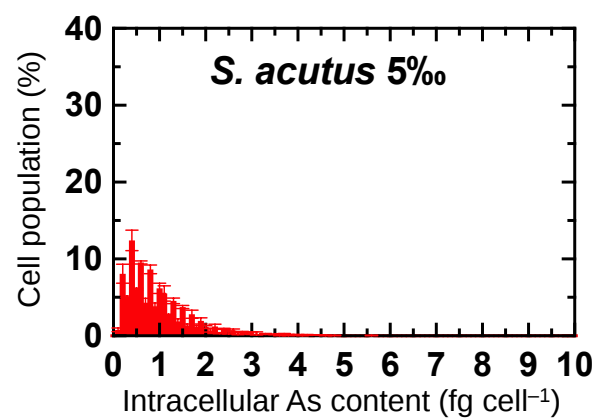


Fig. S2. As mass distribution histograms of *S. acutus* under 5 ‰ salinity stress conditions at 14 days culture. Culture condition: As^V, 0.1 μmol L⁻¹ and PO₄³⁻, 1.0 μmol L⁻¹.

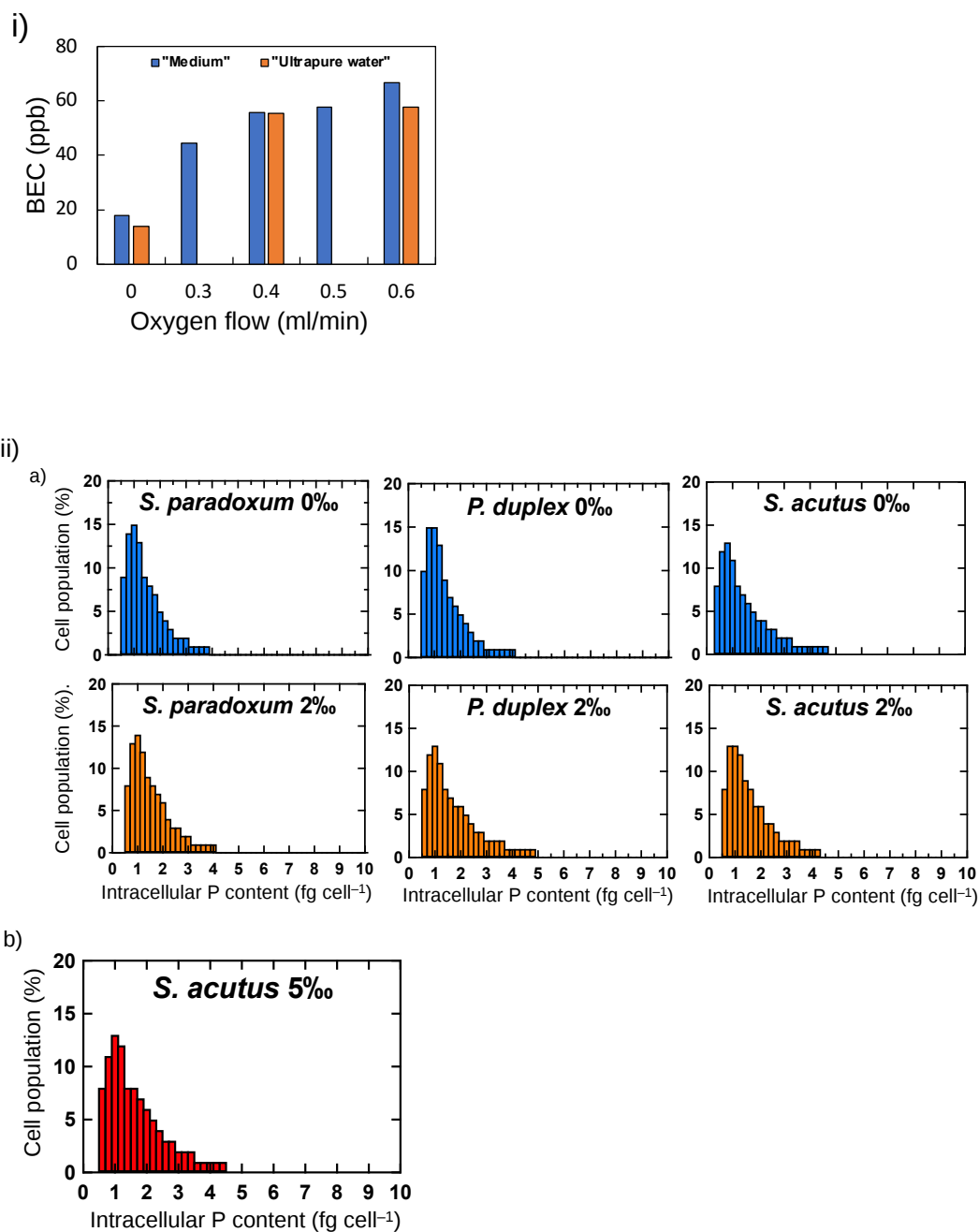


Fig. S3. i) Calibration curve optimization and sensitivity comparison for phosphorus quantification in single cells. The graph illustrates the background equivalent concentration (BEC) obtained through the optimization of measurement conditions. ii) Phosphorus mass distribution histograms of three freshwater phytoplankton under different salinity stress conditions at 14 days culture. a) *S. paradoxum*, *P. duplex*, and *S. acutus* (0 and 2‰ salinity levels), b) *S. acutus* (5‰ salinity level). Culture condition: PO_4^{3-} , $1.0 \mu\text{mol L}^{-1}$.

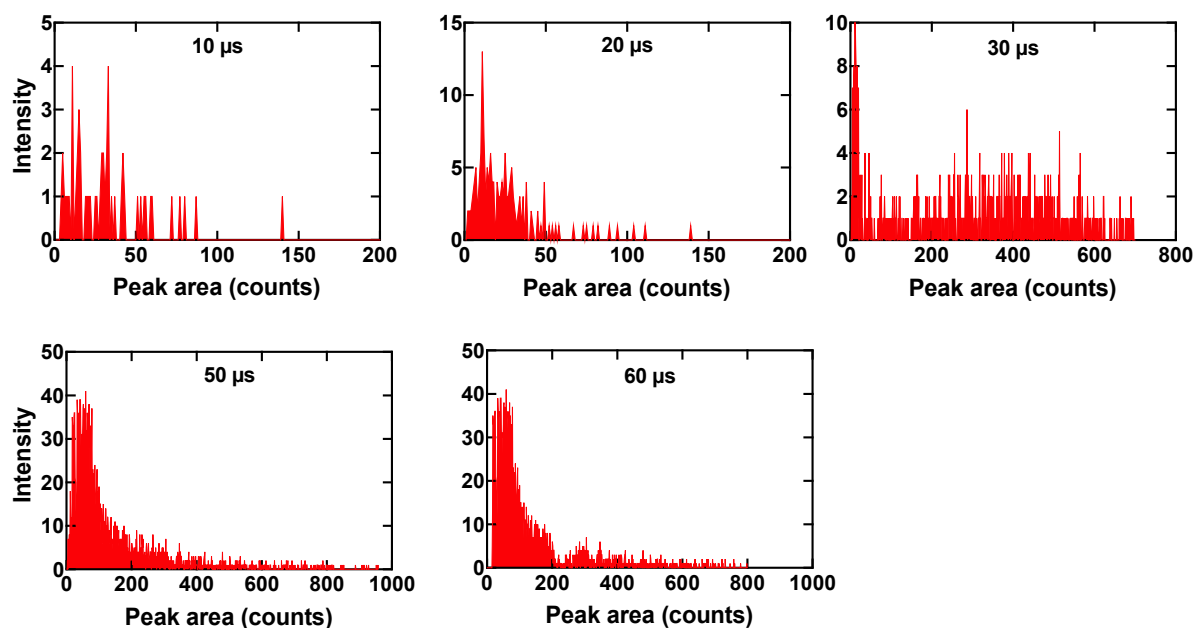


Fig. S4. Time-resolved measurements of arsenic (As) during SC-ICP-MS experiments at 0‰ salinity of *S. acutus*. The graph depicts the intensity of arsenic signals as a function of peak area (counts) obtained at various dwell times (10, 20, 30, 50, and 60 microseconds) at 14 days culture. The y-axis represents signal intensity, while the x-axis represents the peak area. Culture condition: As^{V} , $0.1 \mu\text{mol L}^{-1}$ and PO_4^{3-} , $1.0 \mu\text{mol L}^{-1}$.

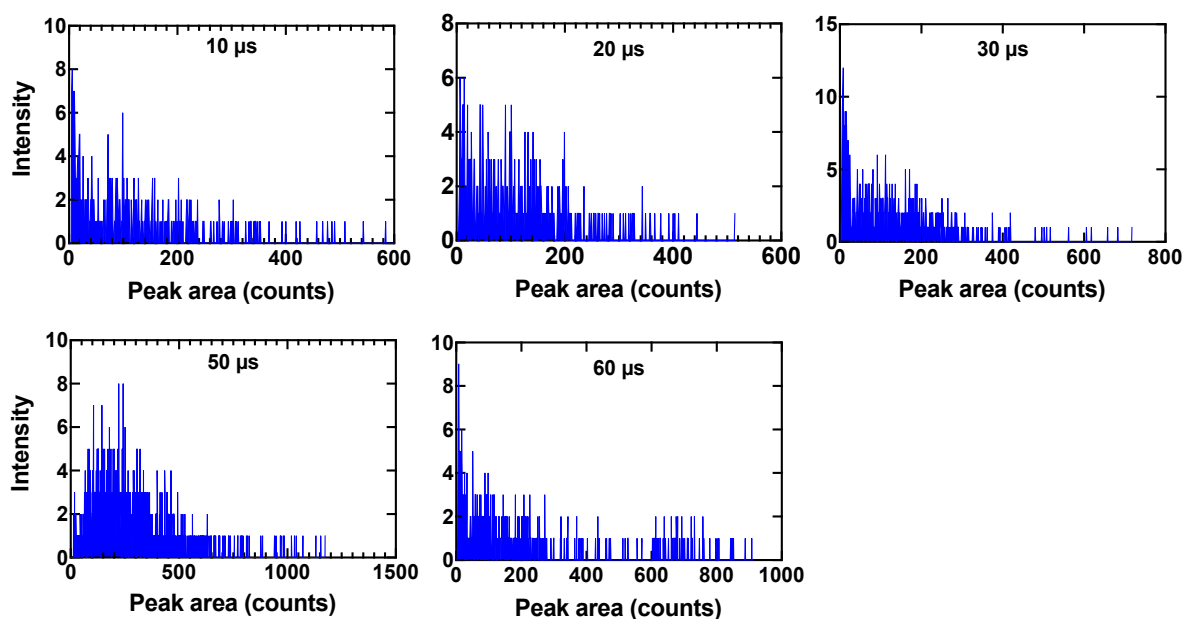


Fig. S5. Time-resolved measurements of phosphorus (P) behavior during SC-ICP-MS experiments at 0‰ salinity of *S. acutus*. The graph showcases the intensity of phosphorus signals as a function of peak area (counts) recorded at different dwell times (10, 20, 30, 50, and 60 microseconds) at 14 days culture. The y-axis denotes signal intensity, while the x-axis represents the peak area. Culture condition: PO_4^{3-} , $1.0 \mu\text{mol L}^{-1}$.