The effect of temperature and salinity on growth rate and azaspiracid cell quotas in two strains of *Azadinium poporum* (Dinophyceae) from Puget Sound, Washington State

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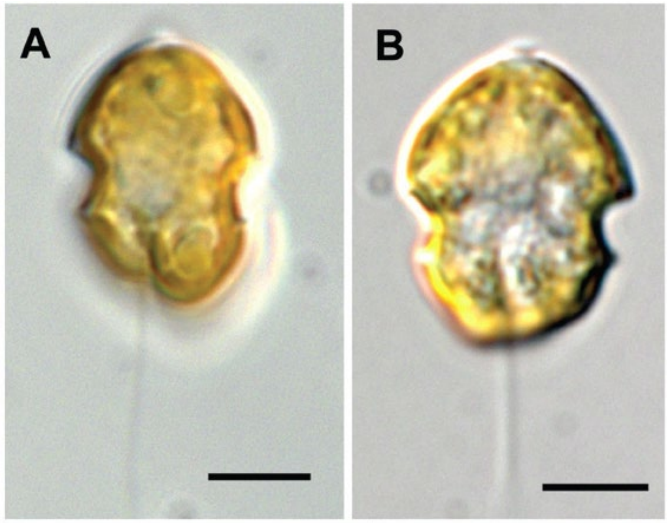
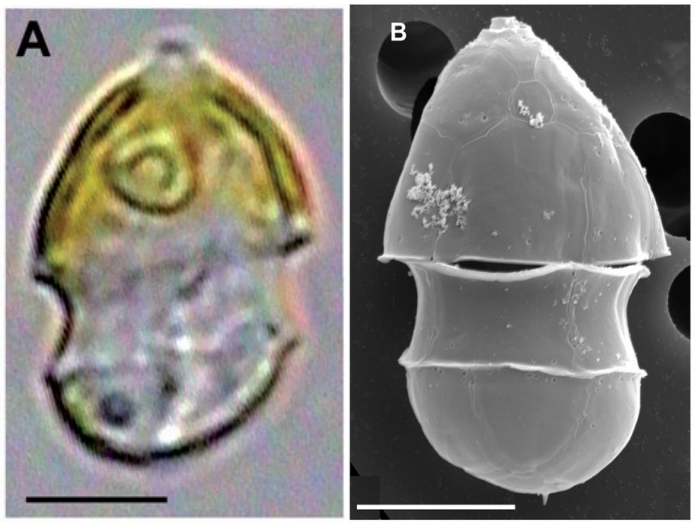
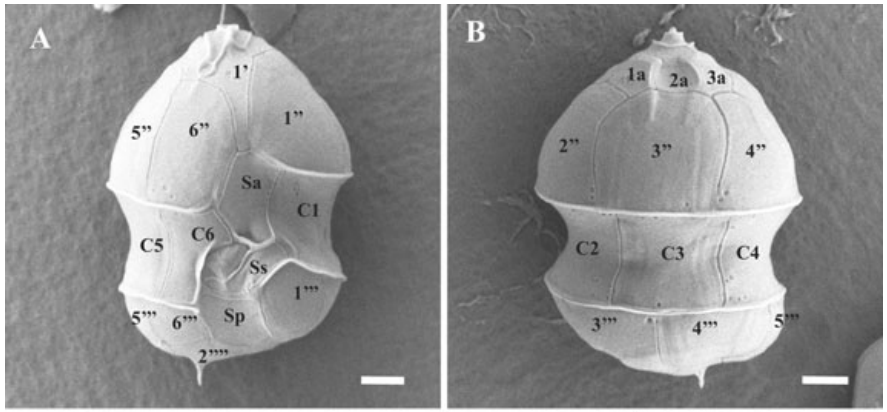
Azaspiracid (AZA) producer



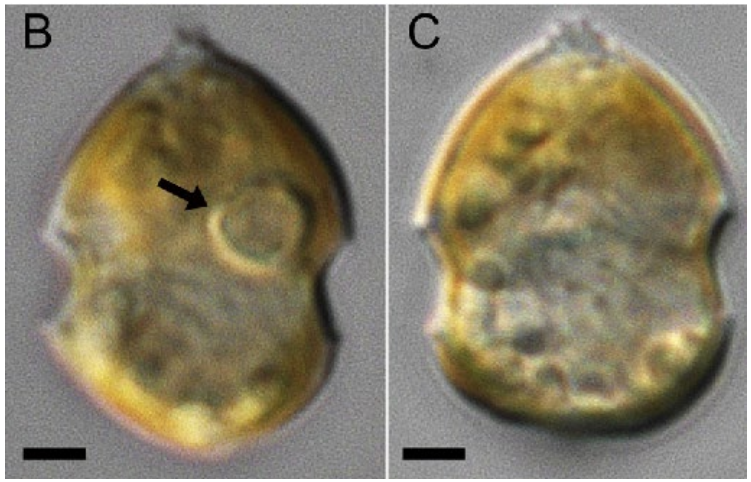
A. spinosum



A. poporum



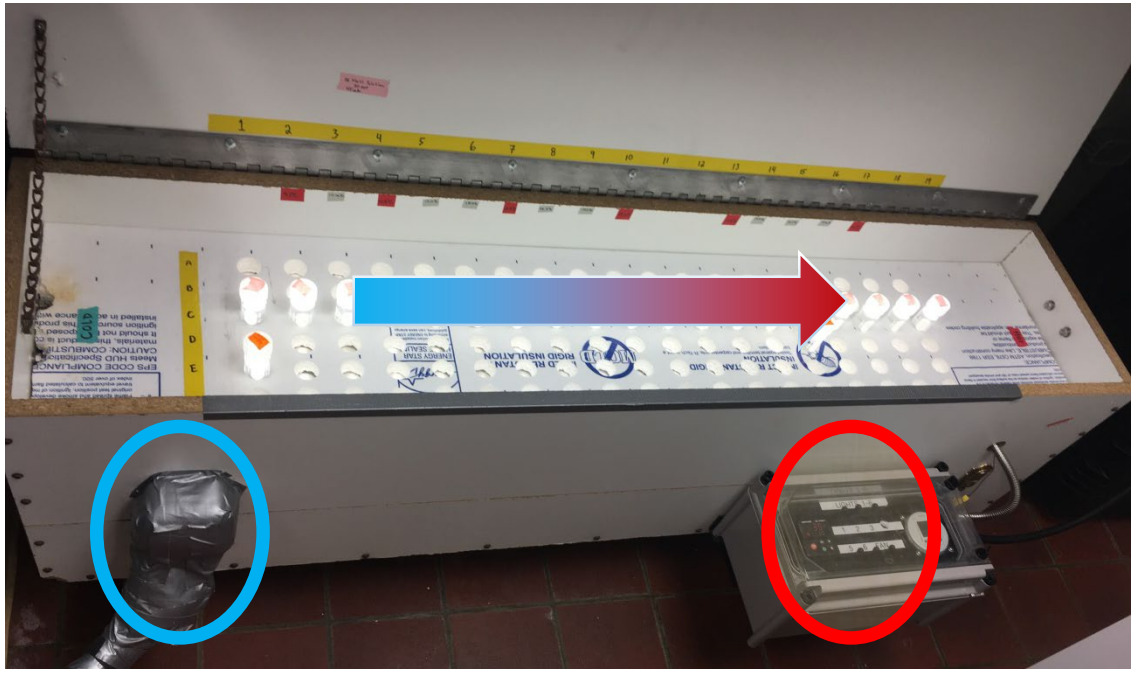
A. dexteroporum



Amphidoma languida

Sediment collected site(16 Jan 2016)

Temperature Gradient Bar



- Chilling/heating elements
- 6 x 19 (114 total) 50ml tubes
- Light/dark cycle: 12/12hr

Light intensity

PPFD:μE/m^2/s
Volume:30mL seawater in 50 mL tube
Measure at center of water column

	1	2	3	4	5	6	7	8	9
A	288	311	320	325	353	342	361	331	344
B	383	416	408	475	436	448	470	459	465
C	430	460	455	465	475	477	489	471	499
D	387	450	453	456	461	486	510	468	478
E	378	420	436	435	466	447	464	456	456
F	297	321	327	339	341	342	381	369	351

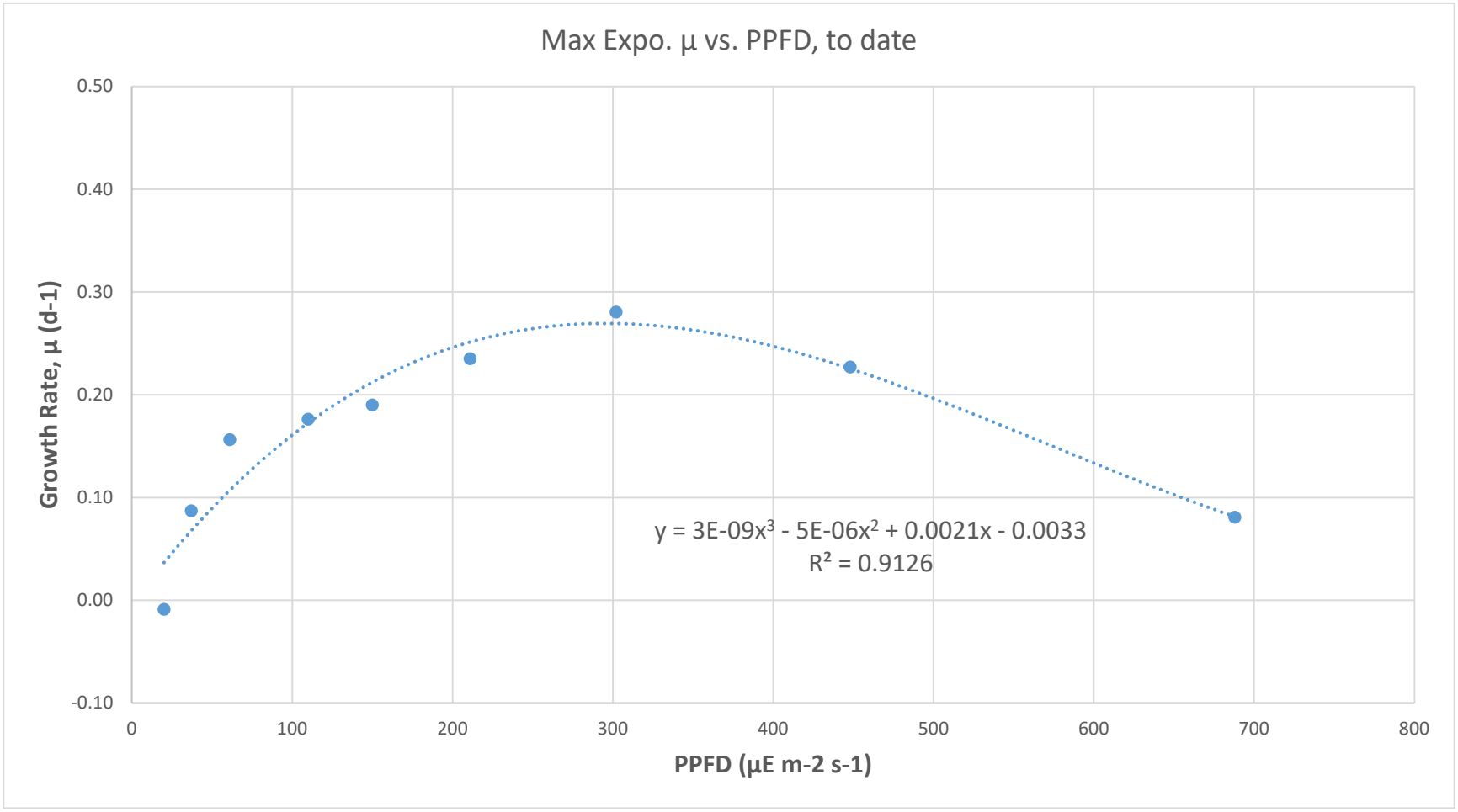
10	11	12	13	14	15	16	17	18	19
334	368	355	360	355	369	360	368	369	320
453	445	466	469	440	475	453	470	472	439
489	473	464	478	481	456	488	471	488	434
487	494	474	473	477	432	484	485	493	433
462	425	424	435	426	434	453	435	445	428
364	324	358	342	336	341	341	355	341	314

Experiment of the effect of light intensity on growth of *Azadinium poporum*

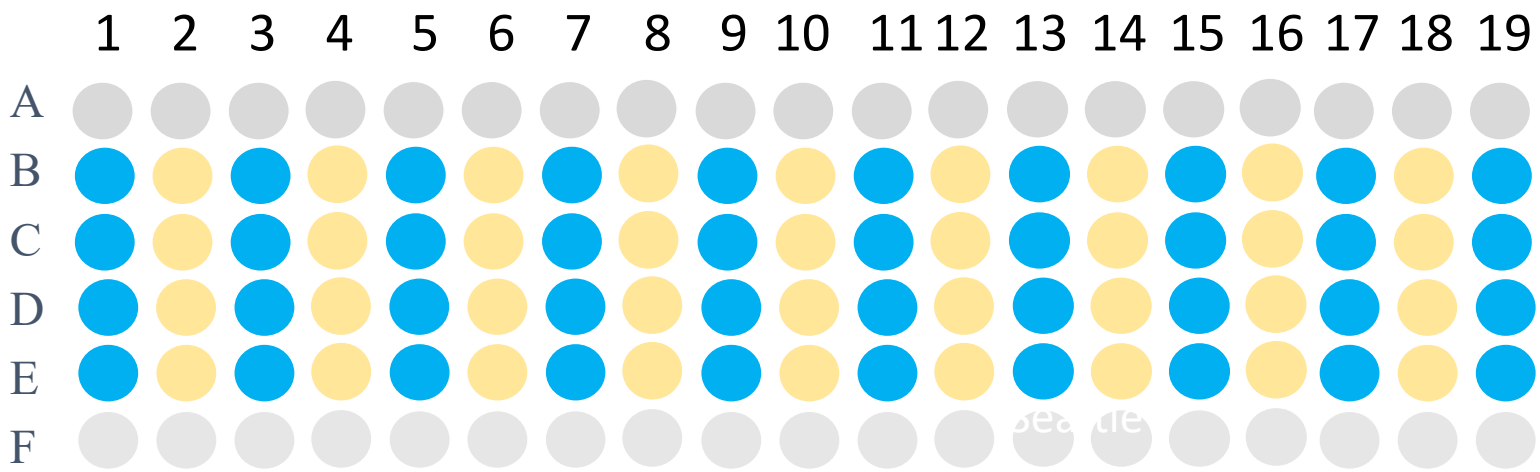
Light intensity gradient $\mu E/m^2/s$

	1	2	3	4	5	6	7	8	9
Tube1	685	446	298	209	150	107	60	38	20
Tube2	690	450	305	212	150	112	62	36	20

The relationship between light intensity and growth rate of *A. poporum* (NWFSC1011)

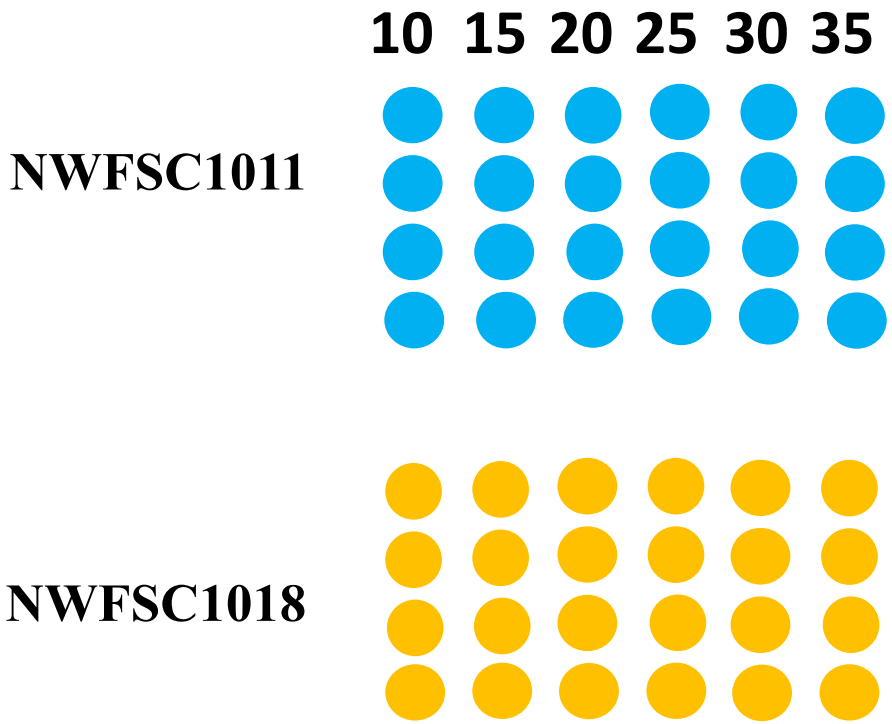


TGB experiment design



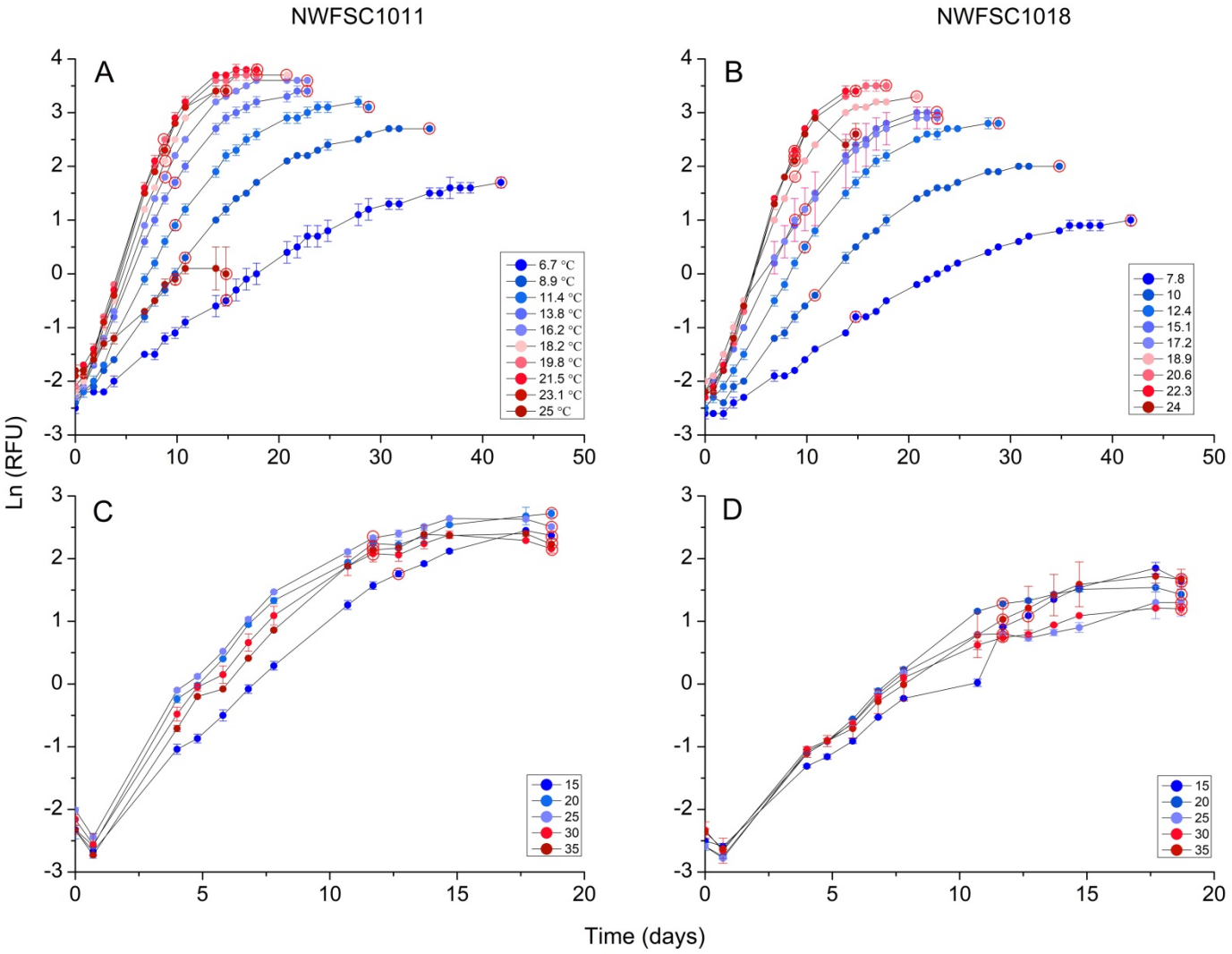
Blue for NWFSC1011 Yellow for NWFSC1018

Salinity Experiment design



qPCR to detect *A. poporum* during 2016-2018

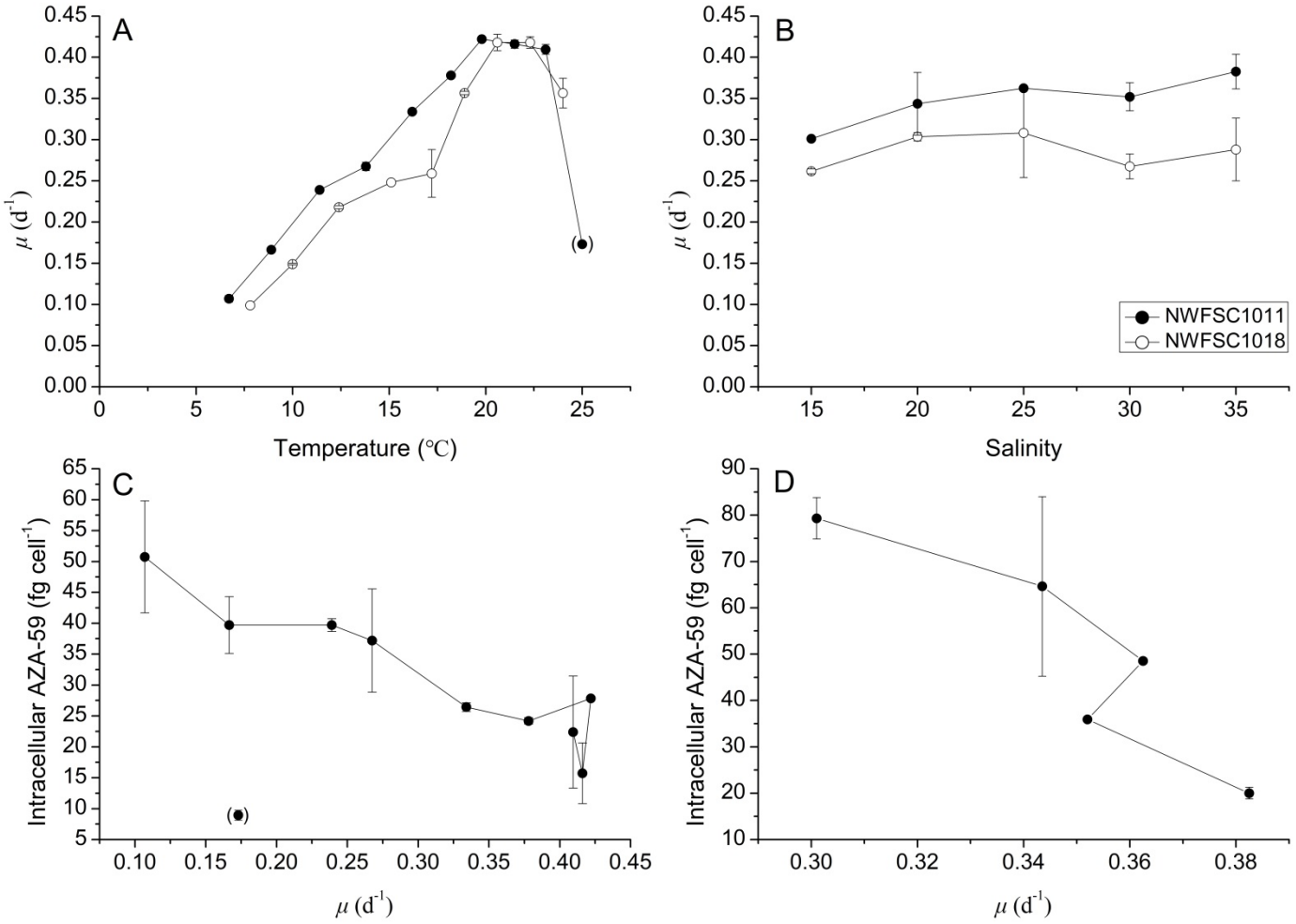
Growth curve



Stationary phase

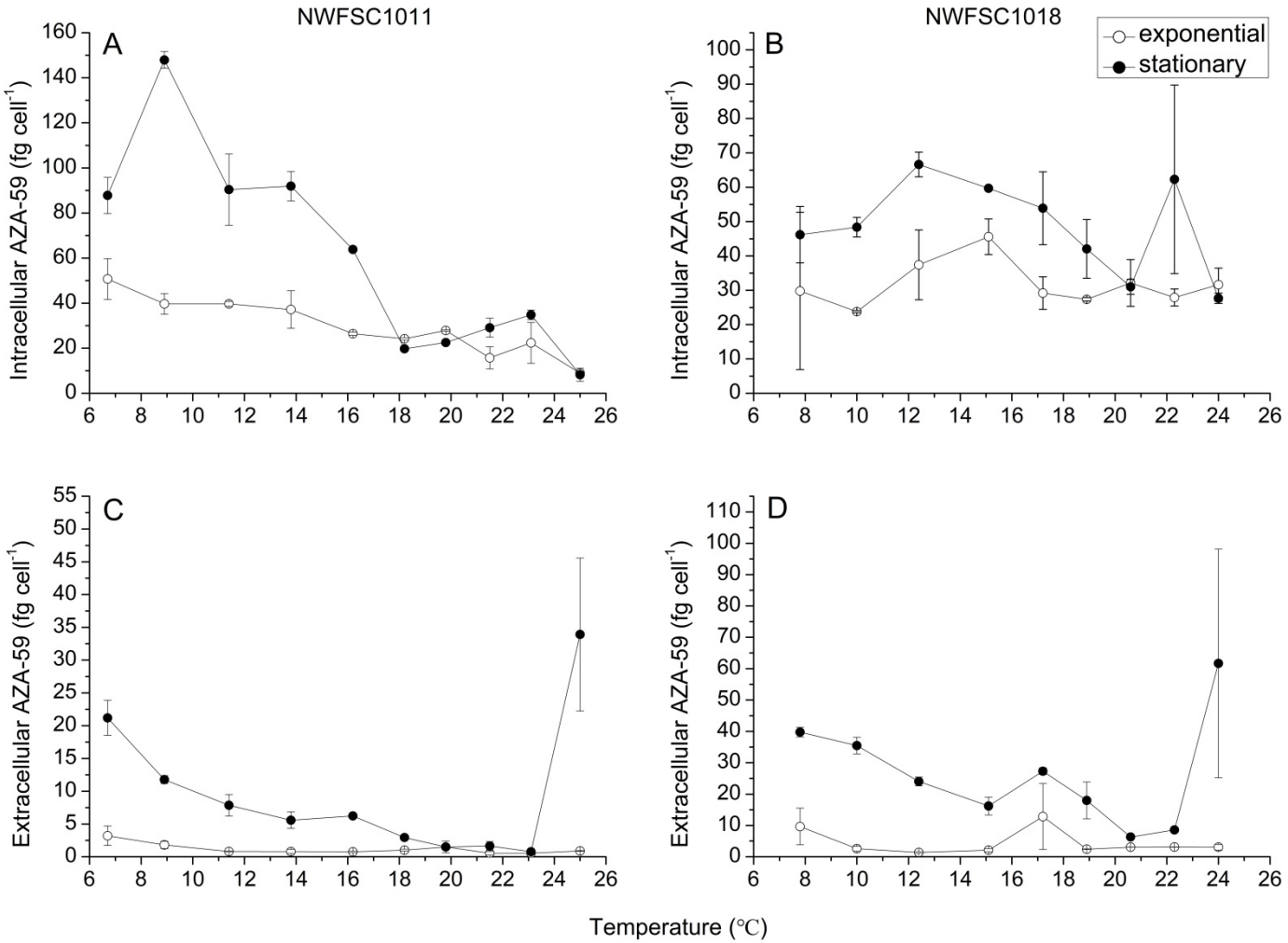
Cell abundance indicated by Ln(RFU)

Growth rate and intracellular AZA-59 quota



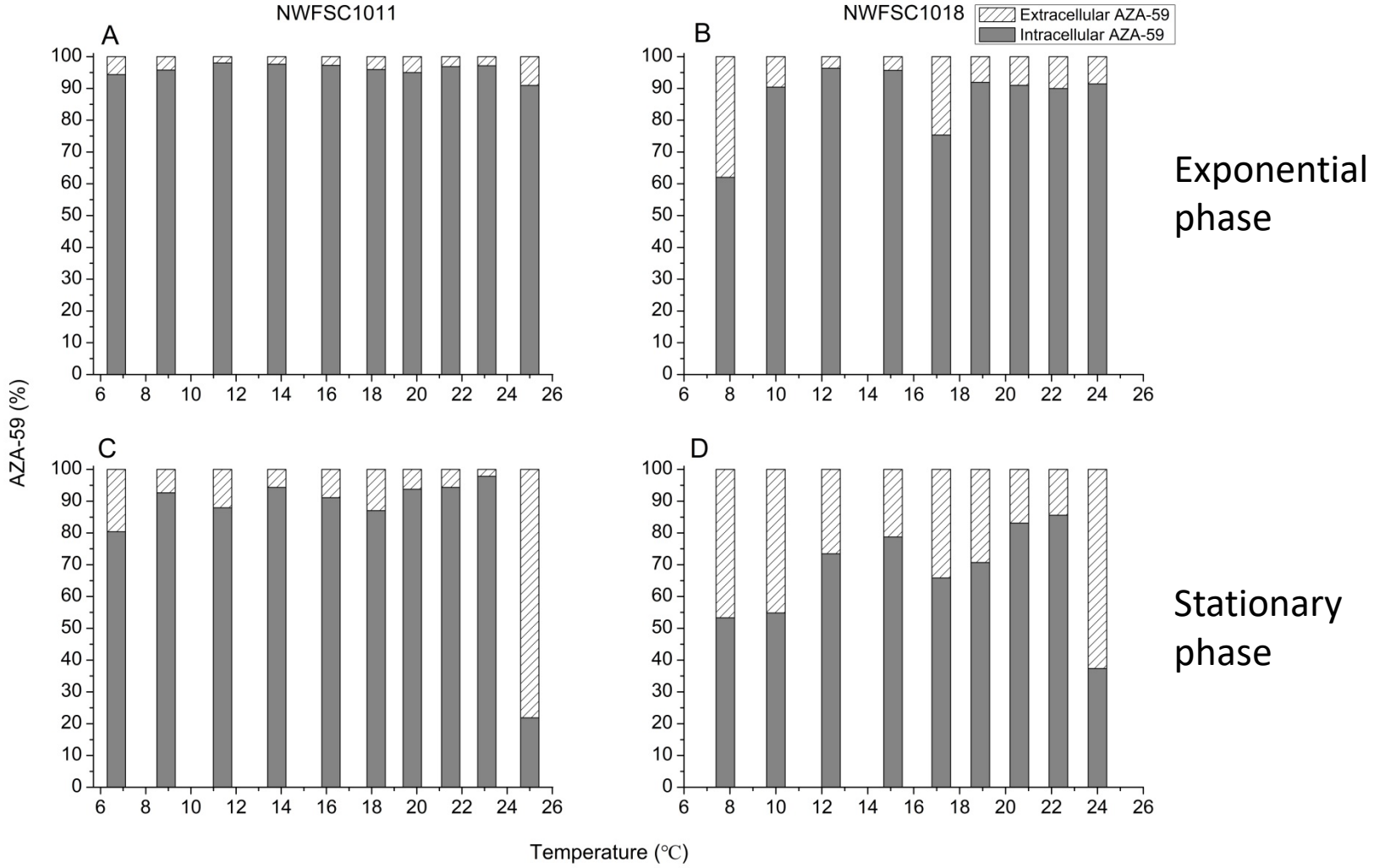
Growth rate (μ) of *A. poporum* along a temperature (A) and salinity (B) gradient. The relationship of various growth rates and intracellular AZA-59 content of both strains

Intracellular and extracellular AZA-59 quota



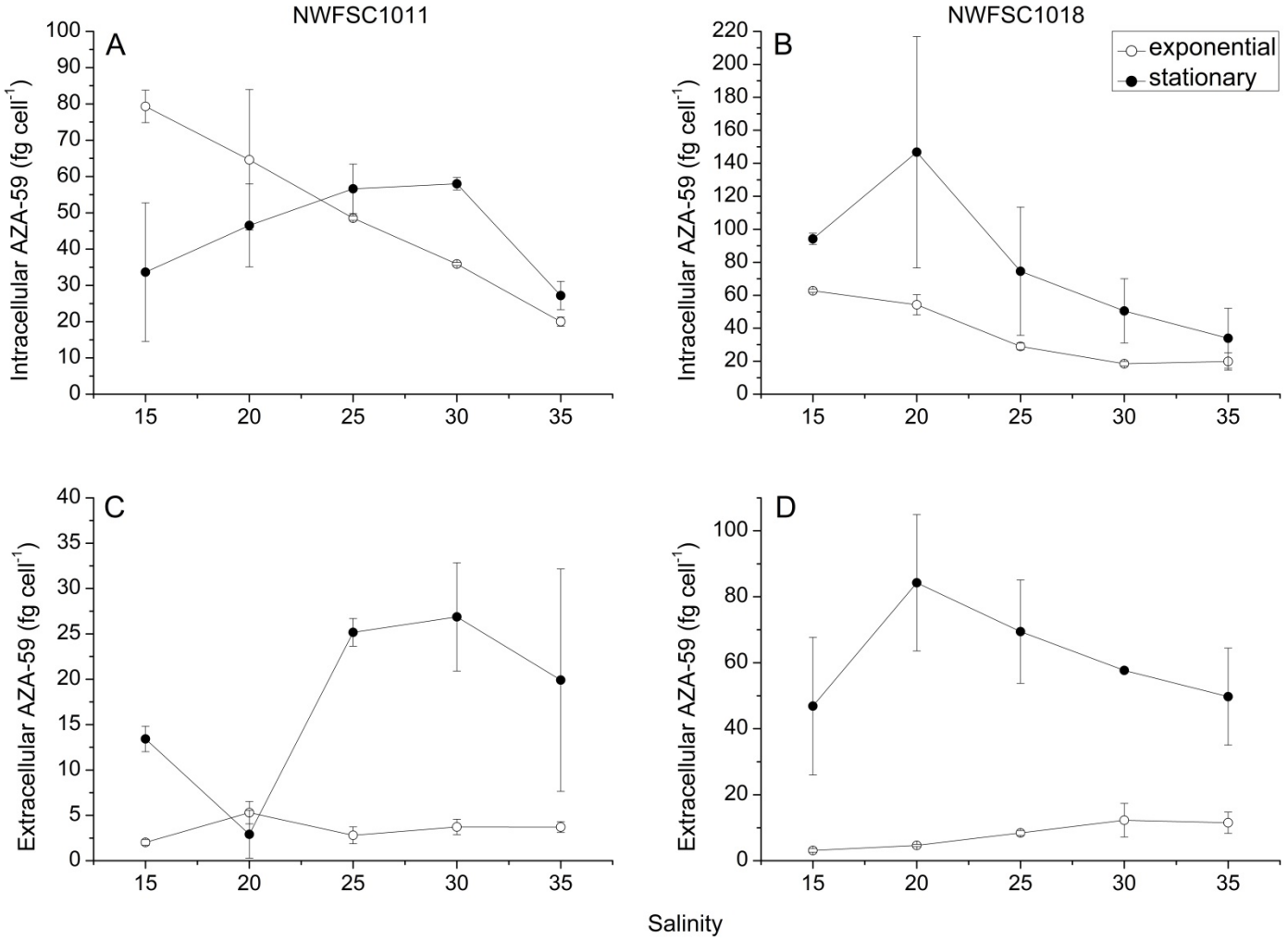
Intracellular (A and B) and extracellular (C and D) AZA-59 cell quota in *A. poporum* strains NWFSC1011 (left panel) and NWFSC1018 (right panel) along a temperature gradient.

Percentage of intracellular and extracellular AZA-59



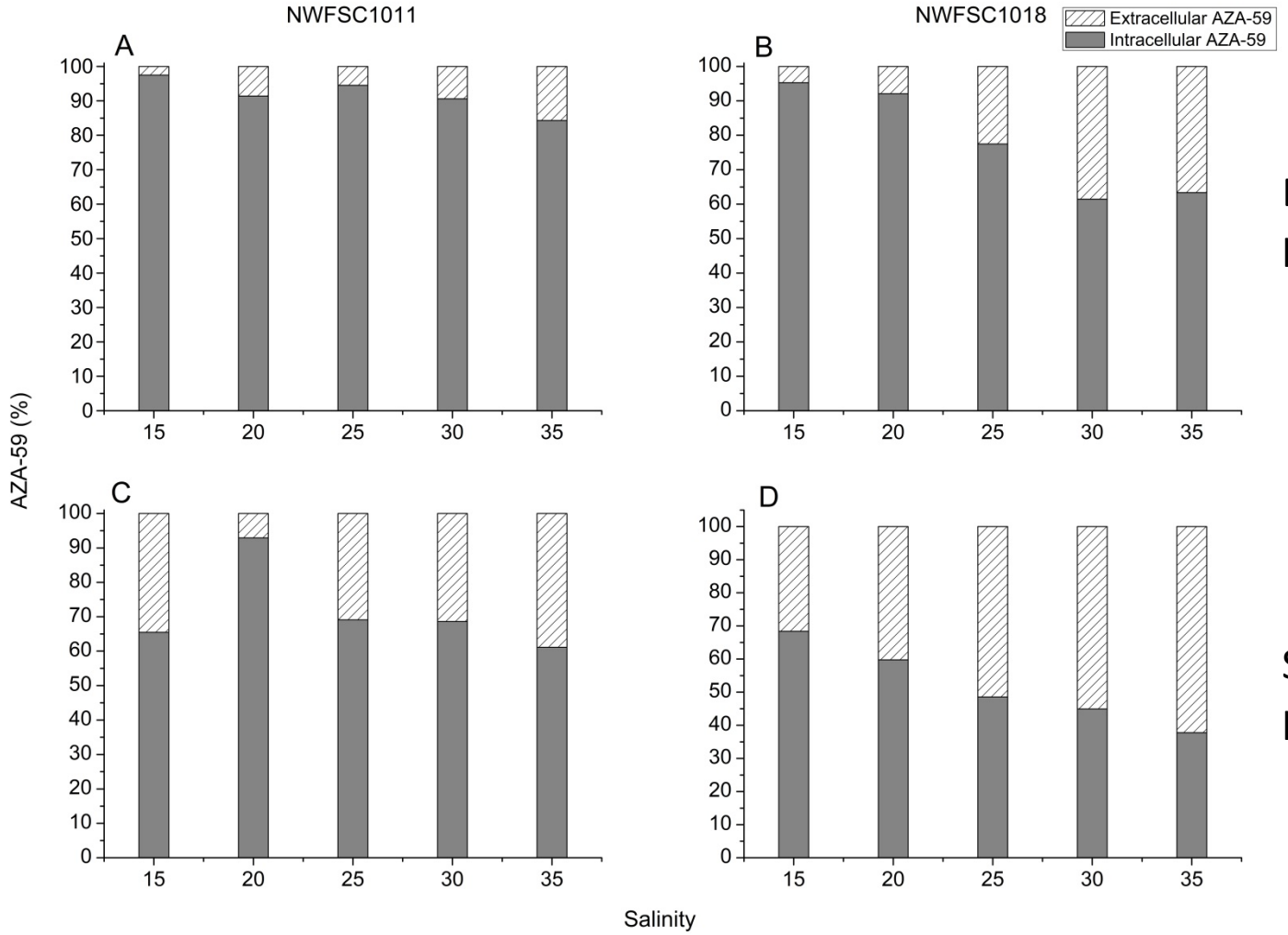
Percentage of intracellular (solid bar) and extracellular (hatched bar) AZA-59 cell quota cell^{-1} in *A. poporum* strains NWFSC1011 (left panel) and NWFSC1018 (right panel) in exponential (A and B) and stationary (C and D) phase along a temperature gradient.

Intracellular and extracellular AZA-59 quota



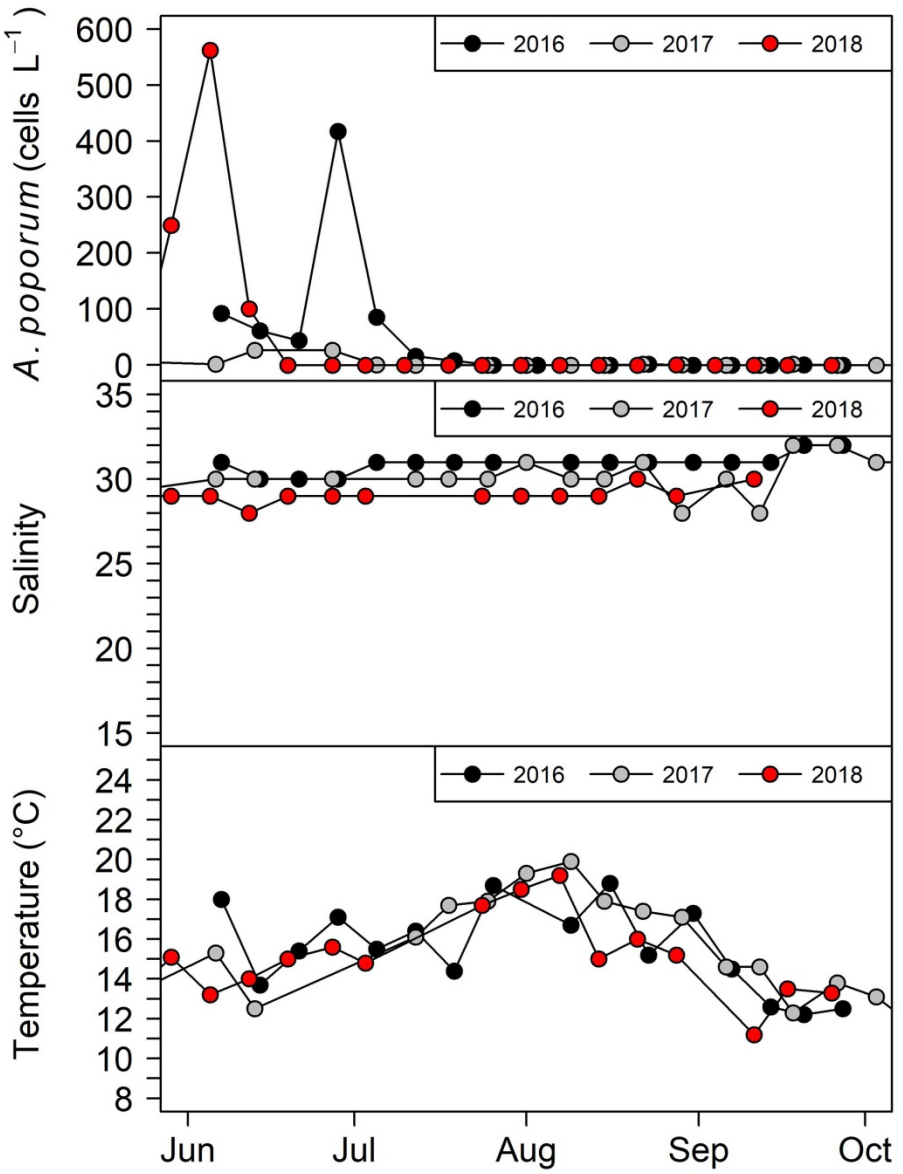
Intracellular (A and B) and extracellular (C and D) AZA-59 cell quota in strain NWFSC1011 (left panel) and strain NWFSC1018 (right panel) in exponential (open circle) and stationary (filled circle) phase along a salinity gradient.

Percentage of intracellular and extracellular AZA-59



Percentage of intracellular (grey bar) and extracellular (hatched bar) AZA-59 cell quota in *A. poporum* strains NWFSC1011 (left panel) and NWFSC1018 (right panel), in exponential (A and B) and stationary (C and D) phase along a salinity gradient.

Azadinium poporum abundance and Temperature and salinity in Sequim Bay, Puget Sound, from late May-Oct, in 2016, 2017, and 2018



summary

- Puget Sound strains of *A. poporum* grew across a wide temperature range from 6.7 ºC to 25.0 ºC and salinities ranging from 15 to 35
- Temperature played a larger role than salinity in determining *A. poporum* growth rates in isolates from Puget Sound.
- Cellular toxin quotas varied by strain in both the temperature and salinity treatments but were highest at the lowest growth rates, especially for strain NWFSC1011.
- *Azadinium poporum* cells in stationary phase almost always had higher intra- and extracellular AZA-59 cell quotas than cells in exponential phase growth at the same temperature and salinity treatments.
- Cellular toxin quotas were strain dependent in both the temperature and salinity treatments. More AZA-59 was released during stationary phase in *A. poporum* than in exponential phase over a range of temperature and salinity treatments.

Acknowledgements

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