Assessment of Ballast Water Management Systems: Science in Support of Policy

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Data from

MacIntyre HL, Cullen JJ (2016) Classification of phytoplankton cells as live or dead using the vital stains fluorescein diacetate and 5-chloromethylfluorescein diacetate. J Phycol 52 (4):572-589

MacIntyre HL, Cullen JJ, Whitsitt TJ, Petri B (2017) Ennumerating viable phytoplankton using a culture-based Most Probable Number assay following ultraviolet-C treatment. J Appl Phycol. doi:10.1007/s10811-017-1254-8

MacIntyre H, Cullen JJ, Rastin S, Miller J, Waclawik M, Petri B (*in prep.*) Estimating viablity in UVC-treated phytoplankton cells with growth assays, vital stains, and variable fluorescence.

The intent: prevent (deleterious) species invasions

Images: NASA, Phys.org, RiverheadLocal

The regulatory response: treat with certified ballast water management systems

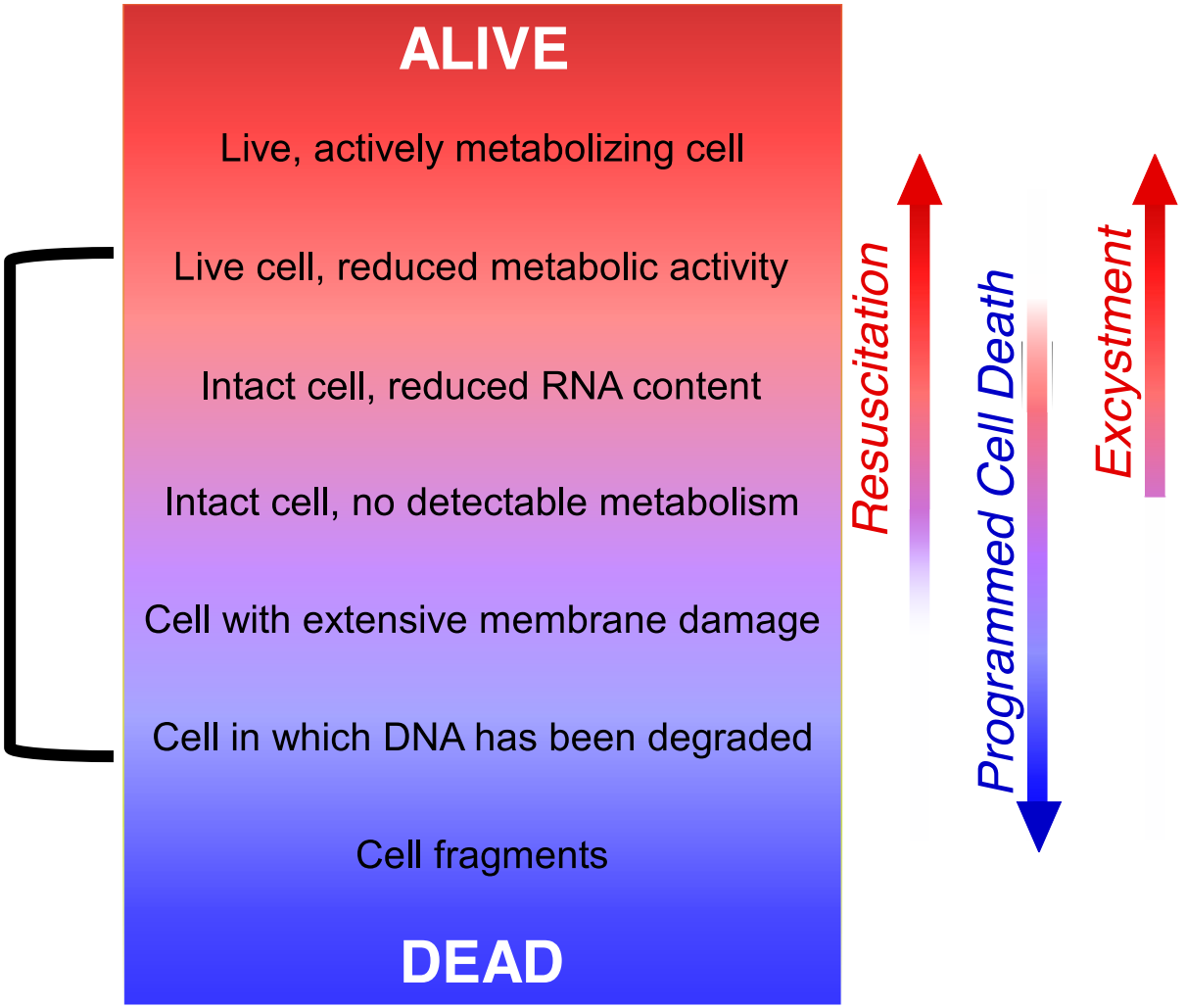
10-50 µm category dominated by phytoplankton

	USCG	IMO
Authority		
Current status	Enacted	Pending
Regulatory criterion	Live/dead	Viable
Test	FDA+CMFDA	FDA+CMFDA SDC-MPN

These can be the same but don't have to be

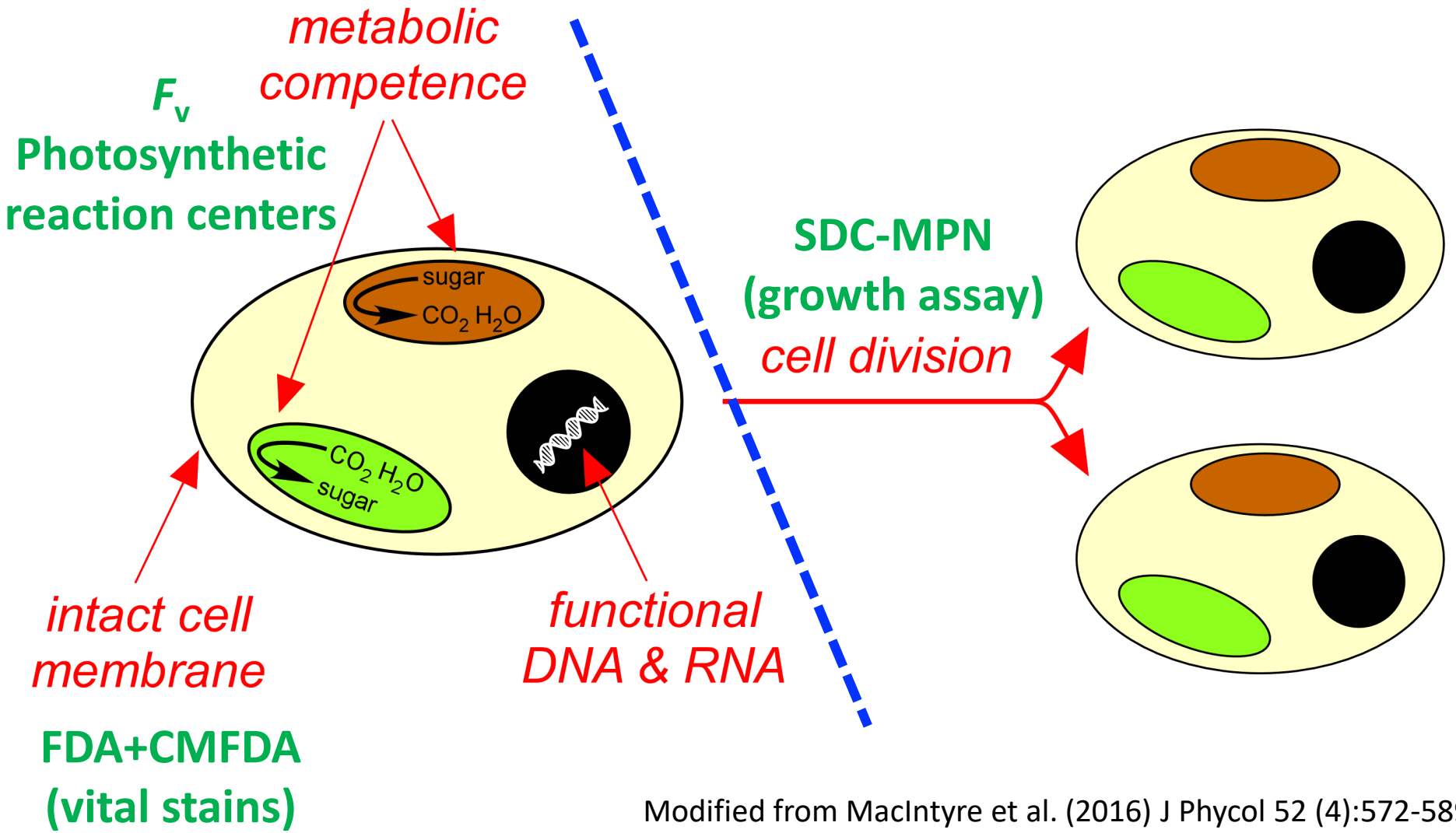
The problem: there is no simple definition of live/dead in bacteria and phytoplankton

Exact point of death unknown



Modified from Davey (2010) *Appl. Environ. Microbiol.* 77:5571-76

Assessing BWMS — vitality vs. viability



Modified from MacIntyre et al. (2016) J Phycol 52 (4):572-589

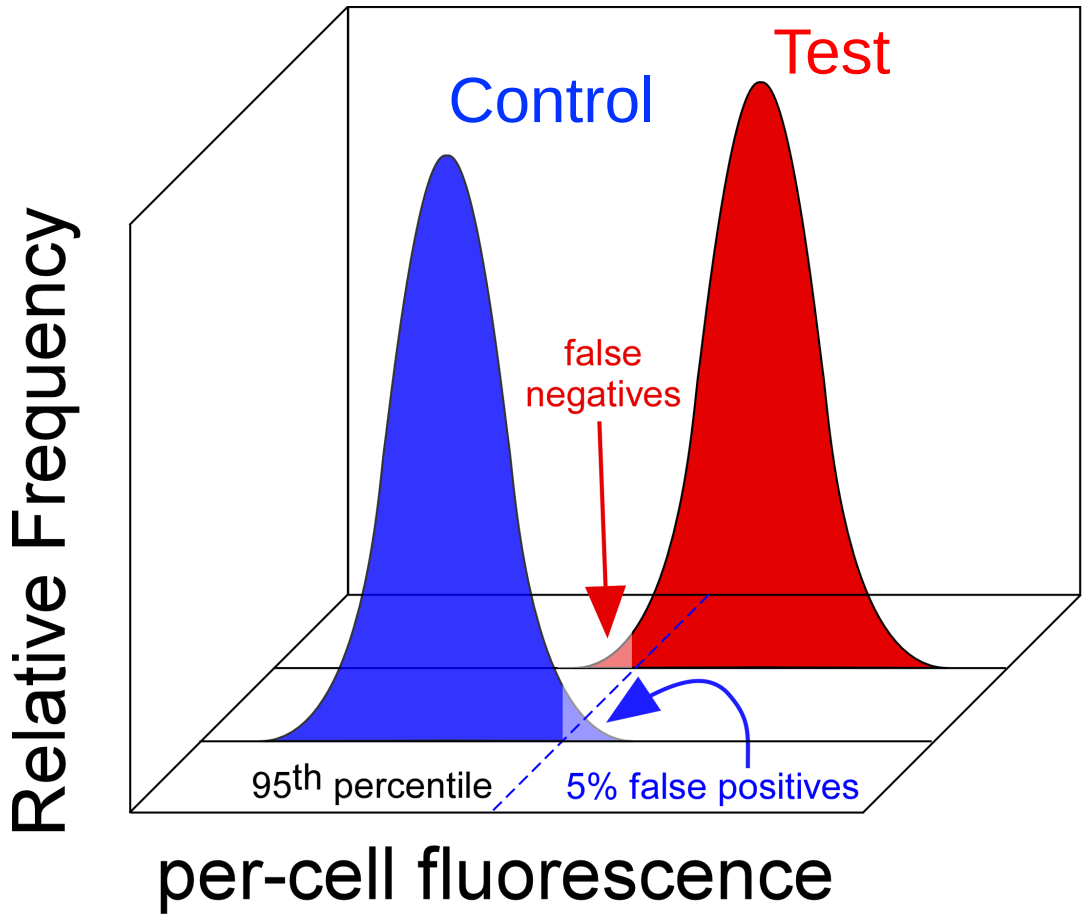
Testing FDA+CMFDA: objective and quantitative classification of live vs. dead by flow cytometry

Non-parametric approach based on 10^3 – 10^5 cells per treatment

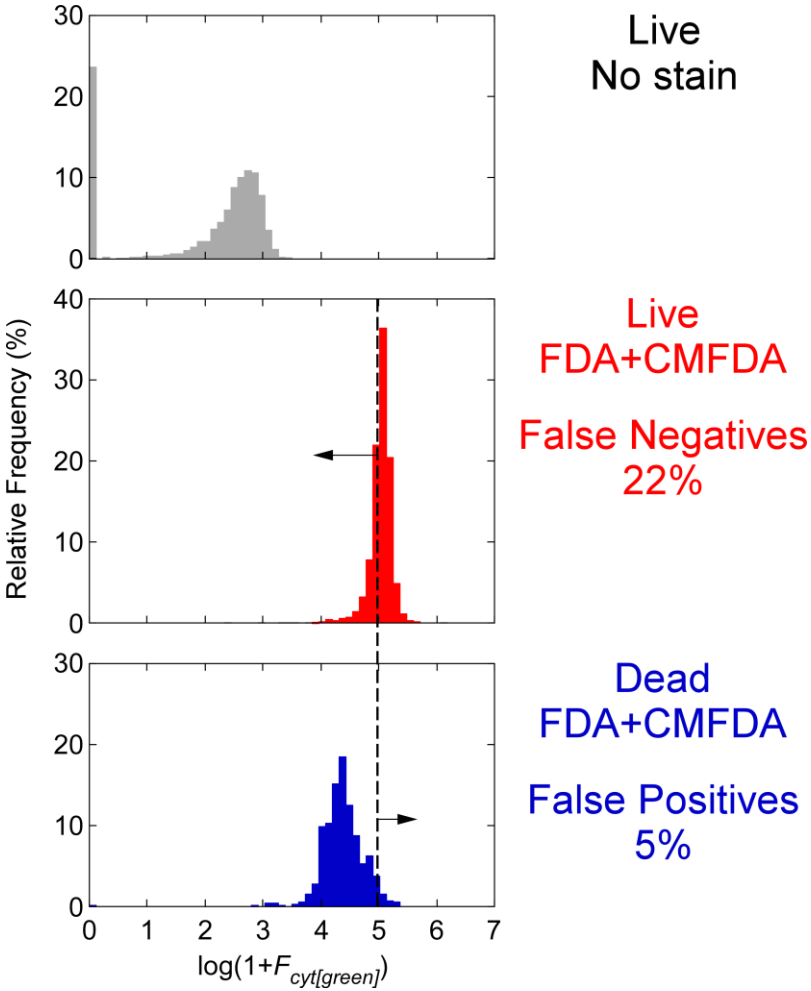
Seperatory threshold set as 95th percentile of distribution of control cells

False positives are control cells with higher fluorescence — 5%

False negatives are test cells with lower fluorescence



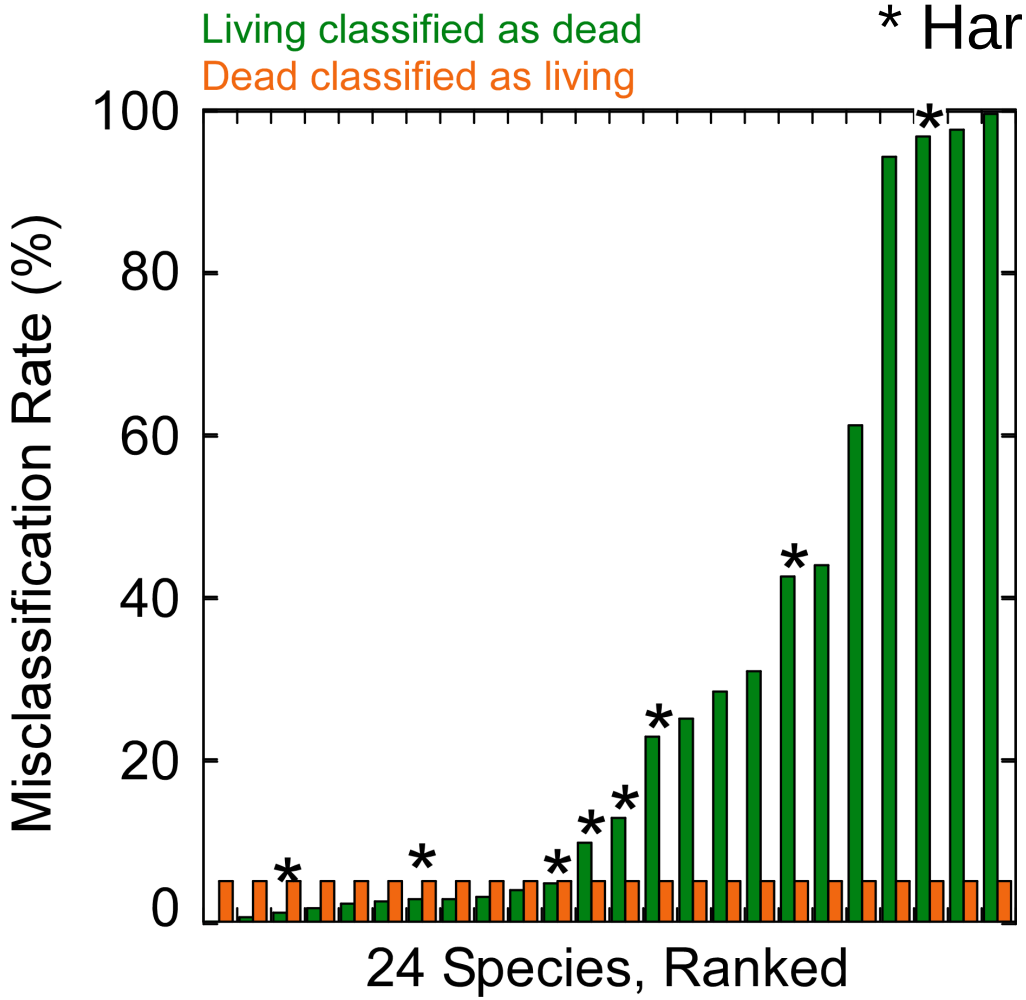
The results: testing against heat-killed, stained cells — statistically-not-dead classification



Eutreptiella gymnastica

False negatives are living cells incorrectly classified as dead — risk of invasion is higher than regulatory threshold

The results: testing against heat-killed, stained cells — statistically-not-dead classification



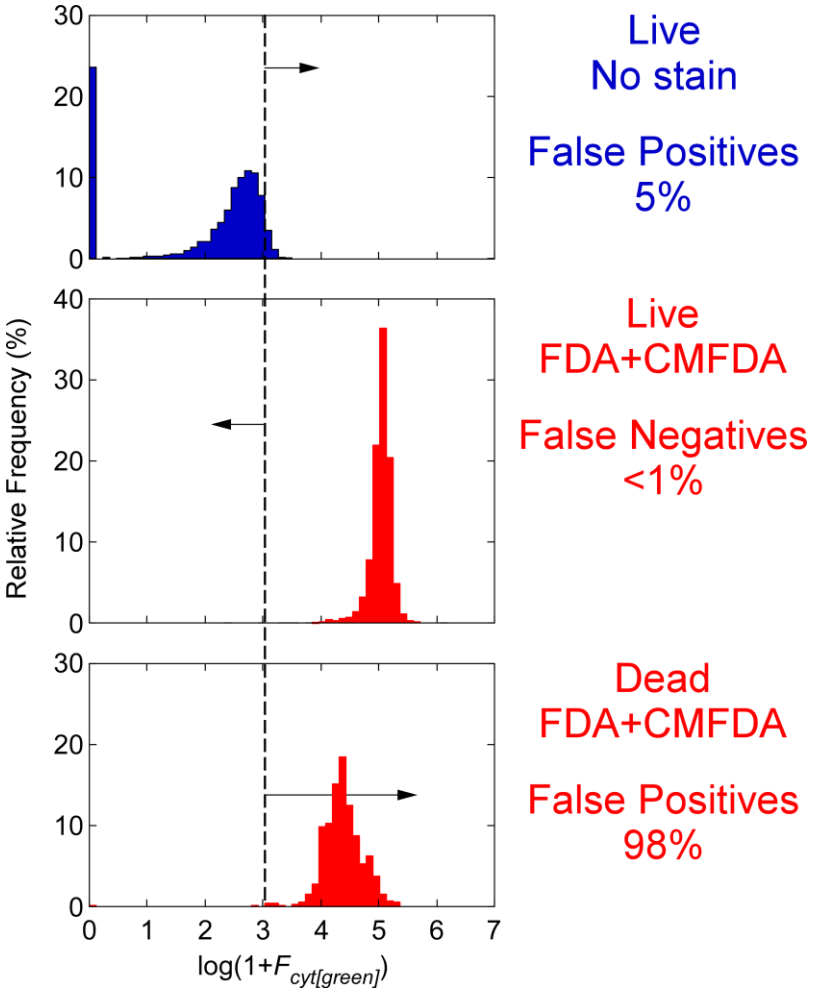
* Harmful Algal Bloom species

9/24 had <10% error
Of those, 8/24 had no significant cells loss on staining

Underestimate of invasive potential

Means of 3-5 replicates

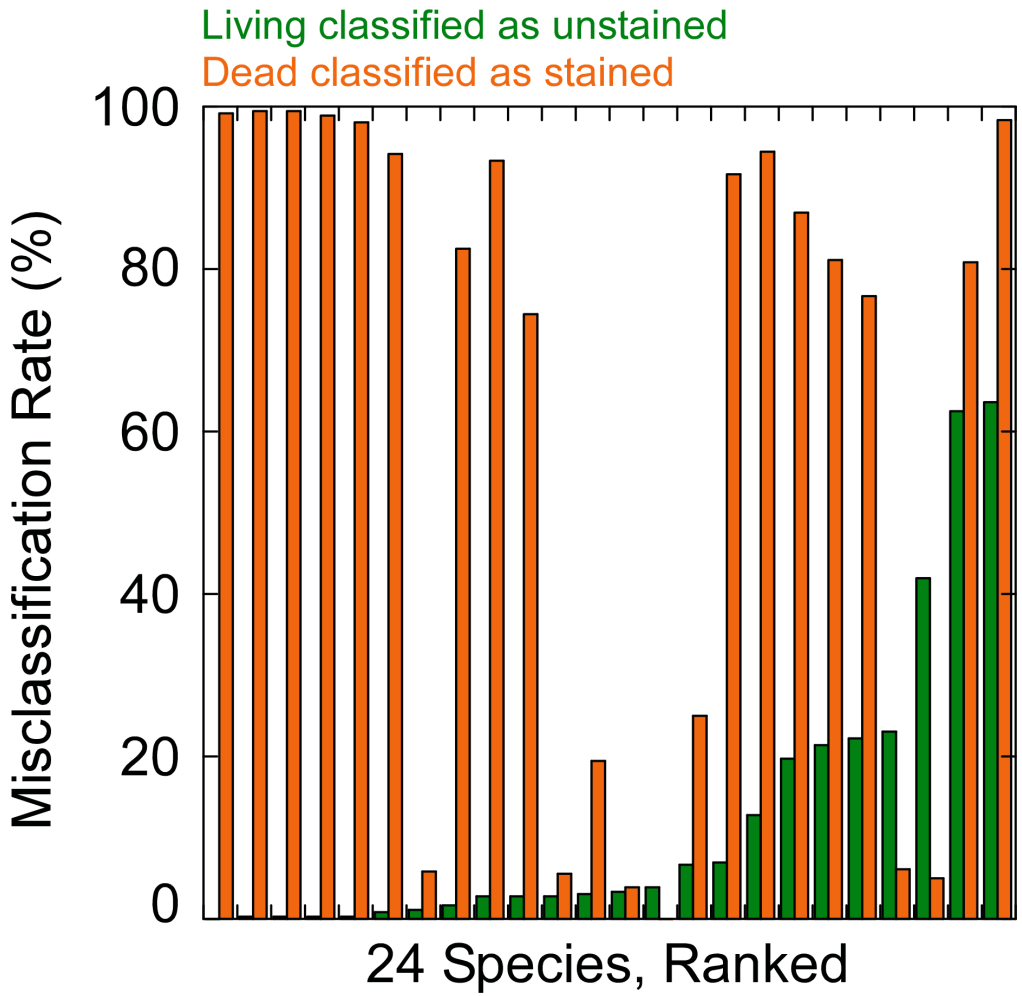
The results: testing against living, unstained cells — statistically-not-stained classification



Eutreptiella gymnastica

False positives are dead cells incorrectly classified as living — risk of invasion is lower than regulatory threshold

The results: testing against living, unstained cells — statistically-not-stained classification

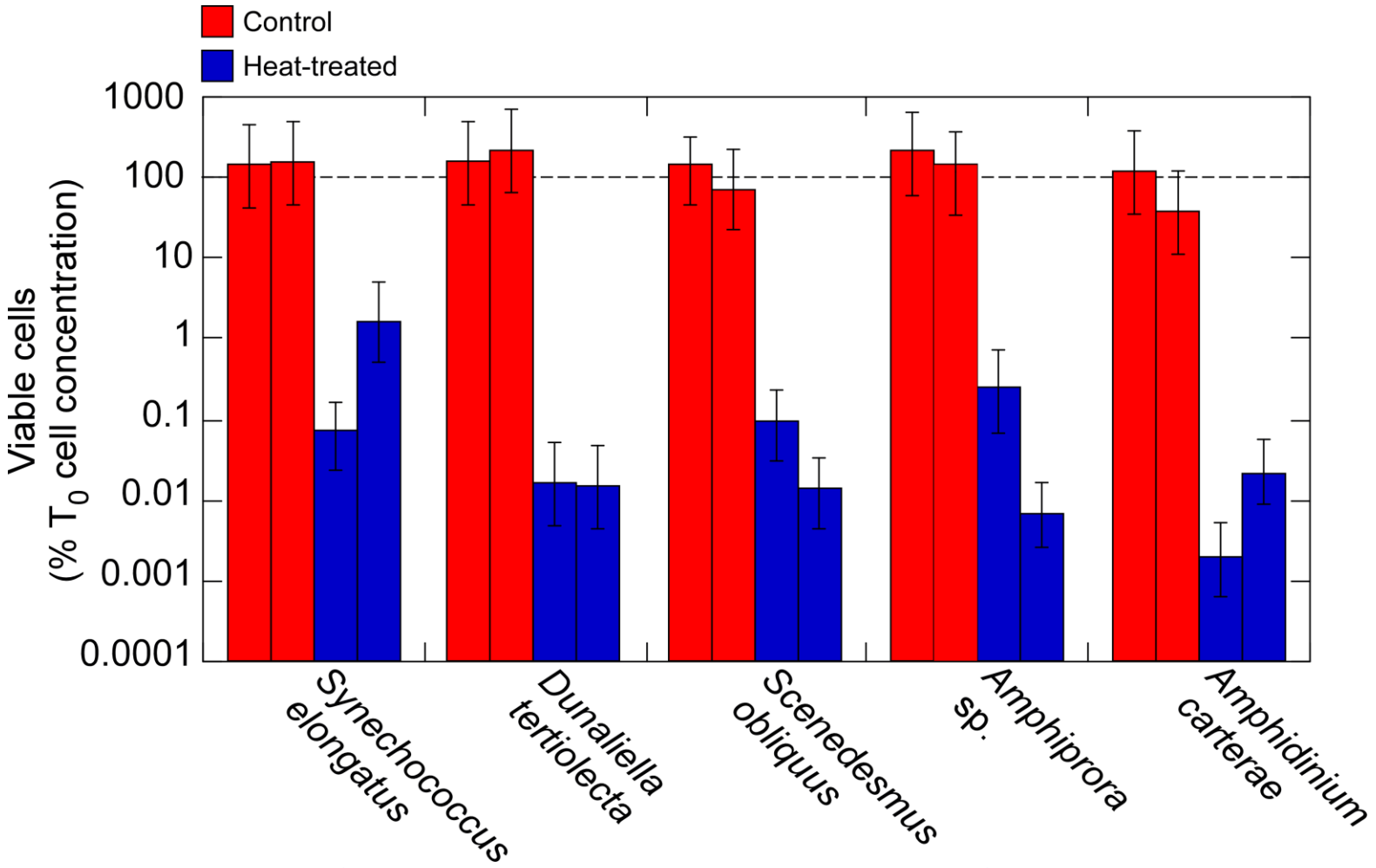


4/24 had <10% error

In 22/24, an overestimate of invasive potential

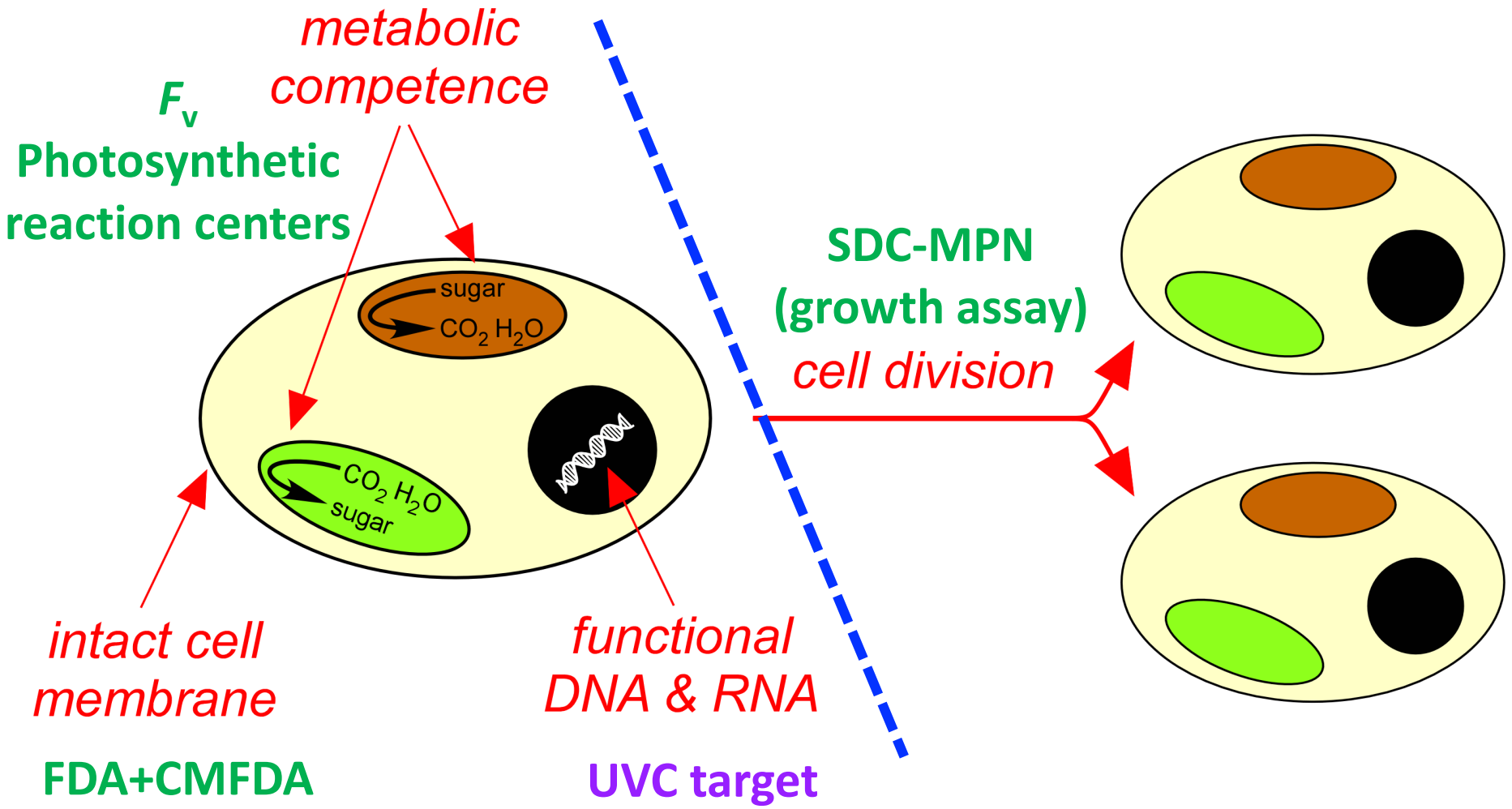
Means of 3-5 replicates

The results: accurate discrimination of worst-case species with Most Probable Number assay



0.002 – 1.2% of cells classed as viable after heat treatment

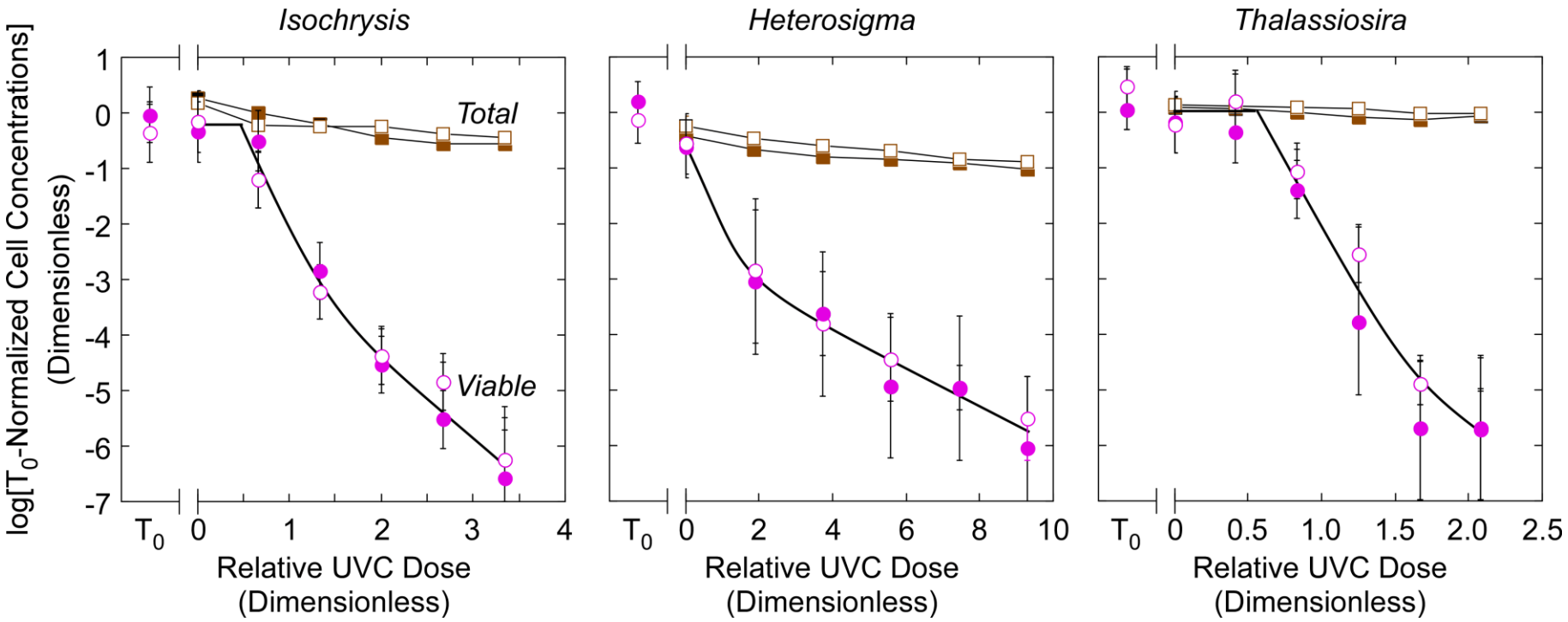
Assessing BWMS when viability is the target



Modified from MacIntyre et al. (2016) J Phycol 52 (4):572-589

Treatment: UVC reduces viability in all species tested

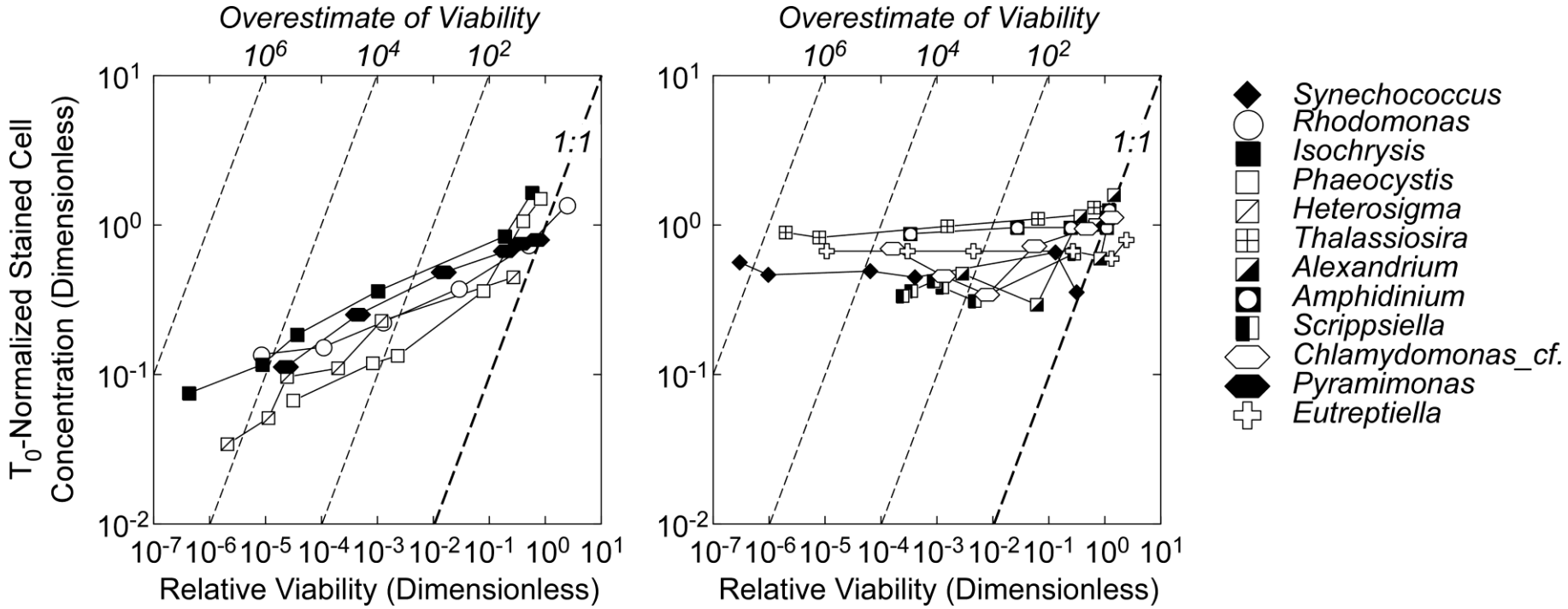
In some species, there is progressive cell loss with dose



Assay: FDA+CMFDA over-estimates viability by orders of magnitude

Correlation
5 species with significant cell loss

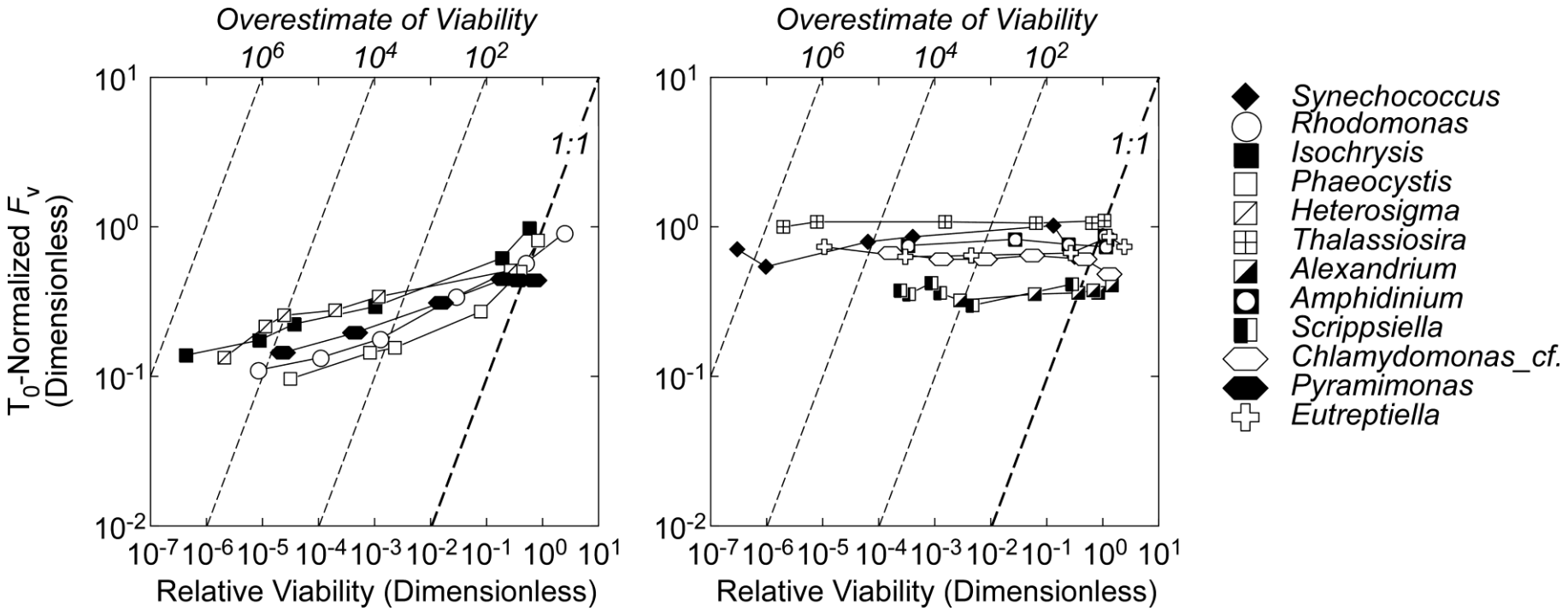
No correlation
7 species without significant cell loss



Assay: F_v over-estimates viability by orders of magnitude

Correlation
5 species with significant cell loss

No correlation
7 species without significant cell loss



Summary

- FDA+CMFDA staining assay was unable to discriminate between live/dead cells in 67-83% of 24 species tested
  - High rates of false negatives (live cells misclassified as dead) with statistically-not-dead classification — less inaccurate, less protective
  - High rates of false positives (dead cells misclassified as live) with statistically-not-stained classification — more inaccurate, more protective
- SDC-MPN growth assay was able to discriminate live vs dead in worse-case taxa (61 – 99% rates of false positives)
- Both FDA+CMFDA and F_v are unable to detect loss of viability in UVC-treated cells
  - Declines in stained cells and F_v were driven primarily by loss of cells