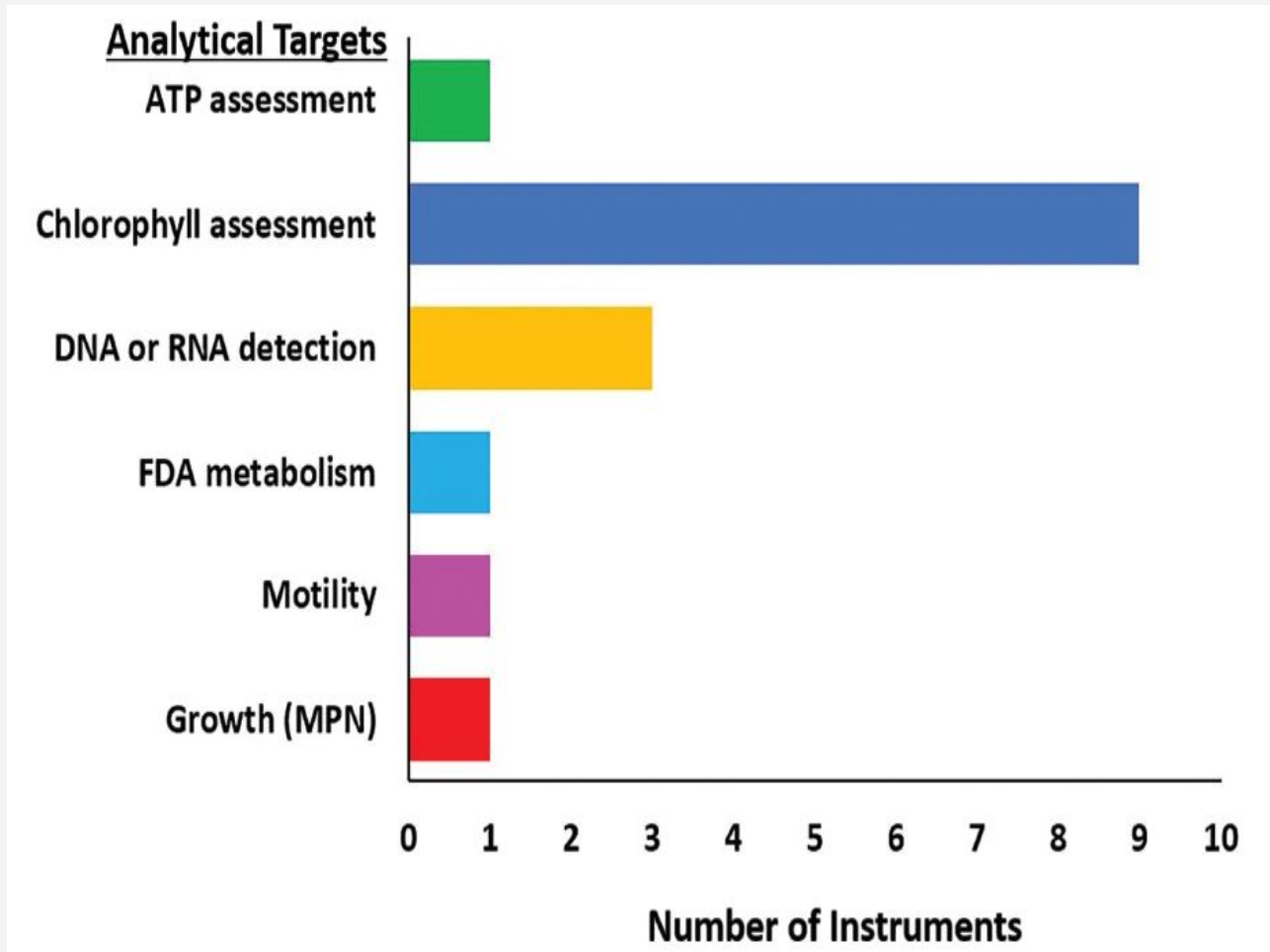


INDICATIVE TESTING, FOR SHIPOWNERS AND COMPLIANCE MONITORING



JEFFREY RAM, WAYNE STATE UNIVERSITY

QUANTITATIVE TARGETS AND INDICATIVE METHODS



Cangelosi, Balcer,
Reavie, and Ram:
*Priorities and
Technologies for
Indicative Testing*

[https://ballastwater.org/
projects/](https://ballastwater.org/projects/)

INDICATIVE MONITORING ANALYTICAL TARGETS/ENDPOINTS

QUANTITATIVE TARGETS/ENDPOINTS

Bulk Biochemical/Biophysical + Algorithm

- Subset of Taxa (e.g., Chlorophyll)
- All Taxa (ATP, Bulk FDA Metabolism)

Direct Counts of Individuals

- Image Motility (Live Motile Only)
- Detect individual fluorescent (chlorophyll) or stained (FDA, mRNA) organisms

Organism Size: Regulated numbers are size-dependent. Can the method determine if the organism is $<10\text{ }\mu\text{m}$, $10 - 50\text{ }\mu\text{m}$, and $>50\text{ }\mu\text{m}$? Or do the organisms have to be separated by size in advance of counting?

Current USCG/IMO discharge standards

$>50\text{ }\mu\text{m}$: <1 organism/cubic meter

$10 - 50\text{ }\mu\text{m}$: $<10/\text{mL}$

$<10\text{ }\mu\text{m}$: specific types of indicator organisms

<1 disease causing *Vibrio cholera*/100 mL

<250 CFU *E. coli*/100 mL

<100 CFU intestinal enterococci/100 mL

Note:VIDA2018 cites standards for the Pacific region that are more stringent than these for certain size organisms

CHLOROPHYLL FLUORESCENCE

- **At Ballast Monitoring Practicums:**

Turner Designs: *Ballast-Check 2*: Fluorescence with multiple turnover Pulse Amplitude Modulated (PAM) analysis

SixSenso: *SX-CYT* Fluorescence with PAM

Chelsea Tech Grp: *FastBallast*: Fluorescence with Single Turnover Active Fluorometry (STAF)

MicroWISE: *BallastWISE*: Fluorescence. Imaged with motility detection.



Other chlorophyll-sensing instruments:

Ballast Water Monitoring A/S: *bw-monitor* Fluorescence

bbe Moldaenke GmbH: *I 0 cells* Fluorescence with PAM, on a filter

Euro Tech: *Ballast Water Checker* Fluorescence with PAM

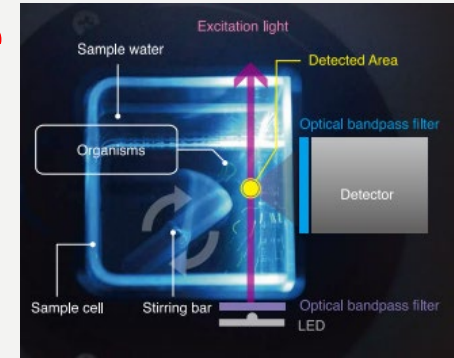
Oceantech: *P. Counter* Fluorescence with imaging

Hach: *BW680* Variable fluorescence (PAM)



FLUORESCCEIN DIACETATE (FDA)

Ballast Eye by Satake: **BallastEye**

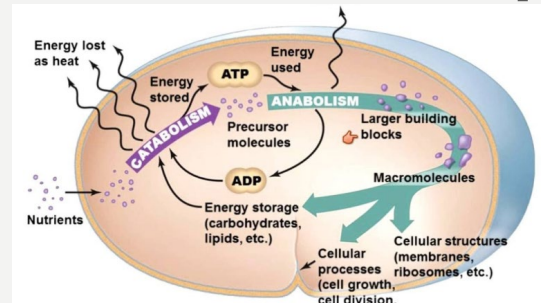


2018 model



Viable Organism Analyzer
(VOA1000K)

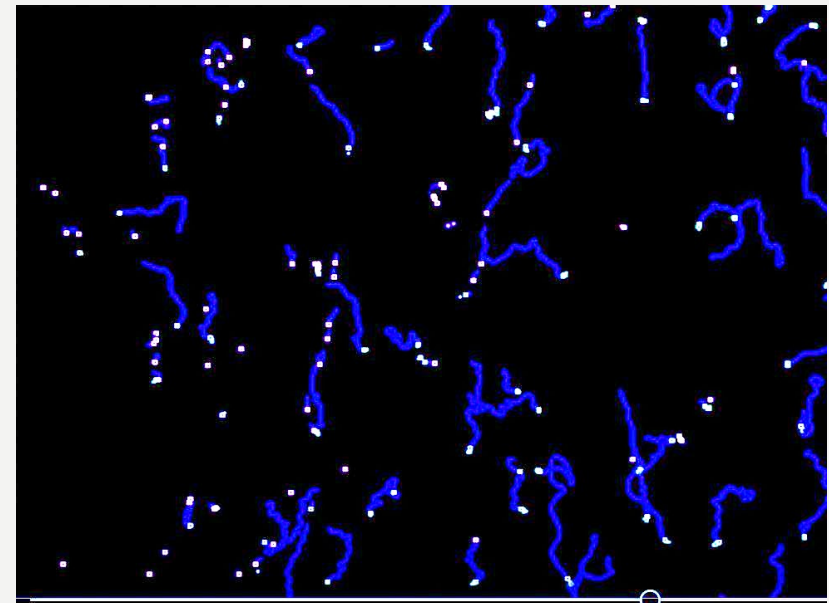
ATP • Method developed by **Luminultra**



ORGANISM MOTILITY

• **BallastWISE** by MicroWISE

Imaged with high resolution
sensitive video cameras



TECHNOLOGICAL APPROACHES

- **Automated (versus load your own samples)**

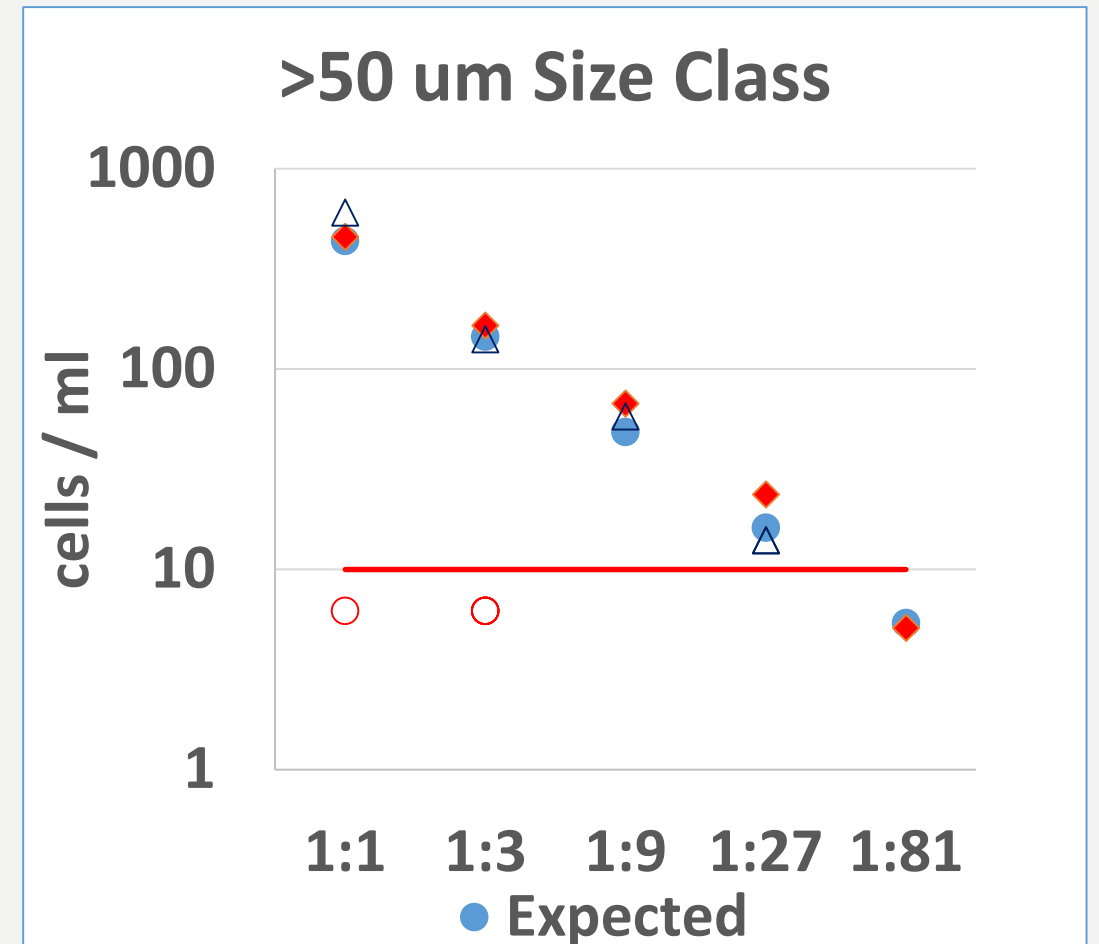
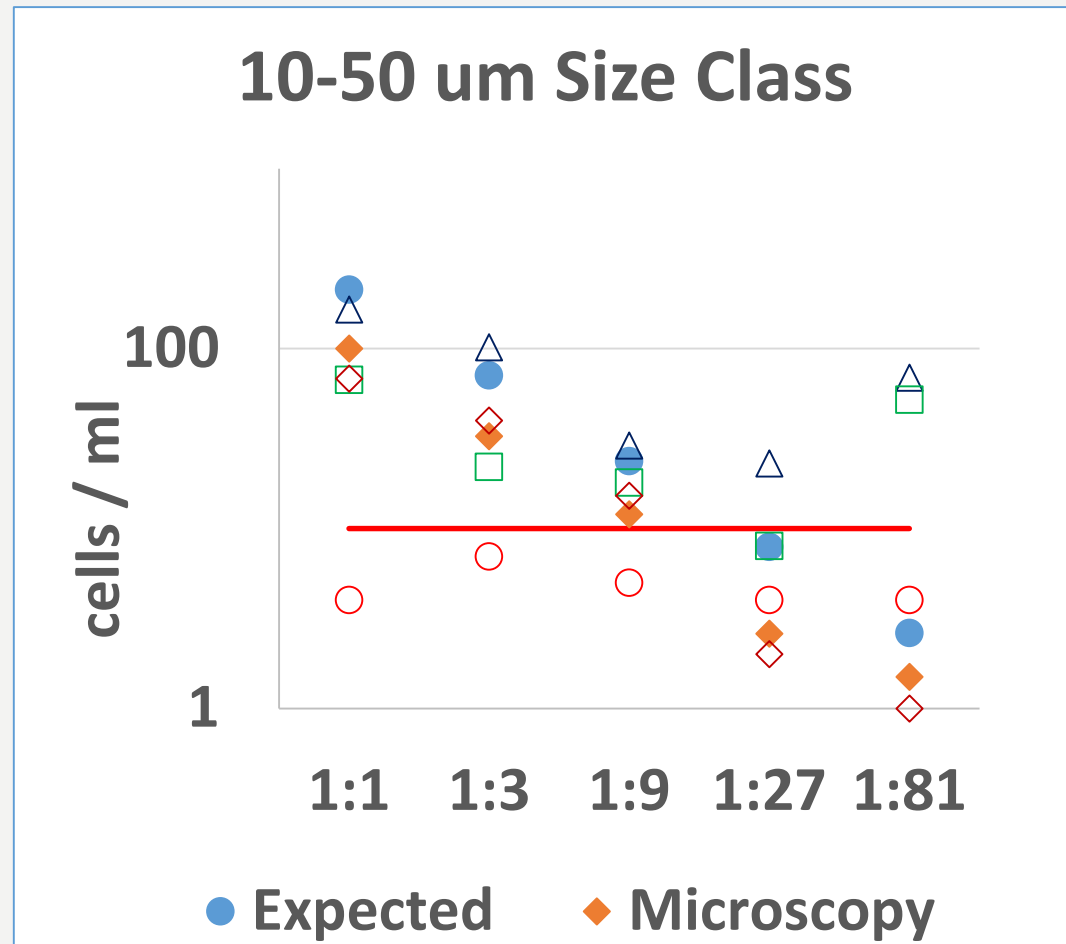
Chelsea Technologies Group Ltd: “FastBallast is a portable ballast water compliance monitor for use with discrete or flow-through samples.”

Microwise: This pumped system has the potential to be adapted to a flow-through configuration.

BW-Monitor: Built into ballast recirculation or discharge plumbing



PRACTICUM COMPARISON OF SEVERAL TOOLS FOR FRESHWATER SYSTEMS (GREAT LAKES)



TAKE HOME: TECHNICAL CHALLENGES IN INDICATIVE MONITORING

Calibration—simple, fast checks to confirm baseline and count calibration are needed


Temperature—enzyme dependent reactions vary with temperature. Correct sample preparation and conditioning temperatures must be used

Sensitivity and size resolution, especially at borderlines of organism sizes. Inadequate resolution or filtration cutoff around 50 μm may prevent accurate counts

These indicative methods measure *vitality* not *viability*

NONVIABLE? YOU ARE DEAD TO ME!

VIDA 2018 REVISED DISCHARGE STANDARD INTERPRETATION

- Viability = Live + Reproductive Capacity
 - “‘non-viable’ ...means...the organism [is] permanently incapable of reproduction”
- Live/Dead = Evidentiary Properties of Living Creatures:
 - Metabolism, Intact Membranes and Motility (mostly for zooplankton); AND
 - Per VIDA 2018: “‘live’ shall not include organisms rendered non-viable.”  Methods-dependent, and TBD