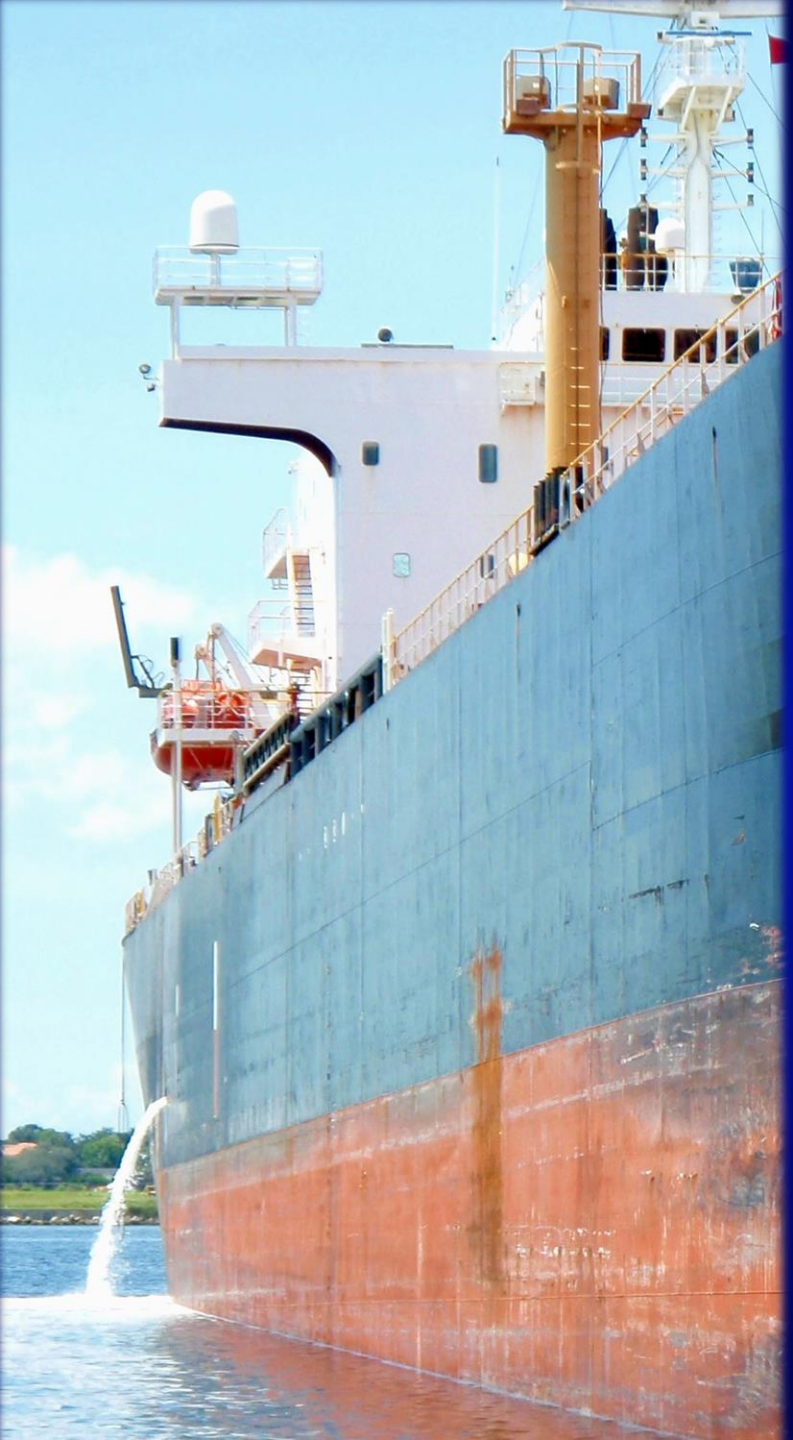
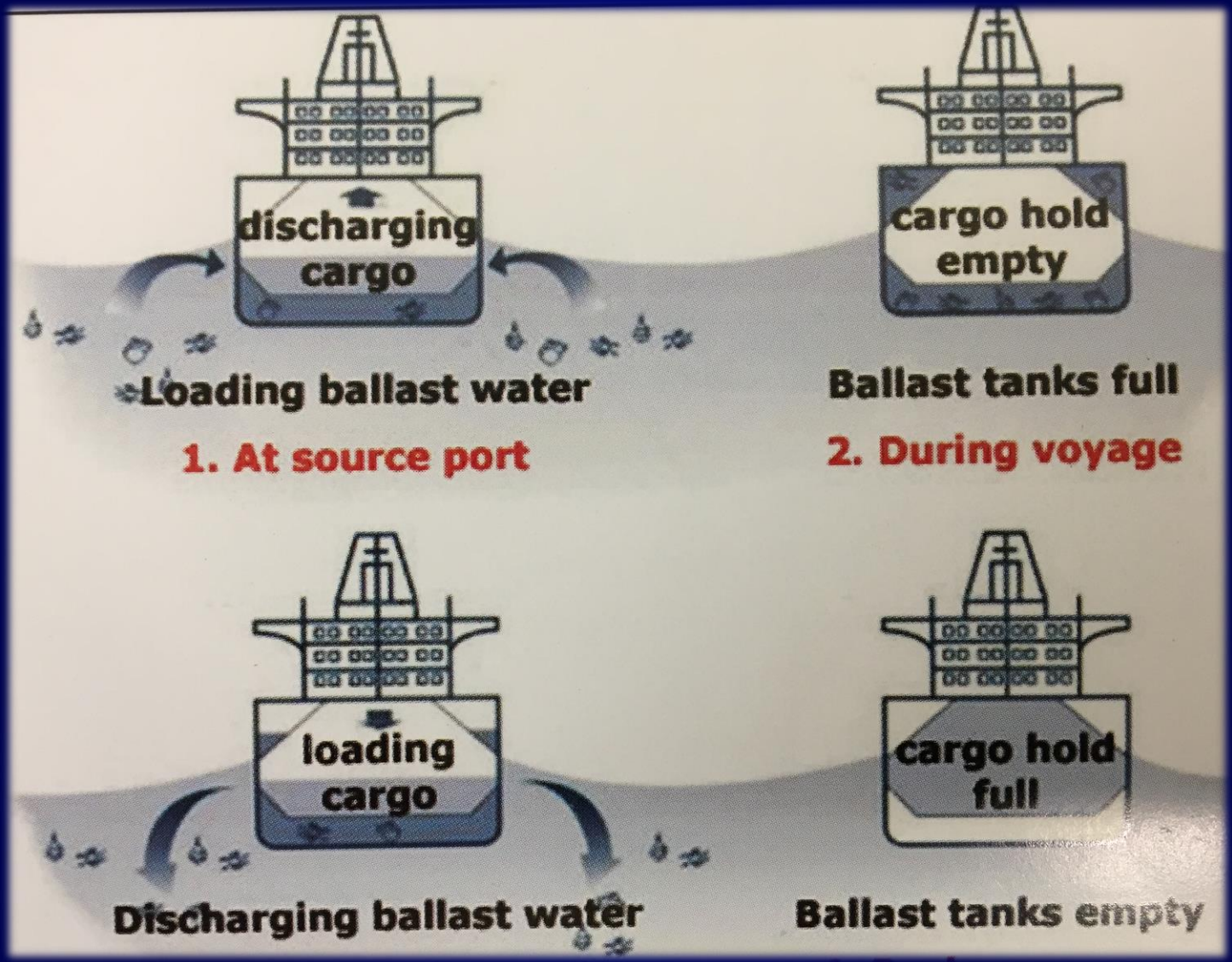


Ballast Check 2 Handheld Pulse Amplitude Modulated (PAM) Fluorometer

Natalie Dunn
Julie Kuo
Division of Aquatic Resources
11/14/2017

Ballast Water



Background

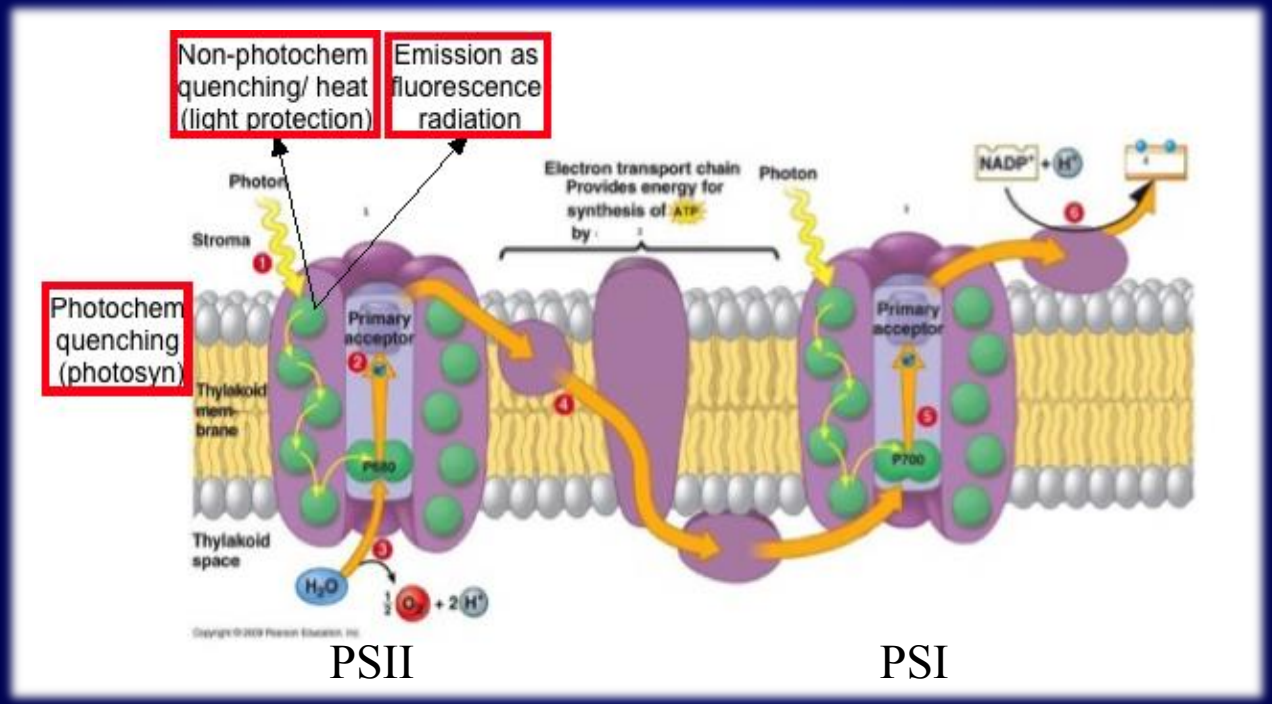
- Requirements of low levels of live organisms in ballast water discharge
  - Treatment systems
  - How to show compliance
- *In situ* active fluorometers measure phytoplankton photosynthetic efficiency
- Ballast Check 2 quick indication of gross exceedance of the compliance standard *in vivo*

Purpose

- Rapid assessment tool to measure ballast water compliance (10-50 µm organism)
- Provides ship operators or port authorities with indication of risk
- Assessment of ballast water treatment systems
- Precision & accuracy optimized for ballast water IMO D-2 regulations

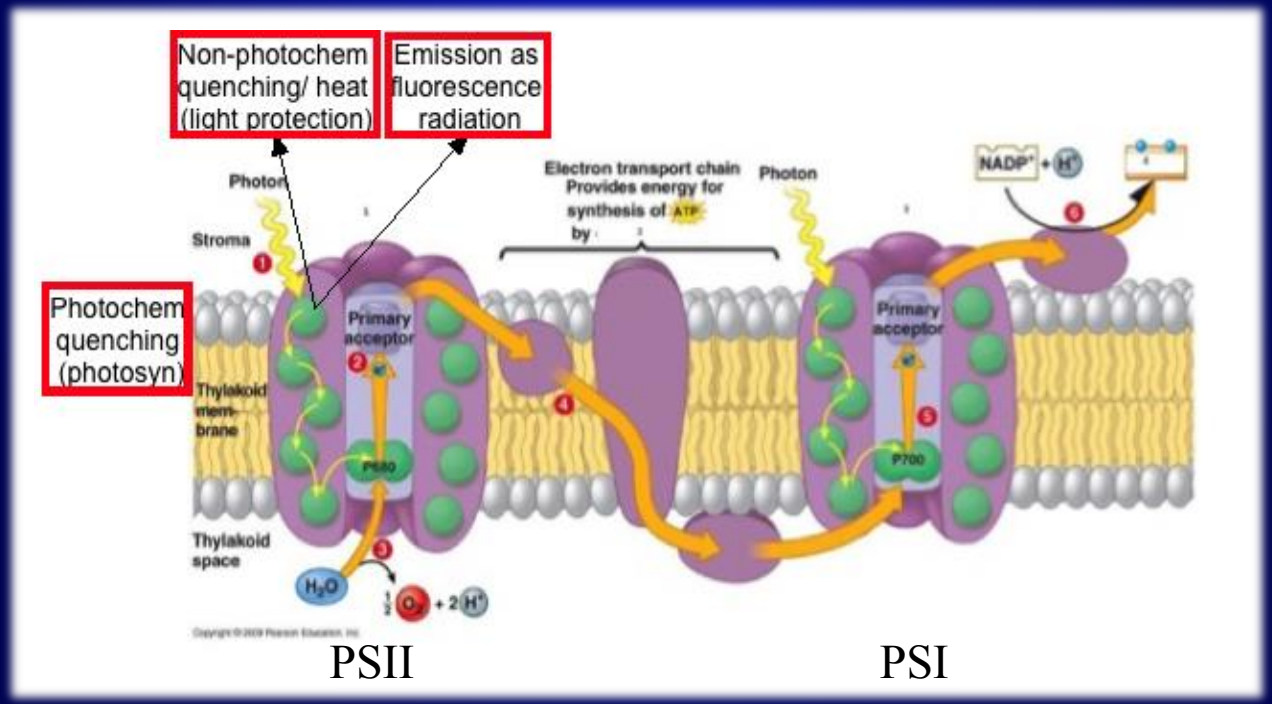
Active Fluorescence

- Light energy → chlorophyll a & b
  1. Photochemical quenching (photosynthesis)
  2. Non-photochemical quenching (heat for light protection)
  3. Emission as fluorescence radiation



Active Fluorescence

- Light energy → chlorophyll a & b
  1. **Photochemical quenching (photosynthesis)**
     Excite electrons $H_2O \rightarrow H^+ + O_2$ (PSII)
     ADP → ATP NADP → $NADPH_2$ CO_2 → sugar
  2. Non-photochemical quenching (heat for light protection)
  3. Emission as fluorescence radiation



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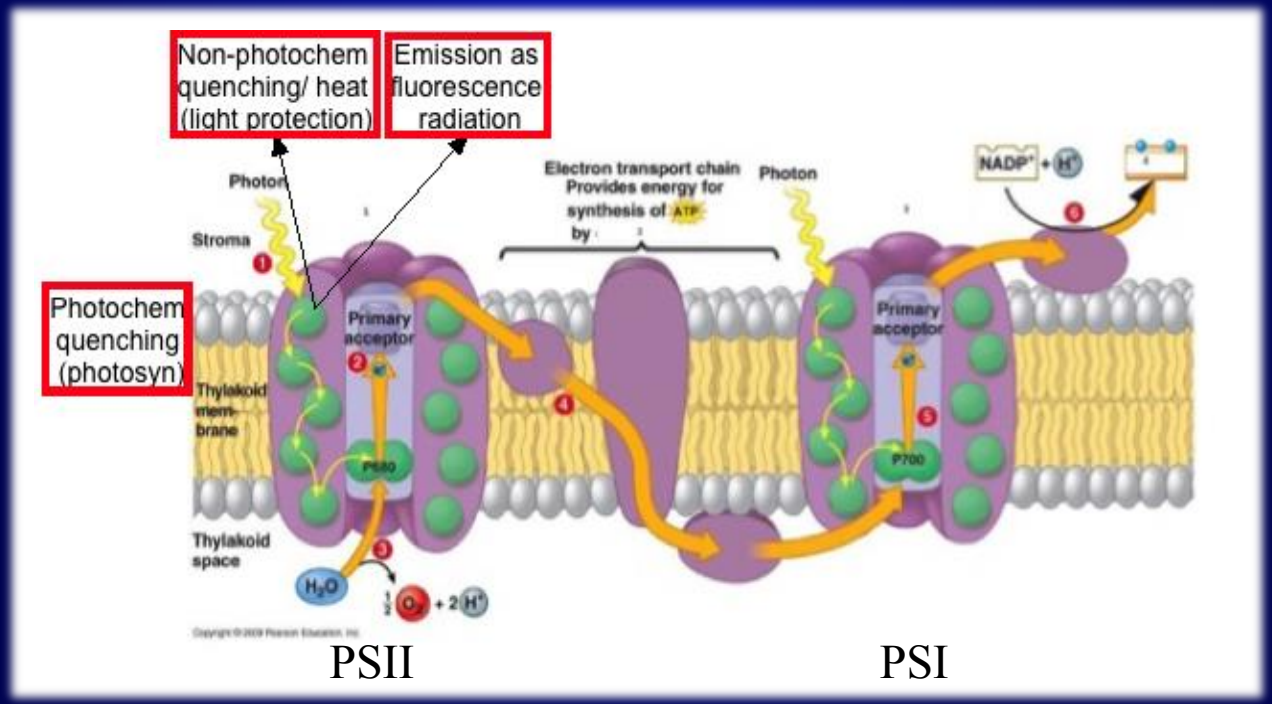
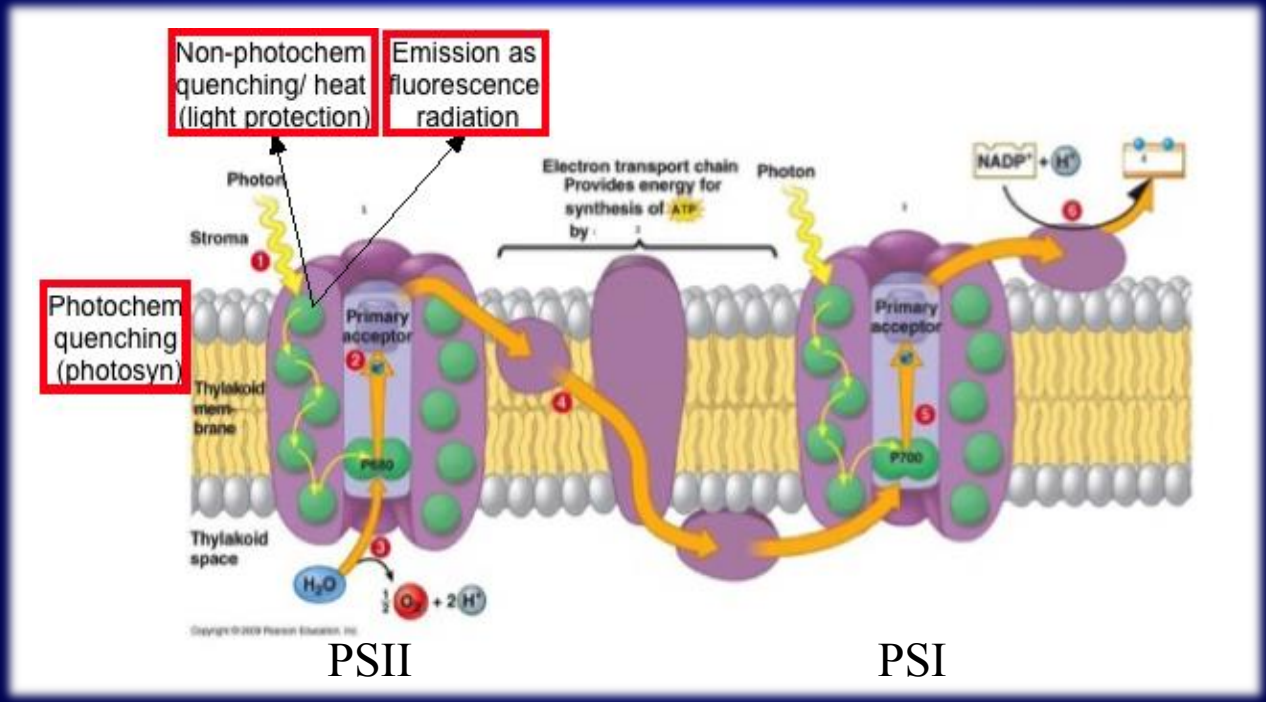


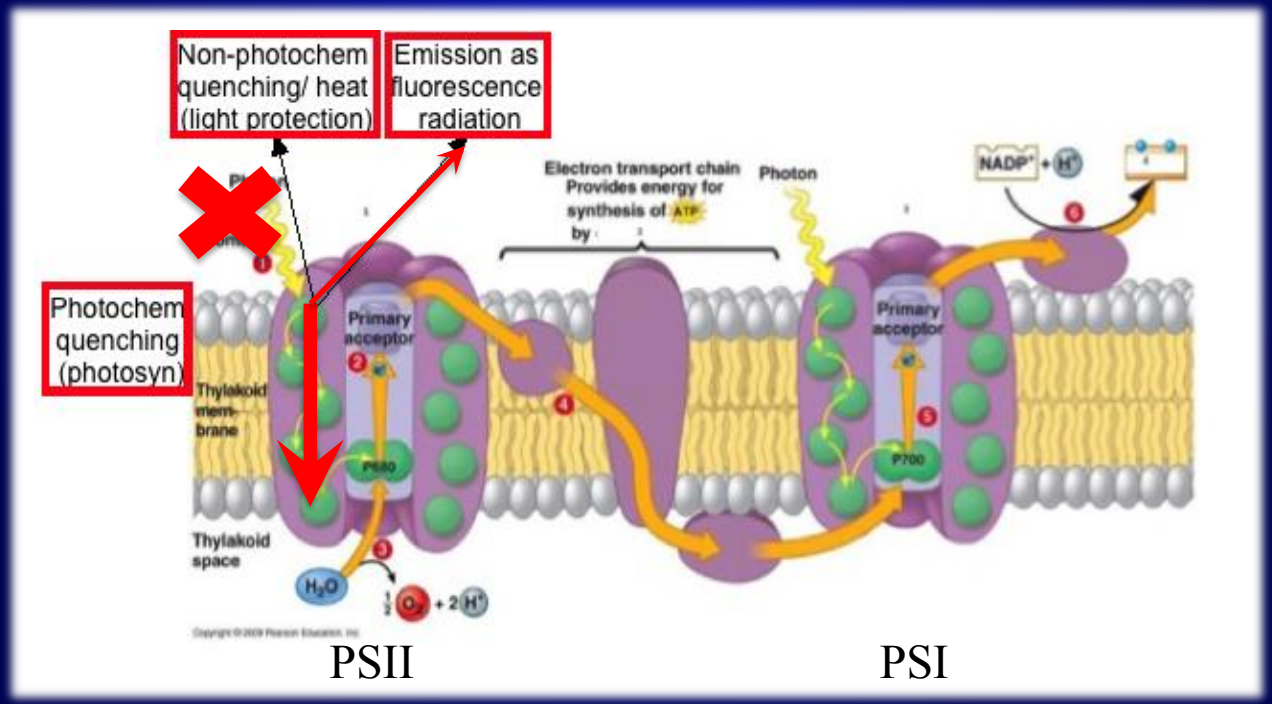
Active Fluorescence

- Light energy → chlorophyll a & b
  1. Photochemical quenching (photosynthesis)
  2. Non-photochemical quenching (heat for light protection)
  3. **Emission as fluorescence radiation**
     - Estimates photochemical efficiency of Photosystem II (PSII) from ratios of fluorescence levels



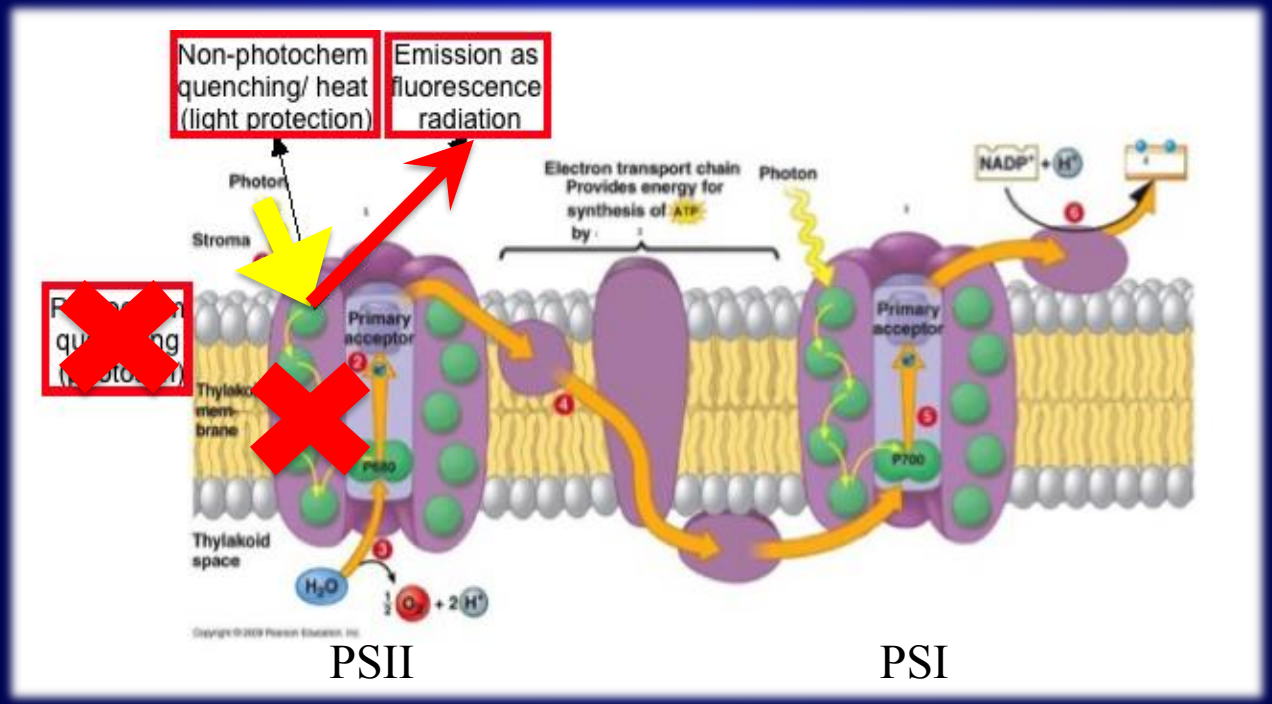
Minimum/*in vivo* fluorescence (F_0)

- Fluorescence in absence of photosynthetic light
- In the dark, limited electrons are used mostly for photosynthesis
- "Background" fluorescence measured



Maximum fluorescence (F_m)

- Fluorescence in absence photosynthesis
  - By high intensity, short wavelength flash of light
  - Electron acceptor plastoquinone (Qa) saturated with electrons (reduced) and closed reaction center
- Excess electrons emitted as fluorescence (max possible)



PhytoFlash

- 9 light emitting diodes (LEDs) (465 nm wavelength peak) arranged circular that evenly saturates sample in optical cell
- 3 LEDs activated to determine minimum fluorescence
- 6 high intensity LEDs activated (actinic light) to determine max fluorescence

Fluorescence Ratio

- Variable chlorophyll fluorescence ($F_v = F_m - F_0$)
  - Difference between fluorescence intensities with closed and open reaction centers
  - Part of the absorbed light energy that would be used in photosynthesis if all reaction centers were in the open state
- Yield (F_v/F_m)
  - Quantum efficiency of primary photochemical reaction of photosynthesis/photochemical quenching (proxy for efficiency of PSII)
  - Algal activity
  - Provides sensitive indicator of cell health

*Fluorometer essentially measures the efficiency of PSII which is an indicator of cell health

High/low Risk

- Algal abundance (# of cells/mL) 10-50 µm size class
- Algal activity (ratio) health/viability
- Filter out <10 µm if high risk (interference)

Measuring a Sample

https://www.youtube.com/watch?v=wruSUZOFOQM

Ballast Water Compliance Monitoring

- Monitoring
  1. Initial inspection
  2. Detailed inspection
  3. Indicative measure
  4. Direct measure of compliance

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  2. Detailed inspection
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  4. Direct measure of compliance

Category	Limit for Discharge
Viable, size > 50 µm	< 10 cells/m^3
Viable, size 10-50 µm	< 10 cells/ml
Vibrio Cholerae	< 1 Colony Forming Units/100ml
Escherichia Coli	< 250 Colony Forming Units/100ml
Intestinal *Enterococci*	< 100 Colony Forming Units/100ml

IMO D-2 Regulations

Ballast Water Compliance Monitoring

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IMO D-2 Regulations

- Indicative measurements (10-50 µm cells)
  - Ideal sample volume
  - Methods simple, quick, no reagents needed
  - Respond to all treatment technologies (chlorination, UV)

IMO Ballast Water Regulations

- Ballast Water Management Convention 2004, 2017
- Vessel-specific Ballast Water and Sediments Management Plan
- Ballast Water Record Book
- International Ballast Water Management Certificate

- D-1 Regulations: ballast water exchange standard
  - ≥ 200 nautical miles from shore AND ≥ 200 m deep
  - Flow-through 3x vol of each ballast tank OR ≥ 95% volumetric exchange

- D-2 Regulations: ballast water performance standard

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Viable, size 10-50 µm	< 10 cells/ml
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Hawai`i

- Vessel-specific Ballast Water Management Plan
- Mid-ocean ballast water exchange or retain all ballast water on board
- Submit a ballast water reporting form to DLNR 24 hours prior to arrival

References

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