# Bioluminescence in Vibrio spp.: A Mechanism for Redox Homeostasis?

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Abstract
Cells rely intimately on dinucleotide cofactors for metabolism. Various metabolic pathways elicited under certain environmental conditions may shift the redox balance of dinucleotide pools, necessitating mechanisms to restore balance. Bioluminescence in *Vibrio* species oxides NADH and NADPH, generating $NAD^+$ and $NADP^+$ for metabolism. This study aimed to test if bioluminescence maintains redox homeostasis under conditions of low oxygen and reductive stress.

Methods

I. **Isolation and Identification of Bioluminescent Microbes**
   Isolate 167 (*Vibrio* sp.) was purified on 'Seawater Complete' medium (SWC) at 30 °C from an environmental sample collected from a boat launch site in Woods Hole, MA. 16s rDNA was amplified using universal PCR primers. 16s rDNA amplificons were sequenced and compared to a non-redundant nucleotide database using BLAST.

II. **Growth of Strains**
   Isolated strain 167 was maintained on SWC agar medium at room temperature. The *Vibrio fischeri* strains ES114 (WT), EVS102, and AMJ2 were obtained from Eric Stabb at the University of Georgia. *V. fischeri* strains were maintained on SWC agar at room temperature.
   For growth of strains, SWC liquid medium was inoculated from a single colony on agar, and grown overnight at 30 °C. Overnight liquid cultures were used to inoculate SWC medium (1:100), and the dilute cell suspension was aliquoted into multi-well plates for use in OD or luminescence measurements (96- or 24-well plates). Mineral oil was applied over liquid in each well to prevent evaporation. Cell suspensions were harvested as needed from wells throughout experiments.

III. **Optical Density and Luminescence Measurements**
   Optical density (OD) and luminescence were measured with a Promega plate reader. OD was measured by absorbance at 600 nm, and luminescence as total light emission with 200 ms integration, or with a 495 BP filter set. Measurements were recorded for 20 hours, at 10 minute resolution. Plates were shaken for 3 seconds prior to measurements with a 2 mm shaking radius.

IV. **Oxygen Electrode Measurements**
   24-well plates were profiled for oxygen concentration as a function of depth. Briefly, a clark-type oxygen microelectrode was used to probe the depth of wells containing SWC medium (inoculated or uninoculated) in 300 $\mu$m step size for a total depth of 8000 $\mu$m.

V. **NAD(P)(H) Measurements**
   NAD+, NADH, NADP+, and NADPH measurements were performed according to the method detailed in Kern, Price-Whelan, and Newman (Springer Science, 2014). Briefly, cells were centrifuged, lysed in 0.2 M HCl or NaOH at 50 °C for 10 minutes, quenched on ice for 5 minutes, neutralized with 0.1 M NaOH or HCl, centrifuged,

and the supernatant containing the dinucleotide cofactor removed and stored at -80 °C. All samples were thawed and processed in parallel using an enzymatic cycling assay. Absorbance at 560 nm was recorded for each reaction in a Promega plate reader.
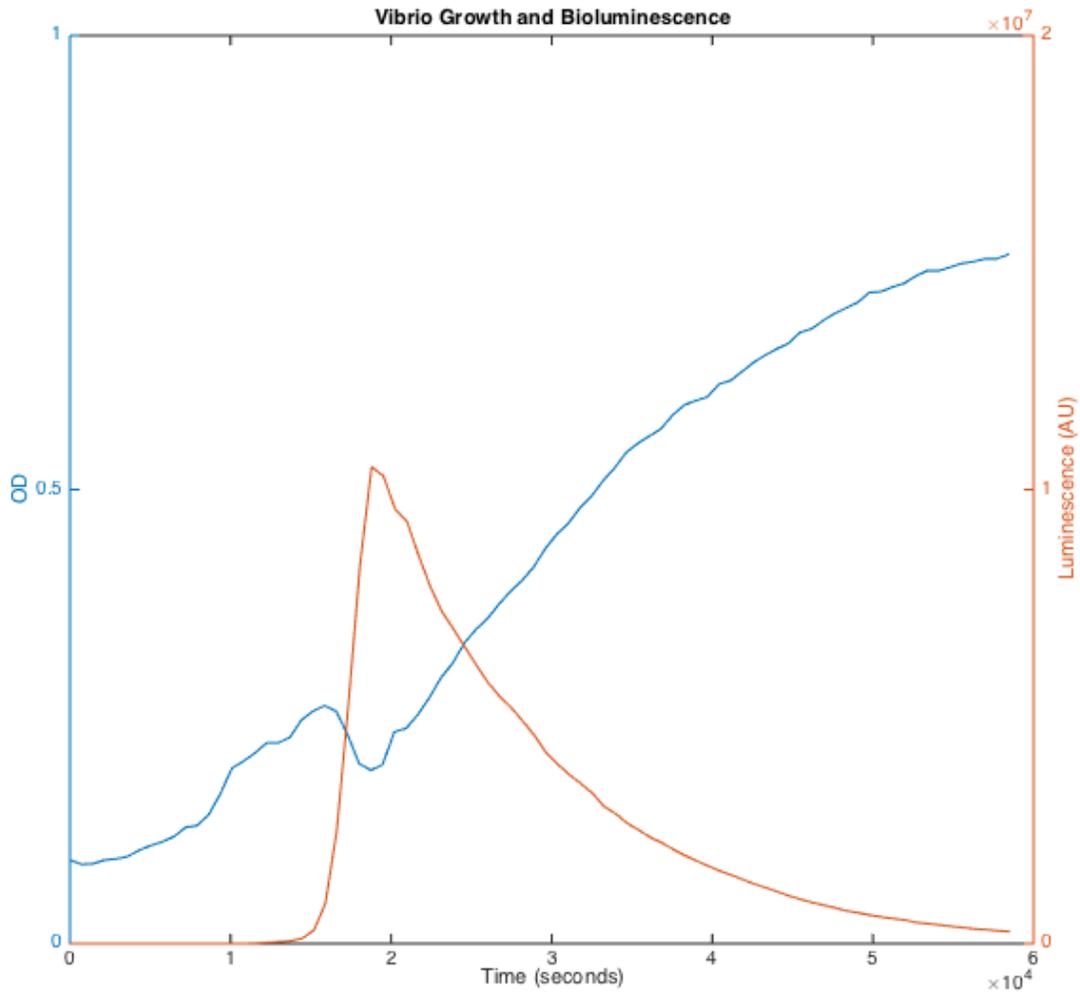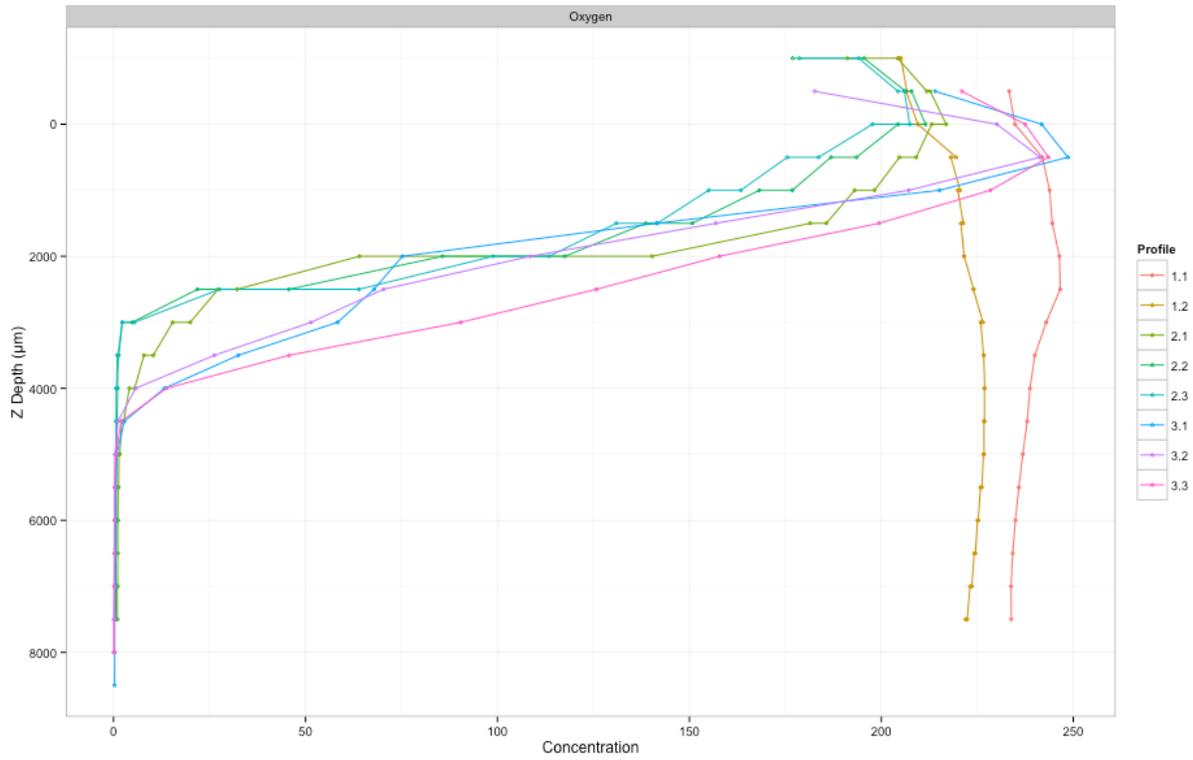
Results
Correlation of Growth with Bioluminescence



Figure 1. Representative traces of OD and luminescence for *Vibrio* isolate 167 grown in 24-well plates (n=95). *V. fischeri* strains exhibited identical behavior.

Figure 2. Oxygen Profiling of Well Dishes



Oxygen is depleted rapidly after inoculation of wells with *Vibrio* strain 167.
Profile series 1, uninoculated wells; series 2, mid-log phase growth; series 3, late-log phase growth.
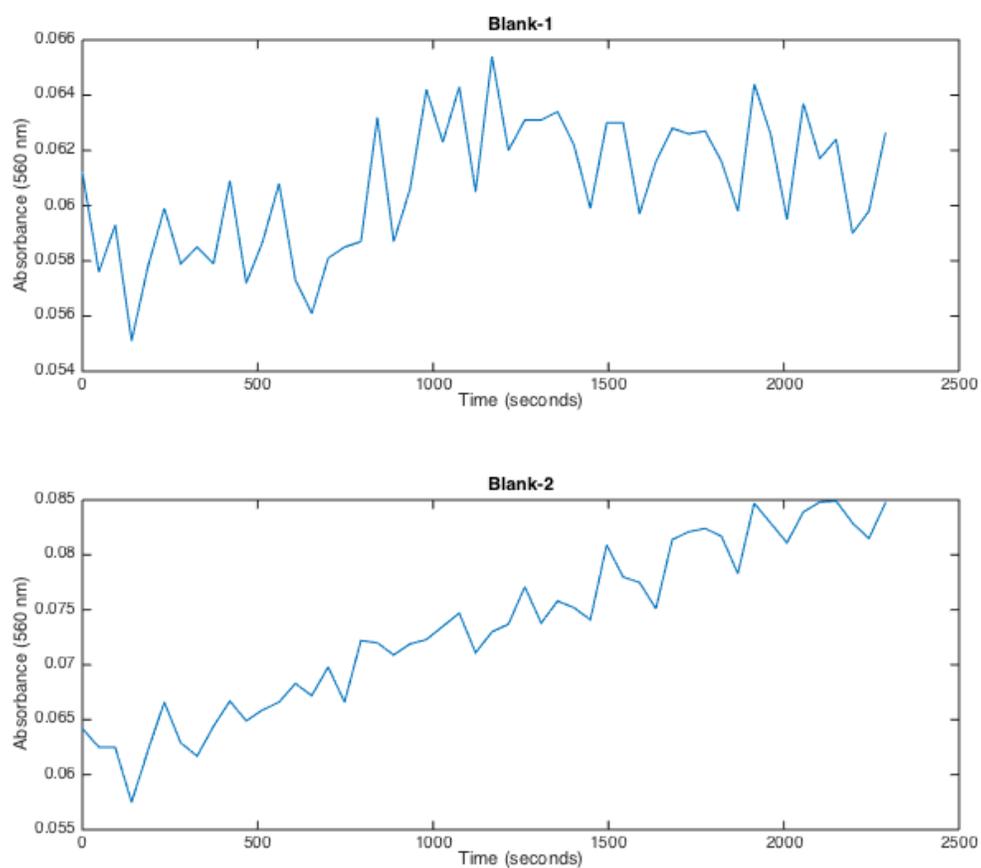
NAD+:NADH Ratiometric Measurements



Figure 3. Absorbance traces at 560 nm of blank wells containing reagent mixture with no added NAD+/NADH or NADP+/NADPH samples.
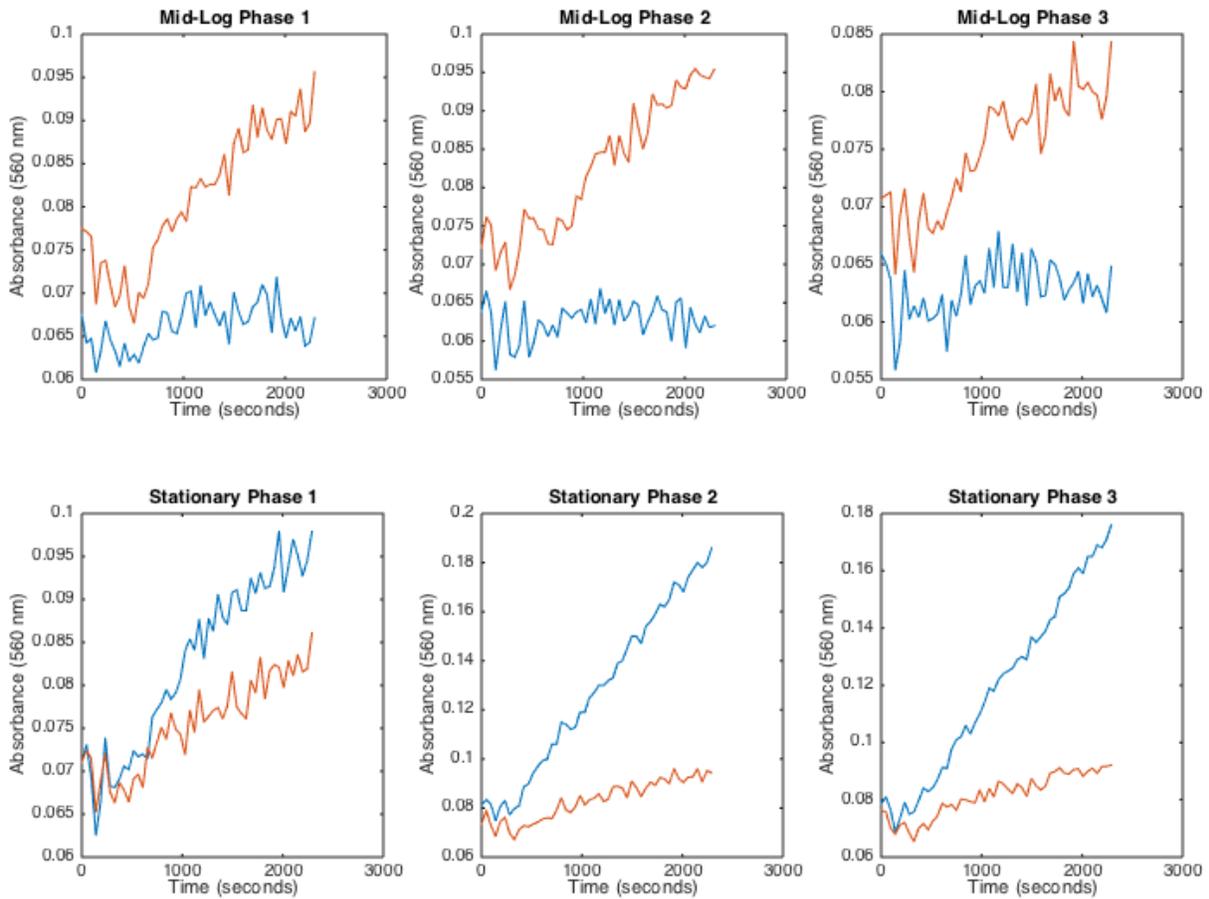
Figure 4. Abs at 560 nm for $NAD^+$ (blue) and NADH (orange) measured in triplicate for 2 conditions (mid-log phase/luminescence peak and late-log/stationary phase).
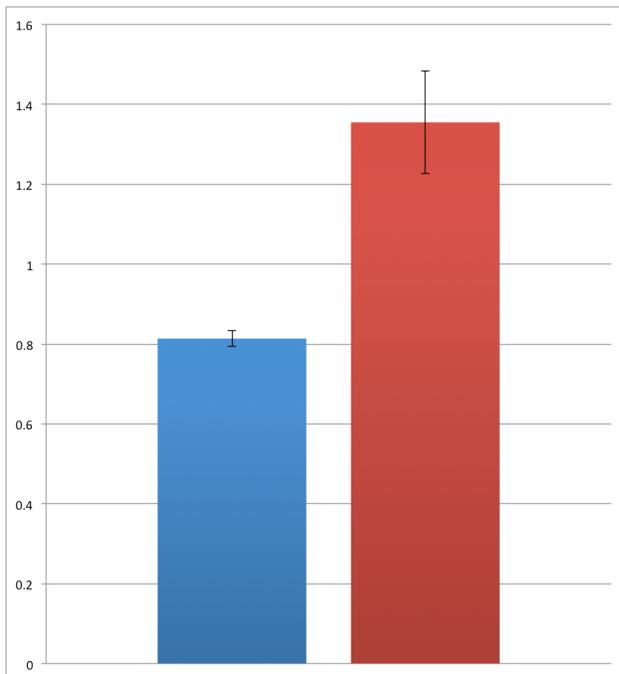


Figure 5. Summary of $NAD^+$:NADH ratio in mid-log phase (blue, 0.81) and stationary phase (red, 1.36). N = 3 (biological replicates) and error bars represent standard error of the mean.
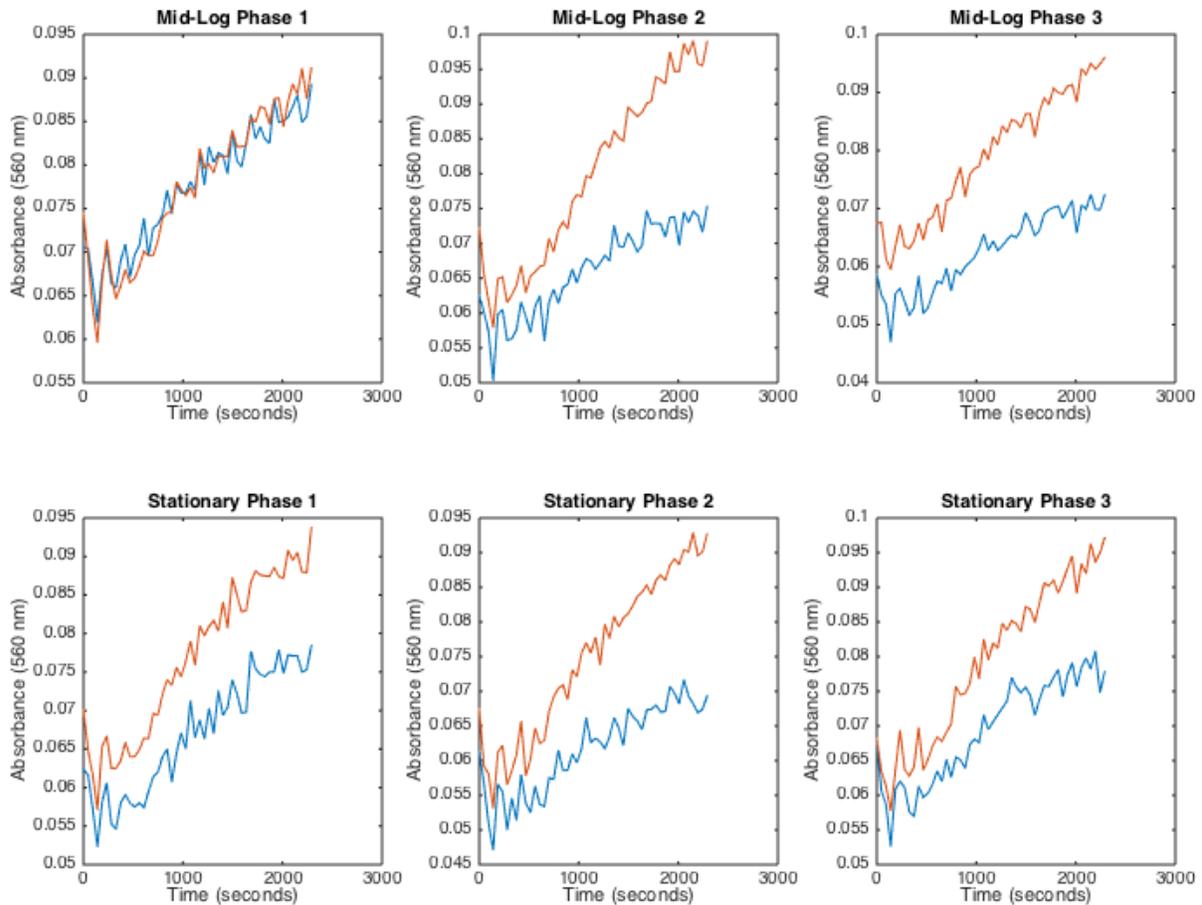
Figure 6. Abs at 560 nm for NADP+ (blue) and NADPH (orange) measured in triplicate for 2 conditions (mid-log phase/luminescence peak and late-log/stationary phase).
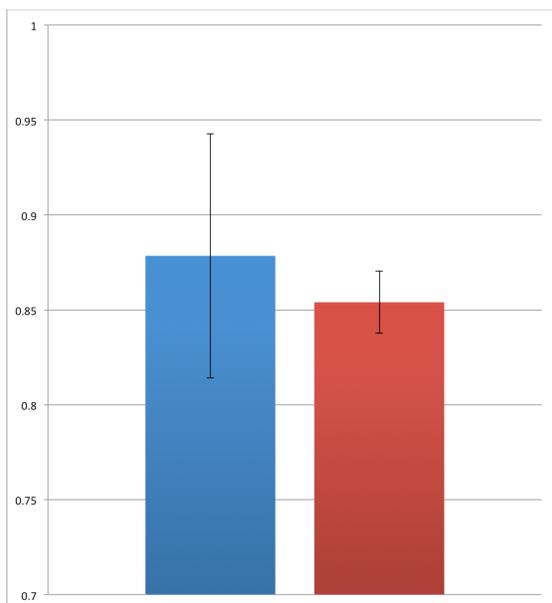


Figure 7. Summary of NADP+:NADPH ratio in mid-log phase (blue, 0.88) and stationary phase (red, 0.85). N = 3 (biological replicates) and error bars represent standard error of the mean.

Discussion

This project aimed to compare $NAD^+$:NADH and $NADP^+$:NADPH ratios between wild type bioluminescent Vibrio spp., and non-luminescent and enhanced-luminescence mutants.

The desired measurements were obtained for isolate 167. To summarize, the ratio of $NAD^+$:NADH in late-log phase cultures was 1.68-fold increased with respect to mid-log phase cultures, at maximal bioluminescence. The ratio of $NADP^+$:NADPH was not significantly different between mid-log and late-log phase cultures.

The mutants obtained from Eric Stabb were derived from *V. fischeri* strain ES114, an isolate from the squid *E. scolopes* light organ. Most unfortunately, strain ES114 does not bioluminescence sufficiently outside of the squid host. C6-Homoserine lactone autoinducer was supplemented to cultures of ES114 and mutants, EVS102 and AMJ2, at ranges of 50 $\mu$M to 50 mM, without any measurable effect on luminescence. Additionally, the ES114 strain and mutants did not grow to high cell density in multi-well plates, preventing high-resolution monitoring of luminescence and optical density. The lack of a non-luminescent mutant in this study severely limited interpretation of the hypothesized role of bioluminescence in redox homeostasis. Future work will aim to generate a non-luminescent mutant in a bioluminescent strain that is luminescent in its free-living form.

Acknowledgements

References

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