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August 19, 2014

The Effect of Redox Potential on Bioluminescence in *Vibrio* spp.

Background

Bioluminescence is currently found in organisms from bacterial and eukaryotic domains. Most bioluminescent organisms are found in the ocean. Many examples of bioluminescence occur in conjunction with symbiotic relationships between ocean-dwelling animals and bacteria (Haddock et al., 2010). The general mechanism of bioluminescence in bacteria involves a reduced substrate, called a luciferin (tetradecanal in *Vibrio* spp.), co-factors ($FMNH_2$ in *Vibrios*), molecular oxygen (O_2), and luciferase. The O_2 is reduced while $FMNH_2$ and tetradecanal are oxidized. These substrates are regenerated using ATP, NADPH, and NADH. Luciferase, which constitutes a substantial fraction of the total soluble protein of the cell, competes with other oxygen-consuming and respiratory enzymes. As luciferase has a very high affinity for oxygen, it is logical that bioluminescent organisms substantially affect environmental oxygen concentrations (Ruby & McFall-Ngai, 1999). Luciferase is also under the control of quorum sensing. The expression of the genes controlling bioluminescence induces further bioluminescence not only within a single organism, but other individuals in a population. Being charismatic, associated with animal hosts, and under control of quorum sensing, bioluminescence in *Vibrio fischeri* has led to the development of a genetic system for this organism; much knowledge of the physiology of *Vibrios* has been gained (Dunn, 2012). This, and the connection of luciferase to redox chemistry and metabolic pathways via reducing equivalents, makes *V. fischeri* a tractable system in which to study questions of redox and energy constraints.

Several genes are necessary for the production and regulation of bioluminescence. The lux operon contains two regulators. LuxI is an autoinducer that is important in quorum-sensing regulation of bioluminescence, together with the signal it generates (3O6C). LuxR is an activator (Dunn, 2012). Further control over the lux operon is provided by ArcA. It represses luminescence by binding to the lux operon. However, ArcA activity is redox sensitive: ArcA represses the lux operon under reducing conditions, but is sequestered and allows LuxR to promote the lux operon under oxidizing conditions (Bose et al., 2007).

Vibrios' use of O_2 is puzzling. *Vibrios* are facultative anaerobes: they use O_2 for respiration when it's available and NO_3^{2-} when it isn't (Dunn, 2012). Why would a facultatively anaerobic chemoorganotroph produce light using oxygen? Why wouldn't such an organism use available oxygen as a terminal electron acceptor? Regeneration of the substrates for bioluminescence is a very energy- and reducing-equivalent-intensive process. At this moment in evolutionary history, bioluminescence is understood in the context of symbiosis. There are many signals provided by the host organism to induce the quorum-sensing pathway that regulates bioluminescence in *Vibrios*. What, though, would be the benefit to organisms to evolve bioluminescence?

Bioluminescence is broadly hypothesized to have evolved in response to reactive oxygen species (ROS). Rees *et al*. (1998) propose that luciferins, which are commonly antioxidant, co-evolved with protein partners to scavenge ROS. In contradiction, Ruby & McFall-Ngai (1999) proposed that bioluminescence evolved as a consequence of scavenging nutrients from hosts by excretion of ROS. They note that some pathogens employ such a strategy, and that use of alternative substrates results in superoxide. Alternatively, it has been proposed that evolutionary pressure for symbiosis has always existed. There is evidence that bioluminescent bacteria are preferentially eaten by zooplankton and subsequently by fish, thereby gaining access to a nutrient rich environment (Zarubin et al., 2012). Finally, it might be that V. fischeri employs bioluminescence to balance the redox state of various electron carriers to keep metabolic cycles moving. Elevated environmental oxygen concentrations could pull electrons away from other carriers and inducing metabolic stress.

Following up on the above hypotheses, Dr. Eric Stabb's research group investigated the effects of redox potential on bioluminescence in *V. fischeri*. The questions driving their research for the past few years has centered on the existence of environmental signals that induce or inhibit bioluminescence. They discovered the role of ArcA in the regulation of the lux operon. Specifically, they found that addition of reductant in the absence of ArcA dramatically increased bioluminescence. They conclude that bioluminescence is under environmental as well as biological control (Bose *et al*., 2007).

This result makes sense if bioluminescence is a way to remove excess oxygen from the cell because as the O_2 concentration increases, the lux operon starts to be expressed and the excess O_2 is removed from the cell as light. Further, in a sub-population of ArcA mutants, production of 3OC6 increased over 100-fold, and induced bioluminescence in neighboring wild-type cells. This underscores the importance of ArcA in mediating bioluminescence (Septer & Stabb, 2012).

If it is true that the environmental redox state regulates bioluminescence via ArcA, then bioluminescence should be observable at low cell density when the appropriate environmental cues are present. It seems straightforward to test this hypothesis by measuring bioluminescence in high redox potential environments and at low cell density.

Methods

I grew *Vibrios* isolated from Garbage Beach in Wood's Hole, Massachusetts under various conditions. I measured the optical density at 600 nm and bioluminescence using the luminometer built into a Promega GloMax.

Cells were grown in "Complete Seawater" (SWC) or "Seawater LB" (SWLB, LB prepared using seawater base) and plated on these media, with agar included (Appendix A). Filter-sterilized resazurin was added to liquid media (100 µL / L) as a visual indicator of approximate redox potential. Catalase was added to specific cultures (16.48 µM) to increase the oxygen concentration upon addition of hydrogen peroxide (20.6 or 41.2 uL / mL). Cultures were grown at room temperature or 37°C. Oxygen concentration in the media was also manipulated by varying aeration of cultures (closed *vs.* open *vs.* shaking tubes). A subset of cultures was incubated with unidentified oxygenic phototrophs originally collected from Sippewissett Salt Marsh phototrophy was confirmed using microscopy.

For each experiment, growth was measured at approximately 25-minute intervals until cultures were visibly turbid and resazurin became colorless.

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Growth curves were adjusted to set the blank for each experiment to zero, which was necessary as absorbance measurements were taken near the detection limit of the instrument. Specific bioluminescence was calculated by dividing bioluminescence by A_{600} (Appendix B).

Results

Initially, I controlled the amount of oxygen in the media by closing tubes, leaving them open, and shaking them. I saw no difference between these treatments, but learned from this that growth on SWC was relatively slow, such that the resolution of the growth curve could be increased (Figure 1).

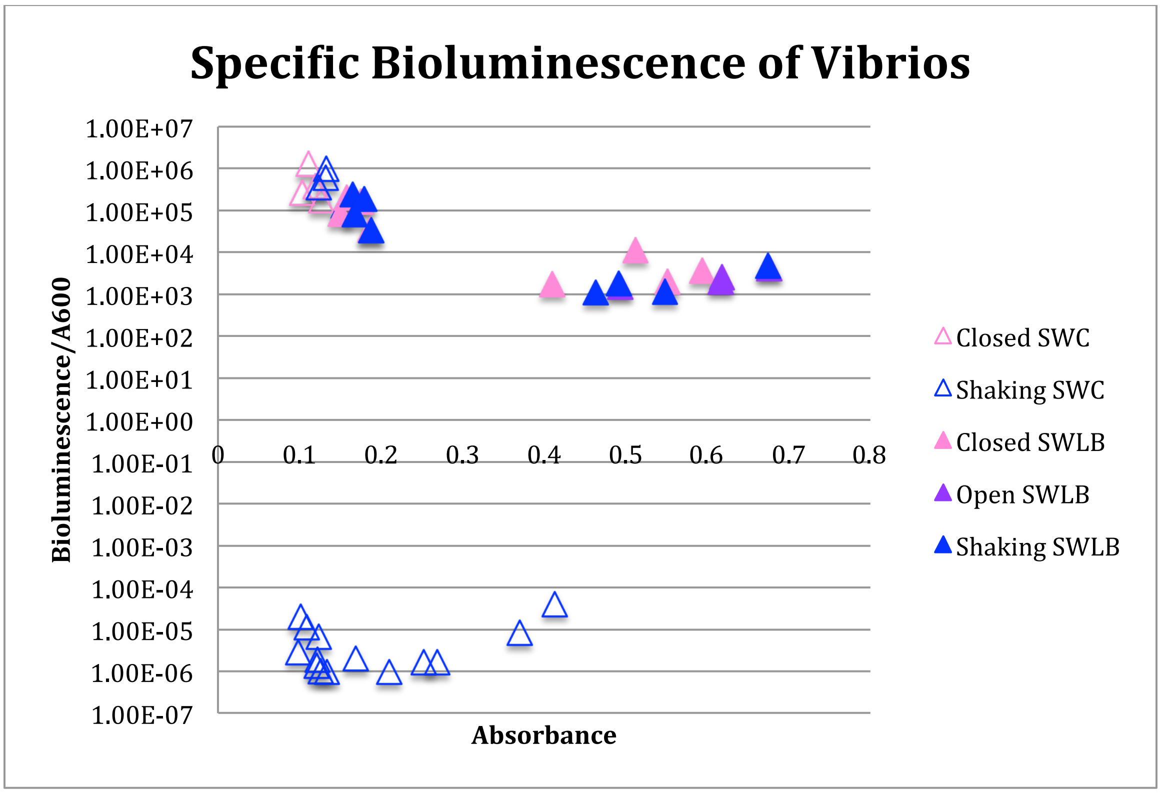

Figure 1. Specific Bioluminescence of *Vibrio* cultures exposed to various oxygen concentrations and grown under various nutrient regimes. A subset of shaken SWC samples is inexplicably far less bioluminescent than the rest.

Ultimately, I manipulated the media to increase the oxygen concentration to 1.5X and 2X that of seawater. Cultures with more oxygen grew more quickly: there is less variation in the A_{600} in the added treatments than in the ambient treatment. However, the luminescence per biomass of the ambient oxygen treatment was much greater at low cell densities. All of these cultures used for this experiment were shaken, so ambient oxygen appears to provide enough oxygen for bioluminescence, and increasing the environmental redox potential doesn't induce increased bioluminescence (Figure 2).

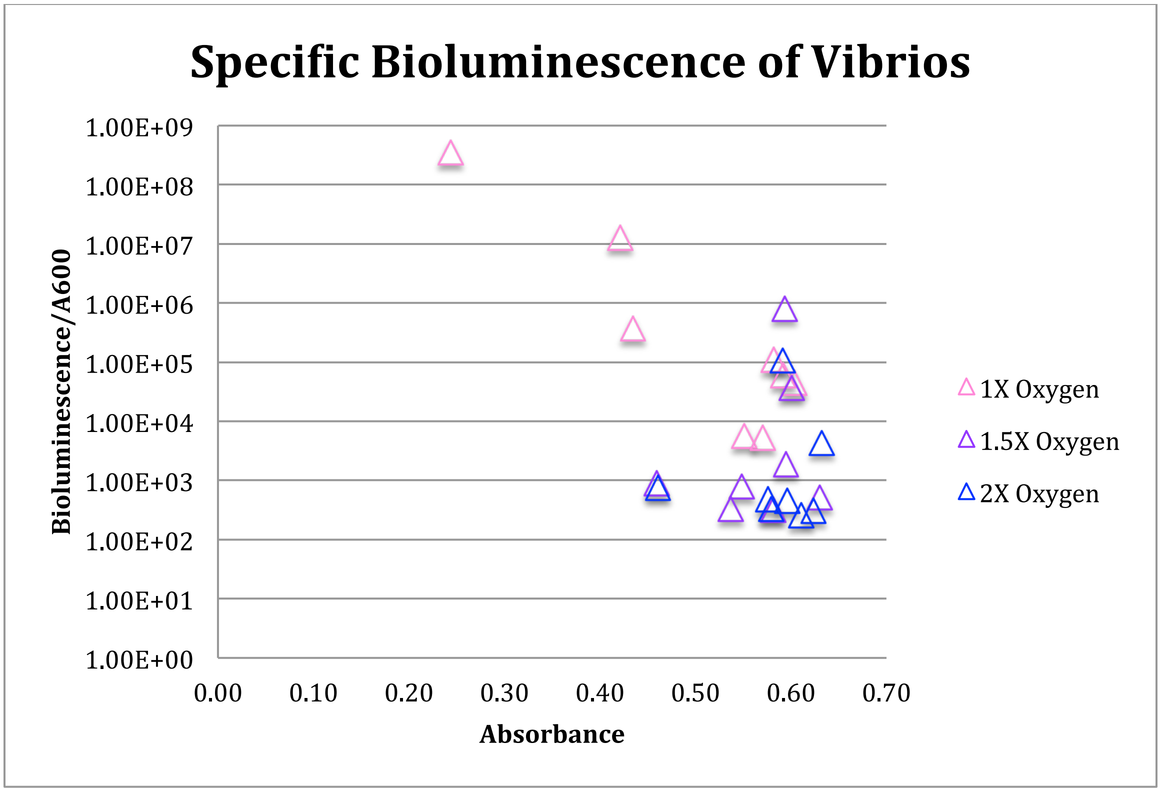

Figure 2. Specific Bioluminescence of *Vibrio* cultures exposed to increased oxygen concentrations. Cultures were grown on SWC. A small but insignificant increase in specific bioluminescence is evident in cultures exposed to ambient oxygen, and less variation in A_{600} exists among cultures exposed to increased oxygen.

The addition of oxygenic phototrophs to *Vibrio* cultures was intended to provide a low but steady supply of oxygen to the *Vibrios*. No difference in bioluminescence was detected between *Vibrios* with and without phototrophs added (Figure 3).

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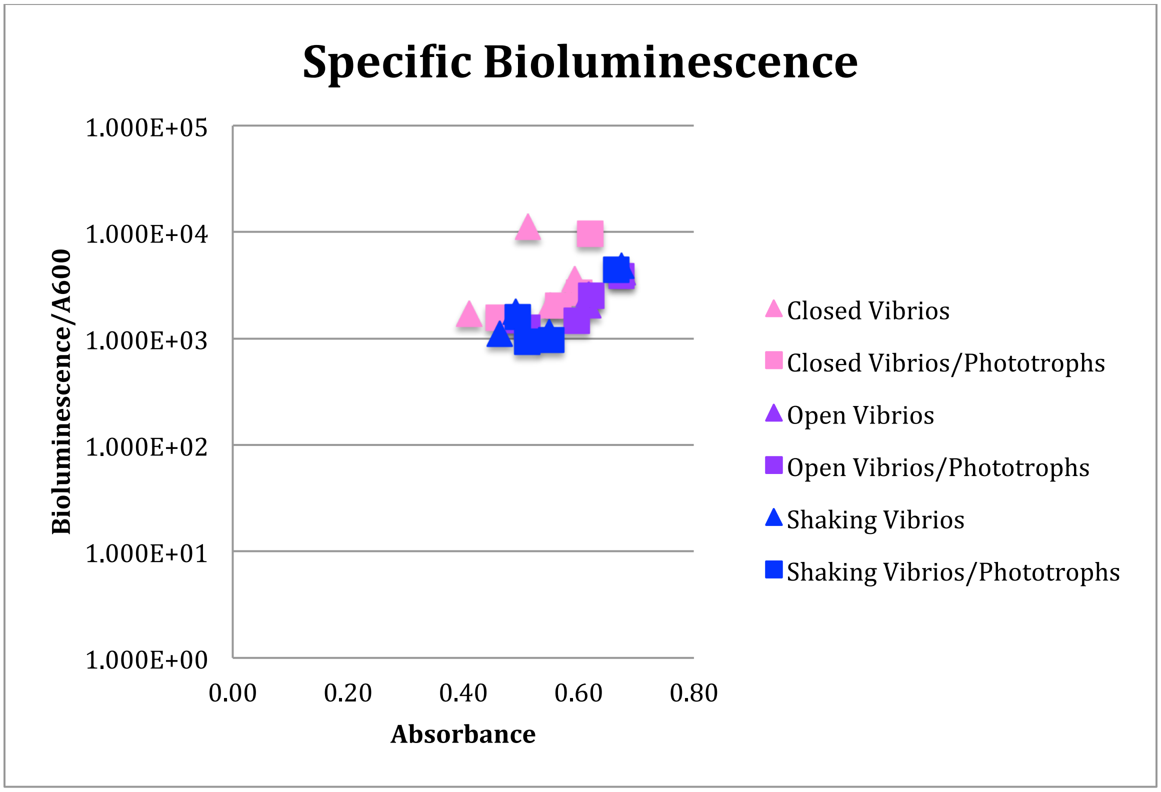

Figure 3. Specific Bioluminescence of mixed *Vibrio* and oxygenic phototroph cultures. Cultures were grown on SWLB. A small but insignificant increase in specific bioluminescence is evident in closed cultures.

Discussion

All of the experiments that were completed suffered from time constraints. With replication and more sensitive instrumentation or more creative techniques, a true conclusion might be drawn about the effect of increased redox potential on bioluminescence in *Vibrios*. The proximal solutions include tuning assays to precisely manipulate the environmental conditions, and including both reducing and oxidizing agents in media. A more sophisticated approach would include working with a series of knockout strains, including ArcA and LuxI knockouts. Further genetic work should include mutagenesis screens to select for up-regulation of bioluminescence where ArcA remains intact.

An additional web of regulators controlling bioluminescence simultaneously acts on the lux operon, confusing results from this series of experiments. Greater bioluminescence is observed under low phosphate conditions (Lyell *et al.*, 2010). FNR, which is necessary for fumarate- and nitrate-respiration, also mediates the repression of lux (Septer *et al.*, 2010). Reduced iron conditions, in contrast, increases bioluminescence via interactions among Fur, LitR, and LuxR (Septer *et al.*, 2013). Bioluminescence in *Vibrios* also requires $MgSO_4$ (Tabei *et al.*, 2012a). Finally, there is evidence that bioluminescence is dependent on the presence of sulfur under nutrient-limiting conditions (Tabei *et al.*, 2012b). None of these confounding factors were addressed here.

Notwithstanding the caveats mentioned above, my results indicate that increased environmental redox potential doesn't induce bioluminescence. I would have expected the opposite result if bioluminescence were a system to remove excess oxygen from cells. It seems reasonable that one or both of the following explanations of the evolution of bioluminescence is consistent with the results I observed and with the current body of literature on bacterial bioluminescence. It should be considered that many molecules and enzymes are slightly bioluminescent; the initial emission of light from an ancient luciferase may have been much less pronounced than today.

First, maintaining redox poise is a crucial cellular function. The ability to make oxidized cofactors available for reduction in anabolic pathways will always be achieved by conceding an energetic loss. Bioluminescence appears expensive, but it may be, or have been in the past, the most efficient way for individuals that are not suffering from ATP starvation to maintain redox balance.

Second, the contention that luciferins may be as or more important in the evolutionary history of bioluminescence is not constrained to their antioxidant properties. It is possible that tetradecanal was an abundant substrate that was metabolized by the ancestor of contemporary luciferases. As symbiotic relationships evolved between bioluminescent bacteria and hosts, the environmental or metabolic source of tetradecanal may have been lost or become unimportant. Thus, perhaps what we see when examining the bioluminescence pathway in *Vibrios* is a broken tool that nature subsequently recycled.

Acknowledgements

I began this class with classical notions of habitability, and I have been struggling to understand a more nuanced, more accurate model, specifically as suggested by Tori Hoehler (2007). Central to these ideas is the concept of redox poise, as oxidation-reduction reactions not only affect specific metabolites present in cells and their environments, but also the energy balance of cells and the energy balance of cells' environments. I discussed the ideas of the absolute energy minimum and redox balance with Drs. Kurt Hanselmann and Dianne Newman at length. I am indescribably grateful for the relatively more sophisticated and generally greater understanding of bacterial cell biology I gained as a direct result of those discussions.

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Appendix A: Media

1X Seawater Base (SW) (per 20 L)

Component	Amount	FW	Final Conc.
NaCl	400 g	58.44	342.2 mM
$MgCl_2 \cdot 6\ H_2O$	60 g	203.30	14.8 mM
$CaCl_2 \cdot 2H_2O$	3 g	147.02	1.0 mM
KCl	10 g	74.56	6.71 mM

SWC

Component	Amount
SW	1000 mL
Bacto tryptone	5 g
Yeast extract	1 g
Glycerol	3 mL
Agar (for plates)	15 g
pH to 7.0	

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Appendix B: Data

Sample	Treatment	Time point	A600	Corrected A600	Bio-luminescence	Corrected Bio-luminescence	Specific Bio-luminescence	Date	Time	Media
Vibrios	Shaking	0	4.733 E-02	1.335E-01	1.010E+05	1.370E+05	9.741E-07	4-Aug	16:06	SWB
Vibrios	Shaking	0	5.279 E-02		1.249E+05			4-Aug	16:06	SWB
Vibrios	Shaking	0	5.297 E-02		5.992E+04			4-Aug	16:06	SWB
Vibrios	Shaking	0	5.306 E-02		1.221E+05			4-Aug	16:06	SWB
Vibrios	Shaking	0	5.346 E-02		1.452E+05			4-Aug	16:06	SWB
Blank	Shaking	0	8.155 E-02		2.639E+04			4-Aug	16:06	SWB
Vibrios	Shaking	1	5.186 E-02	1.257E-01	8.245E+04	1.273E+05	9.875E-07	4-Aug	16:21	SWB
Vibrios	Shaking	1	5.322 E-02		1.080E+05			4-Aug	16:21	SWB
Vibrios	Shaking	1	5.649 E-02		9.254E+04			4-Aug	16:21	SWB
Vibrios	Shaking	1	6.361 E-02		9.168E+04			4-Aug	16:21	SWB
Vibrios	Shaking	1	6.878 E-02		1.217E+05			4-Aug	16:21	SWB
Blank	Shaking	1	6.687 E-02		2.798E+04			4-Aug	16:21	SWB
Vibrios	Shaking	2	5.690 E-02	1.209E-01	6.486E+04	8.667E+04	1.395E-06	4-Aug	16:45	SWB
Vibrios	Shaking	2	5.735 E-02		8.290E+04			4-Aug	16:45	SWB
Vibrios	Shaking	2	5.811 E-02		5.508E+04			4-Aug	16:45	SWB
Vibrios	Shaking	2	6.386 E-02		4.106E+04			4-Aug	16:45	SWB
Vibrios	Shaking	2	6.504 E-02		5.804E+04			4-Aug	16:45	SWB
Blank	Shaking	2	6.064 E-02		2.628E+04			4-Aug	16:45	SWB
Vibrios	Shaking	3	5.035 E-02	1.223E-01	4.574E+04	6.509E+04	1.878E-06	4-Aug	17:17	SWB
Vibrios	Shaking	3	5.637 E-02		4.666E+04			4-Aug	17:17	SWB
Vibrios	Shaking	3	5.918 E-02		5.350E+04			4-Aug	17:17	SWB
Vibrios	Shaking	3	5.941 E-02		4.949E+04			4-Aug	17:17	SWB
Vibrios	Shaking	3	6.174 E-02		2.803E+04			4-Aug	17:17	SWB
Blank	Shaking	3	6.487 E-02		2.041E+04			4-Aug	17:17	SWB
Vibrios	Shaking	4	4.502 E-02	9.828E-02	2.012E+04	3.477E+04	2.827E-06	4-Aug	17:49	SWB
Vibrios	Shaking	4	4.799 E-02		2.498E+04			4-Aug	17:49	SWB
Vibrios	Shaking	4	5.192 E-02		3.092E+04			4-Aug	17:49	SWB
Vibrios	Shaking	4	5.539 E-02		1.971E+04			4-Aug	17:49	SWB

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Vibrios	Shaking	4	5.615 E-02		2.985E+04			4-Aug	17:49	SWB
Blank	Shaking	4	4.698 E-02		9.649E+03			4-Aug	17:49	SWB
Vibrios	Shaking	5	5.027 E-02	1.239E-01	1.196E+04	1.845E+04	6.712E-06	4-Aug	18:15	SWB
Vibrios	Shaking	5	5.194 E-02		1.384E+04			4-Aug	18:15	SWB
Vibrios	Shaking	5	5.487 E-02		1.323E+04			4-Aug	18:15	SWB
Vibrios	Shaking	5	5.760 E-02		8.783E+03			4-Aug	18:15	SWB
Vibrios	Shaking	5	5.804 E-02		1.629E+04			4-Aug	18:15	SWB
Blank	Shaking	5	6.931 E-02		5.631E+03			4-Aug	18:15	SWB
Vibrios	Shaking	7	5.582 E-02	4.135E-01	7.305E+03	1.017E+04	4.067E-05	4-Aug	19:14	SWB
Vibrios	Shaking	7	5.582 E-02		2.200E+03			4-Aug	19:14	SWB
Vibrios	Shaking	7	5.774 E-02		4.844E+03			4-Aug	19:14	SWB
Vibrios	Shaking	7	6.142 E-02		5.644E+03			4-Aug	19:14	SWB
Vibrios	Shaking	7	8.451 E-02		4.664E+03			4-Aug	19:14	SWB
Blank	Shaking	7	3.504 E-01		5.234E+03			4-Aug	19:14	SWB
Vibrios	Shaking	9	4.702 E-02	1.086E-01	5.487E+03	9.524E+03	1.141E-05	4-Aug	20:06	SWB
Vibrios	Shaking	9	5.128 E-02		7.405E+03			4-Aug	20:06	SWB
Vibrios	Shaking	9	5.230 E-02		5.091E+03			4-Aug	20:06	SWB
Vibrios	Shaking	9	5.491 E-02		2.453E+03			4-Aug	20:06	SWB
Blank	Shaking	9	4.899 E-02		4.554E+03			4-Aug	20:06	SWB
Vibrios	Shaking	9	5.773 E-02		4.414E+03			4-Aug	20:06	SWB
Blank	Shaking	12	4.179 E-02	1.019E-01	3.040E+03	5.138E+03	1.984E-05	4-Aug	21:27	SWB
Vibrios	Shaking	12	4.479 E-02		2.403E+03			4-Aug	21:27	SWB
Vibrios	Shaking	12	4.976 E-02		2.380E+03			4-Aug	21:27	SWB
Vibrios	Shaking	12	4.981 E-02		2.780E+03			4-Aug	21:27	SWB
Vibrios	Shaking	12	5.354 E-02		1.406E+03			4-Aug	21:27	SWB
Vibrios	Shaking	12	5.398 E-02		1.523E+03			4-Aug	21:27	SWB
Blank	Shaking	0	1.022 E-01	1.688E-01	1.855E+04	8.478E+04	1.991E-06	5-Aug	11:53	SWC
Vibrios	Shaking	0	6.038 E-02		7.209E+04			5-Aug	11:53	SWC
Vibrios	Shaking	0	6.331 E-02		7.783E+04			5-Aug	11:53	SWC
Vibrios	Shaking	0	6.907 E-02		6.976E+04			5-Aug	11:53	SWC
Vibrios	Shaking	0	7.003 E-02		4.221E+04			5-Aug	11:53	SWC

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Vibrios	Shaking	0	7.035 E-02		6.924E+04			5-Aug	11:53	SWC
Blank	Shaking	1	1.420 E-01	2.102E-01	3.653E+04	2.151E+05	9.775E-07	5-Aug	12:22	SWC
Vibrios	Shaking	1	6.262 E-02		1.088E+05			5-Aug	12:22	SWC
Vibrios	Shaking	1	6.384 E-02		1.873E+05			5-Aug	12:22	SWC
Vibrios	Shaking	1	6.846 E-02		1.793E+05			5-Aug	12:22	SWC
Vibrios	Shaking	1	7.138 E-02		1.468E+05			5-Aug	12:22	SWC
Vibrios	Shaking	1	7.501 E-02		2.705E+05			5-Aug	12:22	SWC
Blank	Shaking	2	1.719 E-01	2.522E-01	2.681E+04	1.540E+05	1.637E-06	5-Aug	12:58	SWC
Vibrios	Shaking	2	6.810 E-02		8.194E+04			5-Aug	12:58	SWC
Vibrios	Shaking	2	7.543 E-02		9.913E+04			5-Aug	12:58	SWC
Vibrios	Shaking	2	7.663 E-02		1.413E+05			5-Aug	12:58	SWC
Vibrios	Shaking	2	8.642 E-02		1.408E+05			5-Aug	12:58	SWC
Vibrios	Shaking	2	9.493 E-02		1.730E+05			5-Aug	12:58	SWC
Blank	Shaking	3	1.009 E-01	2.686E-01	2.862E+04	1.642E+05	1.636E-06	5-Aug	13:30	SWC
Vibrios	Shaking	3	1.451 E-01		1.251E+05			5-Aug	13:30	SWC
Vibrios	Shaking	3	1.454 E-01		9.341E+04			5-Aug	13:30	SWC
Vibrios	Shaking	3	1.563 E-01		1.353E+05			5-Aug	13:30	SWC
Vibrios	Shaking	3	1.916 E-01		1.092E+05			5-Aug	13:30	SWC
Vibrios	Shaking	3	2.001 E-01		2.148E+05			5-Aug	13:30	SWC
Blank	Shaking	4	1.080 E-01	3.700E-01	1.286E+04	4.449E+04	8.317E-06	5-Aug	14:10	SWC
Vibrios	Shaking	4	2.725 E-01		5.397E+04			5-Aug	14:10	SWC
Vibrios	Shaking	4	2.464 E-01		2.312E+04			5-Aug	14:10	SWC
Vibrios	Shaking	4	2.547 E-01		2.156E+04			5-Aug	14:10	SWC
Vibrios	Shaking	4	2.635 E-01		3.042E+04			5-Aug	14:10	SWC
Vibrios	Shaking	4	2.731 E-01		2.908E+04			5-Aug	14:10	SWC
Blank	Closed	0	4.822 E-02	1.107E-01	1.833E+03	1.437E+05	1.298E+06	8-Aug	15:42	SWC
Blank	Shaking	0	5.225 E-02	1.330E-01	1.843E+03	1.311E+05	9.852E+05	8-Aug	15:42	SWC
Vibrios	Closed	0	6.098 E-02		1.725E+05			8-Aug	15:42	SWC
Vibrios	Closed	0	6.245 E-02		9.560E+04			8-Aug	15:42	SWC
Vibrios	Closed	0	6.397 E-02		1.575E+05			8-Aug	15:42	SWC
Blank	Shaking	0	6.455 E-02		1.590E+03			8-Aug	15:42	SWC

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Vibrios	Shaking	0	7.042 E-02		1.365E+05			8-Aug	15:42	SWC
Vibrios	Shaking	0	7.138 E-02		1.649E+05			8-Aug	15:42	SWC
Vibrios	Shaking	0	7.472 E-02		1.190E+05			8-Aug	15:42	SWC
Vibrios	Shaking	0	7.555 E-02		1.133E+05			8-Aug	15:42	SWC
Vibrios	Shaking	0	7.652 E-02		1.172E+05			8-Aug	15:42	SWC
Vibrios	Shaking	0	7.920 E-02		1.252E+05			8-Aug	15:42	SWC
Vibrios	Closed	1	6.328 E-02		6.494E+04			8-Aug	16:19	SWC
Vibrios	Closed	1	6.522 E-02		8.255E+04			8-Aug	16:19	SWC
Blank	Shaking	1	4.757 E-02	1.322E-01	1.710E+03	7.899E+04	5.976E+05	8-Aug	16:19	SWC
Blank	Shaking	1	6.158 E-02		1.416E+03			8-Aug	16:19	SWC
Vibrios	Shaking	1	6.862 E-02		7.907E+04			8-Aug	16:19	SWC
Vibrios	Shaking	1	7.739 E-02		6.940E+04			8-Aug	16:19	SWC
Vibrios	Shaking	1	7.772 E-02		7.521E+04			8-Aug	16:19	SWC
Vibrios	Shaking	1	7.814 E-02		7.625E+04			8-Aug	16:19	SWC
Vibrios	Shaking	1	7.967 E-02		8.418E+04			8-Aug	16:19	SWC
Vibrios	Shaking	1	8.418 E-02		8.047E+04			8-Aug	16:19	SWC
Blank	Closed	1	4.853 E-02	1.031E-01	1.870E+03	2.790E+04	2.705E+05	8-Aug	16:19	SWC
Vibrios	Closed	1	6.073 E-02		7.660E+04			8-Aug	16:19	SWC
Blank	Closed	2	5.462 E-02	1.196E-01	8.200E+02	4.505E+04	3.766E+05	8-Aug	16:57	SWC
Blank	Shaking	2	4.850 E-02	1.238E-01	6.800E+02	4.430E+04	3.577E+05	8-Aug	16:57	SWC
Vibrios	Closed	2	6.236 E-02		3.995E+04			8-Aug	16:57	SWC
Vibrios	Closed	2	6.476 E-02		4.619E+04			8-Aug	16:57	SWC
Vibrios	Closed	2	6.790 E-02		4.655E+04			8-Aug	16:57	SWC
Blank	Shaking	2	5.061 E-02		6.700E+02			8-Aug	16:57	SWC
Vibrios	Shaking	2	6.726 E-02		3.917E+04			8-Aug	16:57	SWC
Vibrios	Shaking	2	7.231 E-02		4.877E+04			8-Aug	16:57	SWC
Vibrios	Shaking	2	7.495 E-02		4.349E+04			8-Aug	16:57	SWC
Vibrios	Shaking	2	7.621 E-02		4.491E+04			8-Aug	16:57	SWC
Vibrios	Shaking	2	7.663 E-02		4.111E+04			8-Aug	16:57	SWC
Vibrios	Shaking	2	7.831 E-02		4.431E+04			8-Aug	16:57	SWC
Blank	Closed	3	4.662 E-02	1.263E-01	3.660E+02	2.222E+04	1.759E+05	8-Aug	17:39	SWC

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Blank	Shaking	3	5.968 E-02	1.539E-01	3.800E+02	1.982E+04	1.288E+05	8-Aug	17:39	SWC
Vibrios	Closed	3	7.863 E-02		2.224E+04			8-Aug	17:39	SWC
Vibrios	Closed	3	7.933 E-02		2.240E+04			8-Aug	17:39	SWC
Vibrios	Closed	3	8.120 E-02		2.092E+04			8-Aug	17:39	SWC
Blank	Shaking	3	6.663 E-02		4.060E+02			8-Aug	17:39	SWC
Vibrios	Shaking	3	8.661 E-02		2.031E+04			8-Aug	17:39	SWC
Vibrios	Shaking	3	8.922 E-02		1.867E+04			8-Aug	17:39	SWC
Vibrios	Shaking	3	8.965 E-02		2.029E+04			8-Aug	17:39	SWC
Vibrios	Shaking	3	9.003 E-02		2.049E+04			8-Aug	17:39	SWC
Vibrios	Shaking	3	9.372 E-02		1.856E+04			8-Aug	17:39	SWC
Vibrios	Shaking	3	9.545 E-02		1.827E+04			8-Aug	17:39	SWC
Blank	Closed	0	7.184 E-02	1.582E-01	9.430E+02	3.216E+04	2.032E+05	8-Aug	15:42	SWLB
Vibrios	Closed	0	7.957 E-02		5.000E+04			8-Aug	15:42	SWLB
Vibrios	Closed	0	8.939 E-02		2.178E+04			8-Aug	15:42	SWLB
Vibrios	Closed	0	9.025 E-02		2.187E+04			8-Aug	15:42	SWLB
Blank	Shaking	0	7.486 E-02	1.655E-01	1.073E+03	3.978E+04	2.404E+05	8-Aug	15:42	SWLB
Blank	Shaking	0	7.490 E-02		8.160E+02			8-Aug	15:42	SWLB
Vibrios	Shaking	0	8.321 E-02		5.885E+04			8-Aug	15:42	SWLB
Vibrios	Shaking	0	8.584 E-02		2.019E+04			8-Aug	15:42	SWLB
Vibrios	Shaking	0	9.196 E-02		2.366E+04			8-Aug	15:42	SWLB
Vibrios	Shaking	0	9.335 E-02		8.037E+04			8-Aug	15:42	SWLB
Vibrios	Shaking	0	9.431 E-02		2.474E+04			8-Aug	15:42	SWLB
Vibrios	Shaking	0	9.498 E-02		2.520E+04			8-Aug	15:42	SWLB
Blank	Closed	1	7.986 E-02	1.756E-01	9.830E+02	2.882E+04	1.641E+05	8-Aug	16:19	SWLB
Vibrios	Closed	1	9.078 E-02		5.020E+04			8-Aug	16:19	SWLB
Vibrios	Closed	1	9.763 E-02		1.762E+04			8-Aug	16:19	SWLB
Vibrios	Closed	1	9.885 E-02		1.569E+04			8-Aug	16:19	SWLB
Blank	Shaking	1	7.865 E-02	1.796E-01	9.330E+02	3.320E+04	1.849E+05	8-Aug	16:19	SWLB
Blank	Shaking	1	7.902 E-02		8.000E+02			8-Aug	16:19	SWLB
Vibrios	Shaking	1	9.705 E-02		1.687E+04			8-Aug	16:19	SWLB
Vibrios	Shaking	1	9.885 E-02		1.619E+04			8-Aug	16:19	SWLB

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Vibrios	Shaking	1	9.919 E-02		1.691E+04			8-Aug	16:19	SWLB
Vibrios	Shaking	1	9.949 E-02		5.542E+04			8-Aug	16:19	SWLB
Vibrios	Shaking	1	1.049 E-01		7.103E+04			8-Aug	16:19	SWLB
Vibrios	Shaking	1	1.051 E-01		1.761E+04			8-Aug	16:19	SWLB
Blank	Closed	2	6.096 E-02	1.504E-01	4.530E+02	1.248E+04	8.299E+04	8-Aug	16:57	SWLB
Vibrios	Closed	2	8.444 E-02		7.305E+03			8-Aug	16:57	SWLB
Vibrios	Closed	2	8.912 E-02		2.135E+04			8-Aug	16:57	SWLB
Vibrios	Closed	2	9.467 E-02		7.425E+03			8-Aug	16:57	SWLB
Blank	Shaking	2	6.381 E-02	1.675E-01	2.330E+02	1.375E+04	8.209E+04	8-Aug	16:57	SWLB
Blank	Shaking	2	8.132 E-02		2.360E+02			8-Aug	16:57	SWLB
Vibrios	Shaking	2	8.782 E-02		7.925E+03			8-Aug	16:57	SWLB
Vibrios	Shaking	2	9.061 E-02		7.735E+03			8-Aug	16:57	SWLB
Vibrios	Shaking	2	9.104 E-02		7.822E+03			8-Aug	16:57	SWLB
Vibrios	Shaking	2	9.784 E-02		7.895E+03			8-Aug	16:57	SWLB
Vibrios	Shaking	2	1.001 E-01		2.822E+04			8-Aug	16:57	SWLB
Vibrios	Shaking	2	1.023 E-01		2.150E+04			8-Aug	16:57	SWLB
Blank	Closed	3	8.443 E-02	1.860E-01	3.060E+02	6.492E+03	3.490E+04	8-Aug	17:39	SWLB
Vibrios	Closed	3	1.011 E-01		3.806E+03			8-Aug	17:39	SWLB
Vibrios	Closed	3	1.011 E-01		1.090E+04			8-Aug	17:39	SWLB
Vibrios	Closed	3	1.025 E-01		3.851E+03			8-Aug	17:39	SWLB
Blank	Shaking	3	7.947 E-02	1.877E-01	1.660E+02	6.394E+03	3.406E+04	8-Aug	17:39	SWLB
Blank	Shaking	3	8.831 E-02		2.360E+02			8-Aug	17:39	SWLB
Vibrios	Shaking	3	9.213 E-02		3.450E+03			8-Aug	17:39	SWLB
Vibrios	Shaking	3	9.870 E-02		3.776E+03			8-Aug	17:39	SWLB
Vibrios	Shaking	3	1.026 E-01		8.753E+03			8-Aug	17:39	SWLB
Vibrios	Shaking	3	1.032 E-01		3.256E+03			8-Aug	17:39	SWLB
Vibrios	Shaking	3	1.077 E-01		3.580E+03			8-Aug	17:39	SWLB
Vibrios	Shaking	3	1.186 E-01		1.434E+04			8-Aug	17:39	SWLB
Blank	Closed-Light	0	3.053 E-01		3.216E+03			11-Aug	12:39	SWLB
Phototrophs	Closed-Light	0	3.287 E-01	6.341E-01	1.260E+02	3.342E+03	5.271E+03	11-Aug	12:39	SWLB
Vibrios	Closed-Light	0	2.068 E-01	5.121E-01	2.500E+03	5.716E+03	1.116E+04	11-Aug	12:39	SWLB

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Vibrios - Phototrophs	Closed-Light	0	3.142 E-01	6.195E-01	2.780E+03	5.996E+03	9.679E+03	11-Aug	12:39	SWLB
Blank	Open-Light	0	3.349 E-01		1.560E+02			11-Aug	12:39	SWLB
Phototrophs	Open-Light	0	3.416 E-01	6.765E-01	1.460E+02	3.020E+02	4.464E+02	11-Aug	12:39	SWLB
Vibrios	Open-Light	0	3.415 E-01	6.764E-01	2.700E+03	2.856E+03	4.222E+03	11-Aug	12:39	SWLB
Vibrios - Phototrophs	Open-Light	0	3.394 E-01	6.743E-01	2.440E+03	2.596E+03	3.850E+03	11-Aug	12:39	SWLB
Blank	Shaking-Light	0	3.253 E-01		1.430E+02			11-Aug	12:39	SWLB
Phototrophs	Shaking-Light	0	3.384 E-01	6.636E-01	1.700E+02	3.130E+02	4.717E+02	11-Aug	12:39	SWLB
Vibrios	Shaking-Light	0	3.494 E-01	6.747E-01	3.103E+03	3.246E+03	4.811E+03	11-Aug	12:39	SWLB
Vibrios - Phototrophs	Shaking-Light	0	3.399 E-01	6.652E-01	2.800E+03	2.943E+03	4.425E+03	11-Aug	12:39	SWLB
Blank	Closed-Light	3	3.139 E-01		1.930E+02			11-Aug	13:12	SWLB
Phototrophs	Closed-Light	3	3.277 E-01	6.416E-01	1.160E+02	3.090E+02	4.816E+02	11-Aug	13:12	SWLB
Vibrios	Closed-Light	3	2.794 E-01	5.933E-01	1.956E+03	2.149E+03	3.622E+03	11-Aug	13:12	SWLB
Vibrios - Phototrophs	Closed-Light	3	2.867 E-01	6.006E-01	1.433E+03	1.626E+03	2.707E+03	11-Aug	13:12	SWLB
Blank	Open-Light	3	3.198 E-01		1.400E+02			11-Aug	13:12	SWLB
Phototrophs	Open-Light	3	3.306 E-01	6.504E-01	1.900E+02	3.300E+02	5.073E+02	11-Aug	13:12	SWLB
Vibrios	Open-Light	3	2.983 E-01	6.181E-01	1.453E+03	1.593E+03	2.577E+03	11-Aug	13:12	SWLB
Vibrios - Phototrophs	Open-Light	3	3.023 E-01	6.221E-01	1.416E+03	1.556E+03	2.501E+03	11-Aug	13:12	SWLB
Blank	Shaking-Light	3	2.170 E-01		1.360E+02			11-Aug	13:12	SWLB
Phototrophs	Shaking-Light	3	2.984 E-01	5.154E-01	1.730E+02	3.090E+02	5.996E+02	11-Aug	13:12	SWLB
Vibrios	Shaking-Light	3	2.744 E-01	4.914E-01	7.430E+02	8.790E+02	1.789E+03	11-Aug	13:12	SWLB
Vibrios - Phototrophs	Shaking-Light	3	2.772 E-01	4.942E-01	6.500E+02	7.860E+02	1.591E+03	11-Aug	13:12	SWLB
Blank	Closed-Light	4	3.180 E-01		1.000E+02			11-Aug	13:35	SWLB
Phototrophs	Closed-Light	4	3.067 E-01	6.247E-01	1.030E+02	2.030E+02	3.250E+02	11-Aug	13:35	SWLB
Vibrios	Closed-Light	4	2.336 E-01	5.516E-01	1.023E+03	1.123E+03	2.036E+03	11-Aug	13:35	SWLB

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Vibrios - Phototrophs	Closed-Light	4	2.464 E-01	5.644E-01	8.800E+02	9.800E+02	1.736E+03	11-Aug	13:35	SWLB
Blank	Open-Light	4	3.252 E-01		1.100E+02			11-Aug	13:35	SWLB
Phototrophs	Open-Light	4	3.226 E-01	6.478E-01	1.230E+02	2.330E+02	3.597E+02	11-Aug	13:35	SWLB
Vibrios	Open-Light	4	2.914 E-01	6.166E-01	1.143E+03	1.253E+03	2.032E+03	11-Aug	13:35	SWLB
Vibrios - Phototrophs	Open-Light	4	2.711 E-01	5.963E-01	7.700E+02	8.800E+02	1.476E+03	11-Aug	13:35	SWLB
Blank	Shaking-Light	4	2.866 E-01		1.630E+02			11-Aug	13:35	SWLB
Phototrophs	Shaking-Light	4	3.014 E-01	5.880E-01	1.660E+02	3.290E+02	5.595E+02	11-Aug	13:35	SWLB
Vibrios	Shaking-Light	4	2.619 E-01	5.485E-01	4.630E+02	6.260E+02	1.141E+03	11-Aug	13:35	SWLB
Vibrios - Phototrophs	Shaking-Light	4	2.656 E-01	5.522E-01	3.700E+02	5.330E+02	9.652E+02	11-Aug	13:35	SWLB
Blank	Closed-Light	5	3.005 E-01		1.230E+02			11-Aug	14:04	SWLB
Phototrophs	Closed-Light	5	2.974 E-01	5.979E-01	1.400E+02	2.630E+02	4.399E+02	11-Aug	14:04	SWLB
Vibrios	Closed-Light	5	1.091 E-01	4.096E-01	5.800E+02	7.030E+02	1.716E+03	11-Aug	14:04	SWLB
Vibrios - Phototrophs	Closed-Light	5	1.609 E-01	4.614E-01	6.000E+02	7.230E+02	1.567E+03	11-Aug	14:04	SWLB
Blank	Open-Light	5	3.036 E-01		1.860E+02			11-Aug	14:04	SWLB
Phototrophs	Open-Light	5	3.102 E-01	6.138E-01	1.100E+02	2.960E+02	4.822E+02	11-Aug	14:04	SWLB
Vibrios	Open-Light	5	1.899 E-01	4.935E-01	5.530E+02	7.390E+02	1.497E+03	11-Aug	14:04	SWLB
Vibrios - Phototrophs	Open-Light	5	2.080 E-01	5.116E-01	4.600E+02	6.460E+02	1.263E+03	11-Aug	14:04	SWLB
Blank	Shaking-Light	5	2.873 E-01		1.730E+02			11-Aug	14:04	SWLB
Phototrophs	Shaking-Light	5	2.897 E-01	5.770E-01	2.060E+02	3.790E+02	6.568E+02	11-Aug	14:04	SWLB
Vibrios	Shaking-Light	5	1.763 E-01	4.635E-01	3.400E+02	5.130E+02	1.107E+03	11-Aug	14:04	SWLB
Vibrios - Phototrophs	Shaking-Light	5	2.233 E-01	5.105E-01	3.000E+02	4.730E+02	9.265E+02	11-Aug	14:04	SWLB
Blank	Shaking-Catalase	0	2.725 E-01		7.600E+01			15-Aug	15:19	SWC
Vibrios	Shaking-Catalase	0	2.784 E-01	5.509E-01	3.063E+03	3.139E+03	5.698E+03	15-Aug	15:19	SWC
Blank-O2-Low	Shaking-Catalase	0	2.955 E-01		8.300E+01			15-Aug	15:19	SWC

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Vibrios-O2-Low	Shaking-Catalase	0	2.978E-01	5.933E-01	1.130E+02	1.960E+02	3.303E+02	15-Aug	15:19	SWC
Blank-O2-High	Shaking-Catalase	0	3.114E-01		8.000E+01			15-Aug	15:19	SWC
Vibrios-O2-High	Shaking-Catalase	0	2.995E-01	6.109E-01	7.600E+01	1.560E+02	2.554E+02	15-Aug	15:19	SWC
Blank	Shaking-Catalase	1	2.829E-01		1.330E+02			15-Aug	15:57	SWC
Vibrios	Shaking-Catalase	1	3.203E-01	6.032E-01	2.660E+04	2.673E+04	4.432E+04	15-Aug	15:57	SWC
Blank-O2-Low	Shaking-Catalase	1	3.025E-01		1.100E+02			15-Aug	15:57	SWC
Vibrios-O2-Low	Shaking-Catalase	1	2.984E-01	6.009E-01	2.030E+02	3.130E+02	5.209E+02	15-Aug	15:57	SWC
Blank-O2-High	Shaking-Catalase	1	3.090E-01		9.600E+01			15-Aug	15:57	SWC
Vibrios-O2-High	Shaking-Catalase	1	3.143E-01	6.232E-01	9.300E+01	1.890E+02	3.033E+02	15-Aug	15:57	SWC
Blank	Shaking-Catalase	2	2.932E-01		4.530E+02			15-Aug	16:27	SWC
Vibrios	Shaking-Catalase	2	2.889E-01	5.821E-01	6.371E+04	6.416E+04	1.102E+05	15-Aug	16:27	SWC
Blank-O2-Low	Shaking-Catalase	2	2.943E-01		2.230E+02			15-Aug	16:27	SWC
Vibrios-O2-Low	Shaking-Catalase	2	3.002E-01	5.945E-01	3.100E+02	5.330E+02	8.966E+02	15-Aug	16:27	SWC
Blank-O2-High	Shaking-Catalase	2	2.914E-01		1.100E+02			15-Aug	16:27	SWC
Vibrios-O2-High	Shaking-Catalase	2	2.844E-01	5.758E-01	1.630E+02	2.730E+02	4.741E+02	15-Aug	16:27	SWC
Blank	Shaking-Catalase	3	3.035E-01		3.930E+02			15-Aug	17:03	SWC
Vibrios	Shaking-Catalase	3	2.883E-01	5.918E-01	3.519E+04	3.558E+04	6.013E+04	15-Aug	17:03	SWC
Blank-O2-Low	Shaking-Catalase	3	2.931E-01		1.660E+02			15-Aug	17:03	SWC
Vibrios-O2-Low	Shaking-Catalase	3	2.883E-01	5.814E-01	2.930E+02	4.590E+02	7.895E+02	15-Aug	17:03	SWC
Blank-O2-High	Shaking-Catalase	3	2.971E-01		1.200E+02			15-Aug	17:03	SWC
Vibrios-O2-High	Shaking-Catalase	3	2.987E-01	5.958E-01	1.530E+02	2.730E+02	4.582E+02	15-Aug	17:03	SWC
Blank	Shaking-Catalase	5	2.933E-01		6.300E+01			15-Aug	18:14	SWC
Vibrios	Shaking-Catalase	5	2.770E-01	5.703E-01	2.903E+03	2.966E+03	5.200E+03	15-Aug	18:14	SWC

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Blank-O2-Low	Shaking-Catalase	5	2.601 E-01		7.300E+01			15-Aug	18:14	SWC
Vibrios-O2-Low	Shaking-Catalase	5	2.882 E-01	5.483E-01	1.030E+02	1.760E+02	3.210E+02	15-Aug	18:14	SWC
Blank-O2-High	Shaking-Catalase	5	2.910 E-01		1.030E+02			15-Aug	18:14	SWC
Vibrios-O2-High	Shaking-Catalase	5	2.883 E-01	5.793E-01	9.000E+01	1.930E+02	3.331E+02	15-Aug	18:14	SWC
Blank	Shaking-Catalase	6	3.112 E-01		5.860E+02			15-Aug	18:45	SWC
Vibrios	Shaking-Catalase	6	1.231 E-01	4.343E-01	1.609E+05	1.615E+05	3.719E+05	15-Aug	18:45	SWC
Blank-O2-Low	Shaking-Catalase	6	2.882 E-01		3.030E+02			15-Aug	18:45	SWC
Vibrios-O2-Low	Shaking-Catalase	6	1.713 E-01	4.594E-01	5.560E+02	8.590E+02	1.870E+03	15-Aug	18:45	SWC
Blank-O2-High	Shaking-Catalase	6	2.924 E-01		1.800E+02			15-Aug	18:45	SWC
Vibrios-O2-High	Shaking-Catalase	6	1.686 E-01	4.609E-01	1.700E+02	3.500E+02	7.593E+02	15-Aug	18:45	SWC
Blank	Shaking-Catalase	7	2.787 E-01		1.586E+04			15-Aug	19:26	SWC
Vibrios	Shaking-Catalase	7	1.426 E-01	4.213E-01	5.375E+06	5.391E+06	1.280E+07	15-Aug	19:26	SWC
Blank-O2-Low	Shaking-Catalase	7	2.964 E-01		5.651E+03			15-Aug	19:26	SWC
Vibrios-O2-Low	Shaking-Catalase	7	2.407 E-01	5.372E-01	1.411E+04	1.976E+04	3.679E+04	15-Aug	19:26	SWC
Blank-O2-High	Shaking-Catalase	7	2.413 E-01		1.133E+03			15-Aug	19:26	SWC
Vibrios-O2-High	Shaking-Catalase	7	3.911 E-01	6.324E-01	1.580E+03	2.713E+03	4.290E+03	15-Aug	19:26	SWC
Blank	Shaking-Catalase	10	2.435 E-01		2.477E+05			15-Aug	21:10	SWC
Vibrios	Shaking-Catalase	10	0.000 E+00	2.435E-01	8.431E+07	8.456E+07	3.472E+08	15-Aug	21:10	SWC
Blank-O2-Low	Shaking-Catalase	10	2.614 E-01		1.005E+05			15-Aug	21:10	SWC
Vibrios-O2-Low	Shaking-Catalase	10	3.685 E-01	6.299E-01	4.008E+05	5.013E+05	7.959E+05	15-Aug	21:10	SWC
Blank-O2-High	Shaking-Catalase	10	2.624 E-01		1.872E+04			15-Aug	21:10	SWC
Vibrios-O2-High	Shaking-Catalase	10	3.292 E-01	5.916E-01	4.509E+04	6.381E+04	1.079E+05	15-Aug	21:10	SWC