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Seasonality in *Vibrio* Bacteria Population Structure: A Practical Application of the Lotka-Volterra Competition Model

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Abstract

The bioluminescent bacteria *Vibrio harveyi* and *Vibrio chagasii* are found in the Gulf Coast of Florida where their relative abundance varies between the winter and summer seasons. This study examines the hypothesis that seasonal variations in relative abundance of *V. harveyi* and *V. chagasii* populations off the coast of Florida are due to changes in growth rate and competitive ability of the individual species caused by seasonal differences in temperature. We conducted growth experiments at six different temperatures to identify temperature dependent growth rates of the two species. Time series population data were also collected in competition experiments at the same temperature range. The experiments conducted qualitatively match population structural changes observed in nature. Experimental results are used to partially parameterize a Lotka-Volterra competition mathematical model to test the presence of factors influencing the competitive dynamics additional to the individual temperature dependent growth parameters.

Keywords: *Vibrio harveyi, Vibrio chagasii*, Lotka-Volterra, Seasonality

1. Introduction

The genus *Vibrio* contains approximately 74 species of bacteria characterized by their curved rod shape, salt tolerance, and ability to digest chitin[1]. Although several *Vibrio* species are associated with infectious disease, members of the genus are also known for their unique quorum sensing mechanisms which result in bioluminescence. Studies of the growth cycles of bioluminescent bacteria have yielded key insights into bacterial communication, and continue to be used as a model of bacterial population dynamics[1,2].

Vibrio harveyi and *Vibrio chagasii* are free swimming marine bacteria found off the Gulf coast of Florida[1,2]. Analyses of surface water samples taken by Dr. Wimpee's lab from Boca Ceiga Bay, Florida show that *V. harveyi* was by far the predominant species of bioluminescent bacteria in summer months, while *V. chagasii* was the dominant bioluminescent species in winter. We hypothesize that the seasonal variation in *V. harveyi* and *V. chagasii* populations is driven by competition, where the competitive ability of the two species is temperature dependent. Assuming that their growth is logistic, we aim to determine whether this seasonality can be explained by competition by using the Lotka-Volterra competition model (LV).

The Lotka-Volterra competition model stems from the Verhulst-Pearl logistic equation, and it has helped develop an understanding of the phenomenon of competition[3]. As the ratio between supply and demand constantly changes in competition, this model developed "competition coefficients, the community matrix, and diffuse competition, that are conceptually independent of the equations" [3].

Development of a modeling framework that fits variations of *V. harveyi* and *V. chagasii* populations in the Gulf coast of Florida may lead to a better understanding of marine microbes, a generally understudied group of organisms.

Furthermore, if our Lotka Volterra model is able to capture variations in the populations of *Vibro* species, it could serve as a basis on which to build models of other microbial relationships.

2. Materials and Methods

All seawater samples were retrieved from the surface of the Boca Ciega Bay. To replicate temperature when samples were harvested, satellite data of surface water temperatures were obtained, and six experimental temperatures were determined: 10°C, 15°C, 20°C, 25°C, 30°C, 36°C [4]. Constant temperatures were maintained in water baths and temperature chambers. Preliminary experiments where nutrient solution was diluted indicated that *V. harveyi* and *V. chagasii*'s competitive ability were not nutrient dependent because the changes were proportional in both species. Due to this, an artificial seawater complete medium was used throughout the entirety of the experiment to simulate salinity and nutrient types found in seawater. Nutrient content in the medium was higher than seawater found in samples, but it is believed that as *Vibrio* species may colonize the gastrointestinal tracts of marine fish where they have access to greater amounts of nutrients they can adapt to this higher concentration[6].

At the beginning of each experiment, two overnight cultures, one each of *V. harveyi* and *V. chagasii*, were created in artificial seawater and allowed to grow for nine hours. Biomass was quantified with colony counts on agar plates to estimate the concentration of cells per milliliter. Colony counts were feasible even when *V. harveyi* and *V. chagasii* were grown on the same agar plate due to visual differences in colony phenotype. Experiments were broken into two phases: assessing first the growth rate and then the competitive ability of the bacteria.

2.1 PHASE 1: Determination of Growth Rate

Twelve flasks of 50 mL artificial seawater were separated and two were placed in the six temperatures mentioned above for nine hours. Overnight cultures were inoculated into species specific flasks in a 1:100 dilution, allowing for only intraspecific competition. Note that cultures were inoculated with only one type of species, thus no interspecific competition would occur, only intraspecific competition; in this paper we will refer to this set up as cultures grown in "isolation". Each experiment was six hours to allow for a full growth phase to complete. Previous experiments proved *V. harveyi* and *V. chagasii* can double biomass in the span of approximately twenty minutes at room temperature. Hourly subsamples were diluted and spread on plates and allowed to grow for 24 hours. When hourly cell counts of each species' growth curves were determined, they were plotted against time on a logarithmic scale in excel and growth rate was determined using a best line of fit function.

2.2 PHASE 2: Competitive Ability

Six flasks of 50 mL artificial seawater were separated into the six temperatures for nine hours. Overnight cultures were inoculated into the same flask at an equal dilution of 1:100 each, allowing for inter- and intraspecific competition. Six replicates were made, one per each temperature. A similar procedure to *PHASE 1* was followed to obtain hourly cell counts of both species for each of the six flasks.

LV requires three types of parameters to be defined. Growth rate values (r) were determined in *PHASE 1* and were relatively easy to estimate, as it could be done using a linear approximation on a log scale. The second type, competitive ability (α), was more difficult to estimate. It was necessary to use the full system of differential equations, and set up a parameter identification problem in MATLAB to find α values that minimized error between laboratory results and model predictions given growth rate and initial population (C_0, H_0) values. The third parameter type, carrying capacity (K), was not relevant in this application (see Discussion). Instead, the parameter estimation code identified a new type of parameter, δ, to express the coefficient α/K. The parameter estimation procedure attempted to find values of δ that minimize the discrepancy between the model and the data in the least square sense. See LV equations used in this process in Table 1.

Table 1: A description of the Lotka-Volterra Competition Model equations.

Eq. 1a: Rate of change of *V. harveyi* population	$\frac{dC}{dt} = r_c \times C\left(1 - (\delta_{cc} C + \delta_{ch} H)\right)$,	$\frac{\alpha_{cc}}{K_c} = \delta_{cc}$ *and* $\frac{\alpha_{ch}}{K_c} = \delta_{ch}$	
Eq. 1b: Rate of change of *V. chagasii* population	$\frac{dH}{dt} = r_h \times H\left(1 - (\delta_{hh} H + \delta_{hc} C)\right)$,	$\frac{\alpha_{hh}}{K_h} = \delta_{cc}$ *and* $\frac{\alpha_{hc}}{K_h} = \delta_{hc}$	
Parameter		**Description**	**Unit**
Growth rates	r_h	Growth rate of *V. harveyi*	cells.milliliter^{-1}.hour^{-1}
	r_c	Growth rate of *V. chagasii*	
Competitive ability coefficients	α_{ch}	Impact of *V. harveyi* cells on *V. chagasii* cells	ratio, unitless
	α_{cc}	Competition between chagasii cells with other harveyi cells	
	α_{hc}	Impact of *V. chagasii* cells on *V. harveyi* cells	
	α_{hh}	Competition between harveyi cells with other harveyi cells	
Carrying capacity	K_h	Carrying capacity of *V. harveyi* population	cells.milliliter^{-1}
	K_c	Carrying capacity of *V. chagasii* population	
Variable		Description	Unit
H		*V. harveyi* population	cells.milliliter^{-1}
C		*V. chagasii* population	

3. Results

In *PHASE 1*, analysis of growth curves showed linear growth when the growth phase of the curve was graphed on a logarithmic scale. *V. harveyi*, the bacteria that is most abundant in summer, experienced mortality at 10°C and minimal growth at 15°C, but grew robustly in 20°C. *V. harveyi* growth rate increased with temperature with a maximum at 30°C, the average sea surface summer temperature. *V. harveyi* was able to sustain a robust growth rate at 36°C. In comparison, the predominant winter species, *V. chagasii*, experienced extreme mortality at 36°C. *V. chagasii* was, however, able to grow at 10°C, albeit at a very low growth rate. *V. chagasii* exhibited a robust growth rate at 15°C, with growth rate generally increasing with temperature to a maximum at 30°C. See Figure 1 for a side-to-side comparison.

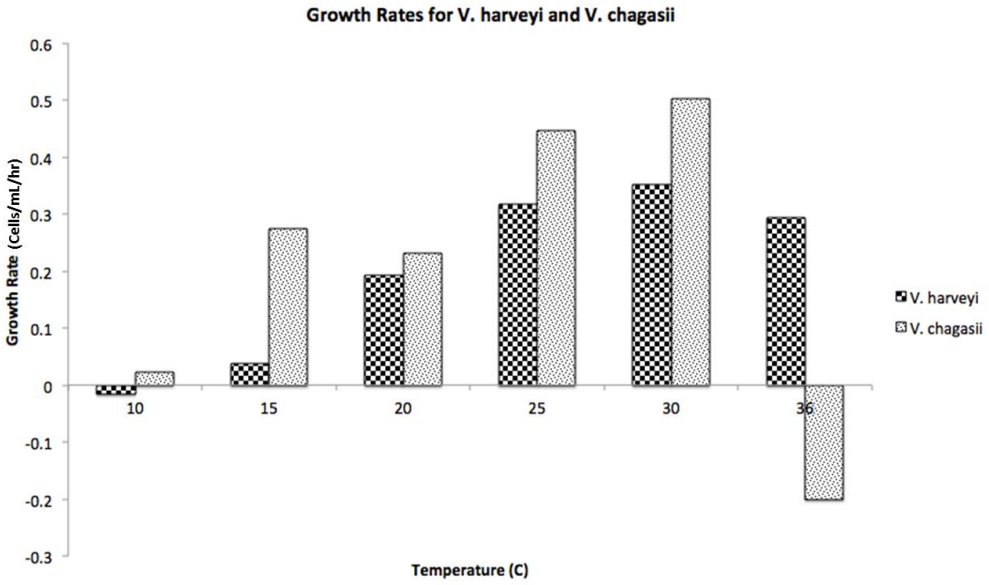
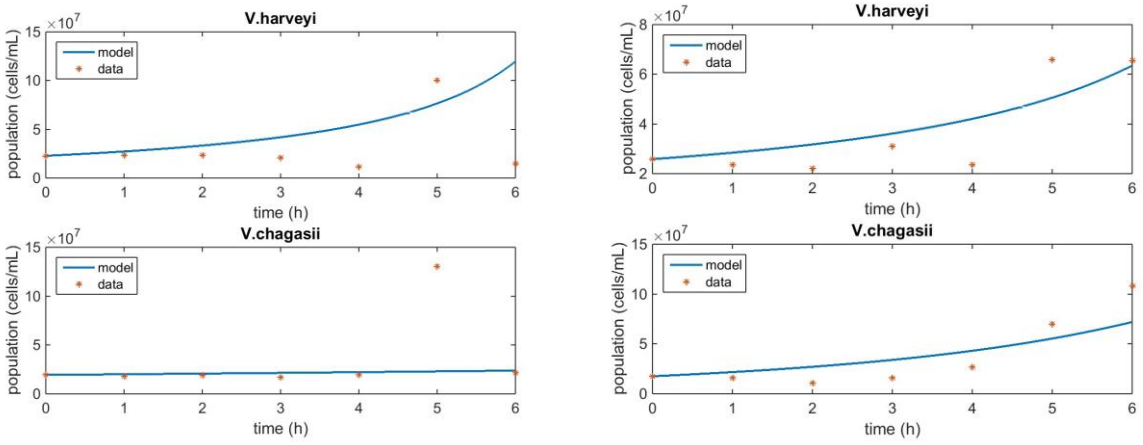
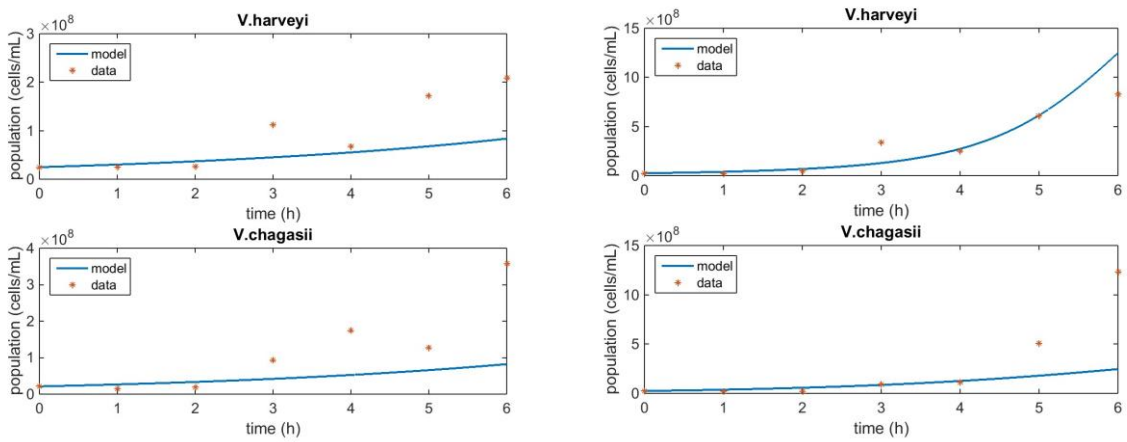


Figure 1. Growth Rates for *V. harveyi* and *V. chagasii*

In examining *PHASE 2* raw data, it becomes apparent that *V. harveyi* accumulates approximately twice as much biomass than *V. chagasii* at any given temperature. Datasets for temperatures 30 and 36°C were flawed and thus could not be used in parameter estimation; δ values were not identified for those conditions. δ values were obtained for 10, 15, 20 and 25°C datasets. See Table 2 for exact values. δ_{cc}, the parameter describing intraspecies competition amongst *V. chagasii* cells, were positive for all four temperatures, whereas δ_{hh}, the *V. harveyi* intraspecies competition parameter, was negative for 10 and 15°C and positive for 20 and 25°C. The interspecies competition parameter describing how the presence of *V. chagasii* cells affects the growth of *V. harveyi* cells, δ_{ch}, was positive for all temperatures. δ_{hc} was negative for all temperatures except for 10°C. To compare laboratory data with model projections, see Figure 2.



(a) (b)



(c) (d)

Figure 2: Model predictions of *V. harveyi* and *V. chagasii* population growth using δ values derived from parameter estimation code (solid line) compared to data obtained in *PHASE 2* laboratory experiments (*) for (a) 10°C, (b) 15°C, (c) 20°C, and (d) 25°C.

Table 2: Competitive ability values obtained using the parameter estimation code. Estimation for temperatures 30 and 36°C was unable to complete due to extreme outliers; see Discussion. See description of notation in Table 1.

Temperature °C	δ_{cc}	δ_{ch}	δ_{hh}	δ_{hc}
10	$10^{-6}\cdot 0.23$	$10^{-6}\cdot 0.35$	$-10^{-7}\cdot 0.22$	$10^{-12}\cdot 0.52$
15	$10^{-9}\cdot 0.41$	$-10^{-7}\cdot 0.80$	$-10^{-7}\cdot 0.18$	$10^{-7}\cdot 0.21$
20	$10^{-14}\cdot 0.51$	$-10^{-8}\cdot 0.18$	$10^{-9}\cdot 0.47$	$10^{-15}\cdot 0.10$
25	$10^{-8}\cdot 0.48$	$-10^{-7}\cdot 0.25$	$10^{-8}\cdot 0.23$	$10^{-16}\cdot 0.19$
30	~	~	~	~
36	~	~	~	~

4. Discussion

In *PHASE 1*, we identified values for growth rates of each species grown in isolation in each of the six temperatures (Figure 1). Previous analysis done by the Wimpee lab throughout several years consistently showed *V. harveyi* was the dominant bioluminescent bacterial species present in summer months and *V. chagasii* dominant in winter months. From examining growth curves, it is obvious that both *V. harveyi* and *V. chagasii* have trouble surviving at the extreme winter sample temperature, 10°C, but *V. harveyi* experiences mortality whereas *V. chagasii* exhibits minimal growth. This ability to survive at extreme winter sea surface temperature is an indication that *V. chagasii* may have an adaptive advantage to survive harsh winter conditions. Further evidence that *V. chagasii* has an adaptive advantage in cold temperatures is that cultures exhibited a much higher growth rate at 15°C than *V. harveyi*. When grown in isolation, *V. chagasii* has a higher growth rate than *V. harveyi* at all temperatures except 36°C, the extreme summer temperature. At 36°C *V. chagasii* experiences mortality. This similarly suggests that *V. harveyi* has an adaptive advantage in warmer temperatures.

Although *PHASE 2* δ estimation is incomplete, we can draw some conclusions. As mentioned in Methods and Materials, even though LV calls for a carrying capacity parameter, *K*, to be identified, determination of carrying capacity is difficult with our experimental setup. We therefore decided to forego an attempt to pinpoint a carrying capacity experimentally for the two species, and instead designed a parameter estimation code that would find a new parameter, δ, which is defined as competitive ability divided by carrying capacity (α/K) (Table 1).

Choosing to define δ as a parameter to describe the relationship between *V. harveyi* and *V. chagasii* can still distinguish between intra- and interspecific competition when examining δ_{ch} and δ_{hc}, compared to δ_{cc} and δ_{hh}. For example, if $\delta_{ch} > \delta_{cc}$, then the presence of *V. harveyi* cells affects the growth of *V. chagasii* more than the presence of

V. chagasii cells. In other words, interspecific competition would be stronger than intraspecific competition. If $\delta_{ch} < \delta_{cc}$, then intraspecific competition would be greater than interspecific. (To see the wide variety of ecological relationships LV can describe with different values of α, see Table 4.) The model can also distinguish between competition and facilitation. If δ_{ch} is positive, then interspecific competition is occurring, while if δ_{ch} is negative, then the presence of *V. harveyi* in fact facilitates *V. chagasii* growth, which would disprove our hypothesis. Note that if $\delta_{ch} = 0$, *V. harveyi* has no effect on *V. chagasii* growth.

Table 3: Relationships that can be expressed with various values of α, the competitive ability parameter[6]

α_{ch}	α_{hc}	**Relationship**
-	-	Mutualistic
-	0	Commensal
0	-	Commensal
+	-	Parasitic
-	+	Parasitic
+	+	Competitive

Table 2 contains computationally identified δ values in *PHASE 2*. Results for three of the four datasets that were useable did not produce δ values we expected. We predicted that *δ* values would either be positive or zero for all temperatures, but this was only the case for one temperature dataset. We can see that δ_{cc} is positive at all temperatures, indicating that *V. chagasii* experiences intraspecific competition year-round. Compare to the negative δ_{hh} values at 10 and 15°C, which suggests *V. harveyi* is facilitating itself in what may be quorum sensing. Keeping in mind that *V. harveyi* experiences mortality at 10°C and minimal growth at 15°C, facilitation described by δ_{hh} at this temperatures may indicate some sort of survival mechanism to help *V. harveyi* cells that survive outlier winter temperatures to continue to do so. At 20 and 25°C, δ_{hh} is positive; *V. harveyi* experiences intraspecific competition at mid-range temperatures, which is to be expected, considering *V. harveyi* growth rate increases at those temperatures to produce a dense population. Intraspecific competition increases between 20 and 25°C. If 30 and 36°C datasets were available, δ_{hh} would likely continue to increase with temperature. A similar trend cannot be observed with δ_{cc} as temperature increases.

Both δ_{ch} and δ_{hc} are positive for the 10°C dataset in *PHASE 2*. This suggests that *V. harveyi* and *V. chagasii* experience some competition at this temperature. Since $\delta_{ch} > \delta_{cc}$ interspecific competition is stronger than intraspecific competition for *V. chagasii*. An interesting relationship occurs at 15, 20 and 25°C. δ_{ch} is negative and δ_{hc} is positive, indicating that *V. harveyi* growth rate is improved by the presence of *V. chagasii* while *V. chagasii's* growth is hindered by the presence of the other species. This would suggest a parasitic relationship, where *V. harveyi* draws resources from *V. chagasii.* It is interesting to note that δ_{hc} is extraordinarily close to zero for temperatures 20 and 25°C, possibly meaning that the parasitic relationship is very weak and not very beneficial to *V. harveyi* at those temperatures. See these relationships summarized in Table 4. Finally, note that *V. harveyi* accumulates more biomass in all temperatures when grown in the same culture as *V. chagasii.* This further supports the idea that *V. harveyi* is the greater competitor or parasite at all temperatures examined.

Table 4: Summary of inter and intraspecific relationships amongst *V. harveyi* and *V. chagasii* for 10, 15, 20, and 25°C.

		Intraspecific relationship amongst	
Temperature, °C	**Interspecific relationship**	***V. harveyi***	***V. chagasii***
10	Competition	Competition	Facilitation
15	Parasitic		
20			Competition
25			

5. Future Work

Collection of more data in the lab is needed to complete parameter estimation of α values for the model at 30 and 36°C. Two of our three replicates were flawed during the execution of *PHASE 2* procedures due to inconsistent temperature and impure agar plates. The single replicate in these experiments had extreme outliers and the parameter estimation code could not identify reasonable parameters.

Future work could also focus on replicating the seasonal changes in temperature to determine whether or not a population can survive a winter-to-summer transition and back in a laboratory setting. For example, if *V. harveyi*, the dominant summer species, is first cultivated at 30°C, then at 15°C, could it return to the more bountiful summer population if it is again placed in a 30°C chamber? Could *V. chagasii* survive a similar transition? To perform this experimentally, the procedure should be consistent with inoculation methods used in Phases I & II as explained in the Methods section. Future work could also include constructing a LV model with parameter values identified in this paper, and compare results with data obtained in this new experiment.

Surface water samples could be taken and analyzed from Boca Ciega Bay in the coming years with special attention to the population structure of *Vibrios*. This is especially important as sea surface temperatures are predicted to change as climate change progresses, which may affect which species are present during various months of the year. It would also be beneficial to begin taking subsurface samples offshore to compare results to previous samples taken. Future work could also expand on Ruby and Morin's work on *Vibrio* presence in the digestive tracts of marine fishes to further classify what species are present and in what proportions, and whether population structure within enteric contents also display a similar seasonal shift as has been observed in surface water samples. This study did not examine what fish species were present in winter versus summer, or at what abundances, which may also affect nutrient availability to *Vibrios* in the different seasons.

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