# Overyielding in Marine Heterotrophic Bacteria

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## Abstract

We test the theory of overyielding with marine heterotrophic bacteria . Cell biomass was compared in polyculture versus cell biomass in monoculture. Also, we took media samples and compared the remaining DOC (dissolved organic carbon) in polycultures and the remaining DOC in monocultures. All polycultures non-trangressively over-yielded (outperformed the weighted average of the component monocultures), while most showed a slight tendency to transgressively overyield (outperform the most productive monoculture. In addition, polycultures tended to use more of the carbon in the media than the monocultures. This seems to be due to both increased cell biomass in polyculture and probable increased competition, leading to decreased efficiency.

### Introduction

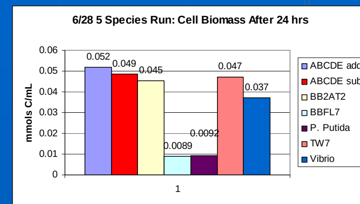
The relationship between diversity and productivity has long been debated. Some ecologists propose that a greater number of species leads to an increase in community biomass. This theory is called overyielding. There are two kinds of overyielding. The first kind is non-transgressive, in which the biomass of the polyculture exceeds the weighted average of the constituent monocultures, and it is considered the more liberal definition. Transgressive overyielding occurs when the biomass of the polyculture is greater than the biomass in the most productive monoculture, and it is considered to be the more definitive sign of overyielding (Hector et al, 2002). Degrees of overyielding are calculated with the formula D= (O-M)/M, where D is the deviation (or overyield), O is the observed total yield of the polyculture, and M is the monoculture yield of the comparison species (or weighted average in the case of non-transgressive overyielding) .Almost all of the research in this area has been done in the plant community, and most results indicate that overyielding does occur due to niche complementarity and positive species interactions (Tilman 1999 and Naeem, 1994). But these results are highly debated. Some ecologists argue that a Sampling Effect could be taking place. In other words, placing more species in a plot means a greater chance of a particular species having extreme traits which will drive a community toward greater productivity. We eliminated this effect by running polycultures simultaneously with the bacterial monocultures. In addition, it has been argued that overyielding increases with increasing time (Hooper and Dukes, 2004), and by using marine heterotrophic bacteria (bacteria which utilize free organic carbon in the oceans) with very short generation times, this issue was rendered moot.

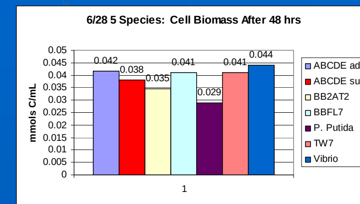
## Methods

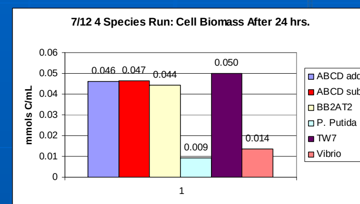
We used 5 species of marine heterotrophic bacteria isolated by Dr. Kay Bidle off the coast of California. They are BB2AT2, BBFL7, TW7, Pseudomonas Putida, and Vibrio NAP. All runs were done in liquid 100% ZoBell (5g peptone/L, 1g yeast extract/L) media at full seawater salinity. The final run was done at half seawater salinity. For every run, 4 reps of each type of culture were made, with 2 beakers sampled after 24 hrs, and 2 beakers sampled after 48 hrs. Also, two kinds of polycultures were run, additive, in which the intraspecies density was kept constant, and substitutive in which the interspecies density was kept constant.

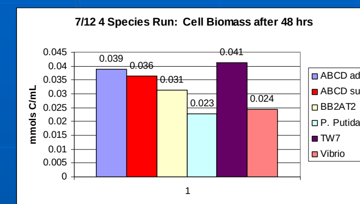
Initially, base cultures of 25 mL of ZoBell media were inoculated with plated colonies of bacteria. These base cultures were then placed in a 27 degree Celsius room, on a 100 rpm shaker table for 72 hours to grow. Then, 10 mL of the culture was centrifuged at 7000 rpm for 12 minutes. The media was poured out, and the cells were resuspended in a 3% salt solution. Then, .5 mL of the bacterial salt solution was added to each monoculture flask (a 50 mL flask with 10 mL of ZoBell). Next, 3% salt solution was added to the monocultures and substitutive polycultures to maintain the same volume and nutrient concentration as the additive polyculture. Every 24 hours, a 1 mL sample was taken from a flask and vacuum filtered through a Whatman GFF filter (.7 micron). Another 1 mL sample was then centrifuged until the cells pelleted out. Then, the media was collected and evaporated. The evaporated media and Whatman filters were then run through a Carlo Erba Elemental Analyzer. For the final species run, samples were taken every ten hours instead of every 24, only 2 reps of each culture were run (with 25 mL in a 150 mL flask), and flasks were resampled. The salt inoculum solutions were vacuum filtered as well.

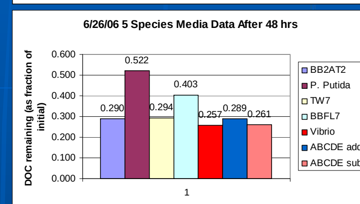
## Results

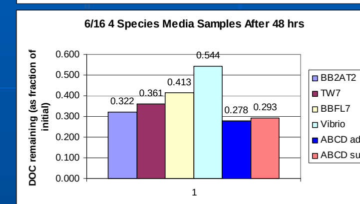


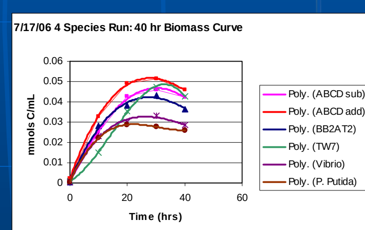



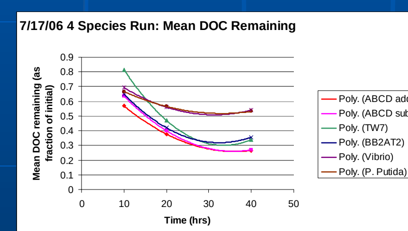



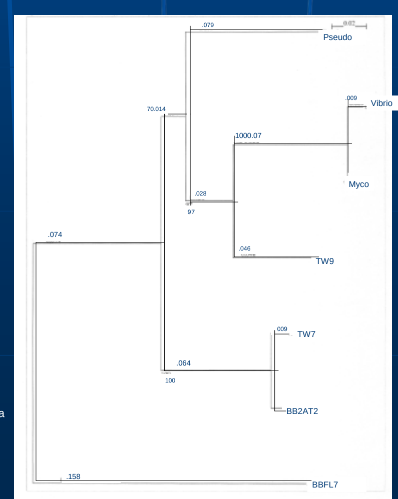




Phylogenetic Tree of Utilized Bacteria from 16s gene sequences




## Discussion

As we can see, all species grew in the 100% ZoBell solution, and all species peaked within 48 hours. Also, it is evident that the both polycultures non-transgressively overyield during every run (exceeds weighted average, D>0). In addition, both polyculture conditions overyielded transgressively on two different runs (7/16 and 6/26). The overyield can be seen on the peaks of the 6/26 run, but a 3rd degree polynomial regression was run on the 4 data points of the 7/16 run. Then, the time average was obtained via integration over the 40 hour period. Both polycultures overyielded relative to the highest grossing monoculture (BB2AT2) with D values of .198 for the additive and .0305 for the substitutive polyculture. In addition, the polycultures were taking much more carbon from the media than the monocultures (the Vibrio numbers from 6/26 are most likely due to allowing the media to sit for hours before sampling). This indicates that not only is there more bacterial biomass over the 40-48 hour interval, but also that there may be less efficiency (particularly in the substitutive monoculture) due to the competition between the different bacterial strains. Note also, that the additive cultures grew significantly more than the substitutive cultures. The additive culture did receive 4-5 times the innoculum, but this alone does not explain the increased growth of the additive cultures. Interestingly, there is more than 20% of the original DOC left in the media as growth declines, indicating that it is not the lack of carbon, but perhaps the buildup of some metabolic waste product that leads to the death of the bacteria. Given this fact, it would be intriguing to see if there is greater overyielding under more nutrient strained conditions, such as those given by a 5 or 10% ZoBell solution.

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