

Proceedings of the Berkeley Carroll



INDEPENDENT RESEARCH CONFERENCE

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In addition, we would like to acknowledge the support and assistance of the following members of the Berkeley Carroll Administration, without whom this program would not have been possible:

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## Welcome from the Editor

ow do you know if an experiment "works"?

In a typical high school classroom, that's easy—your experiment works if you measure the expected result. The acceleration of gravity is always 9.8 m/s<sup>2</sup>.

Real science, on the other hand, is messy.

For one thing, you may not have any idea what your result should be. Ernest Rutherford, for example, was famously shocked when some of his alpha particles bounced back from a thin piece of gold foil nearly exactly the way they went in.

Furthermore, experimental design is complicated. Ideally, you vary one quantity, measure another, and control everything else. But that's easier said than done—some variables are quite difficult to control. And when you introduce humans into the mix, all bets are off. How can you put a number to something like motivation?

This year, in our third edition of the Proceedings of the Independent Research Conference, our students have begun to realize how messy science can be.

Before completing his study on the relationship between sleep and academic performance, for example, Andrew Colon had to redesign his study from scratch twice *and* conduct a pilot study before he could get final approval.

Caleb Gordon, in his investigation of the effect of grazer length on algae consumption, had to deal with grazers that seemed to feed on each other rather than the algae that were the subject of the experiment.

Rebecca Bender struggled for months before she found an appropriate way to measure selfconfidence in her paper "Is Oxytocin all it seems?"

To be sure, our students have tackled very important topics and worked very hard. Yanai Feldman, Charlotte Pierce, and Rebecca Ennis all looked at novel approaches to the treatment of disease, David Colon delved deep into the brain as he tried to understand the relationship between concentration and binaural beats, and Olivia Cucinotta wrestled with the ethics of prenatal genetic testing.

However, I think it's fair to say that not one of them measured a result that they expected. By the standards of a typical high school lab report, that means none of the experiments presented in this journal "worked".

Where they expected straight lines, statistical differences between sample means, and clear categories in survey answers, they found random clusters, conflicting data, and overlapping and un-interpretable results.

In other words, they started to see that science works because it's messy.

Until next year Scott W. Rubin Upper School Chair of Science

The Berkeley Carroll School



**Charlotte Pierce** is a senior at Berkeley Carroll, and she has been researching the effects of infectious diseases for several years. Her paper is a detailed literature review of several of the most

threatening global diseases facing us today.



**Yanai Feldman** is a senior at Berkeley Carroll. He has been researching quorum sensing in bacteria since his sophomore year. This ability of bacteria to seemingly communicate with one another over a distance is on the cutting

edge of science. In his paper, Yanai argues that quorum quenching, in which this communication is interfered with intentionally, could be an effective way to fight modern diseases.



**Rebecca Bender** is a senior at Berkeley Carroll. She has been researching the hormone Oxytocin and how it affects confidence in kids and adults since sophomore year. She came across a scientist

by the name Dr. Paul Zak and from there has moved on to how Oxytocin can be enhanced or reduced due to social media webpages, such as Facebook. This year, Rebecca conducted an experiment to test if using Facebook would affect how an individual feels about himself or herself.



**Olivia Cucinotta** is a senior at Berkeley Carroll, and she has been researching changing attitudes toward prenatal genetic testing.



**Rebecca Ennis** is a senior at Berkeley Carroll. Since the end of her sophomore year, Rebecca has been researching antibiotics. During her time in the Science Research and Design Program, she

has specifically concentrated on antibiotic resistance, and the emergence of superbugs.

Rebecca has focused on the work of Dr. Christopher T. Walsh, Dr. Herbert Ennis, and Dr. Carlos F. Amábile-Cuevas. This year she developed a survey to determine how informed the general public is about antibiotics and antibiotic resistance.



**David Colon** is a senior at Berkeley Carroll. He has been researching brain waves during sleep for several years, and he has conducted a study in which he addressed the experimental question of whether or not binaural beats can improve concentration during a so-

called "chess test". In his paper, he presents the results of his study.



**Caleb Gordon** is a senior at Berkeley Carroll. By the start of his junior year, Caleb chose to focus on Harmful Algal Bloom (HAB) proliferation and control methods. He studied biological control agents used to mitigate blooms in the

hopes of finding an 'ideal grazer' species, and finally selected the branchiopod Artemia salina as a likely candidate. In June 2013, Caleb interned at the Gobler Lab in South Hampton (headed by Dr. Christopher Gobler, of the School of Marine and Atmospheric Sciences of Stony Brook University), which provided him with pivotal lab experience. Then, collaborating with Dr. Gobler and BC faculty, Caleb devised an experiment early fall 2013 to conduct on the 181 Lincoln campus this spring. In the experiment Caleb tested how the age of the grazer Artemia salina affected its ability to consume the harmful algae Cochlodinium polykrikoides. The experiment was still in progress when this research journal was printed, but Caleb finished it and collected data in March 2013.



Andrew Colon is a senior at Berkeley Carroll who has been researching the relationship between sleep and motivation for several years. After several attempts at pilot studies and a great deal of refinement in experimental design, he has completed

an independent study in which he addresses the experimental question of whether or not an increase in sleep correlates with higher academic performance as measured by grades. He presents the results of his study in his paper.

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## Examination of the Growing Global Infectious Disease Threat

by Charlotte Pierce

n recent years, infectious diseases have been reemerging with greater virulence and resistance to drugs, while new, untreatable, lethal diseases have emerged with great prevalence.<sup>1</sup> Changes in human behavior, including lifestyles, land use patterns, cultural and social values, increased trade and travel, and more widespread, and often inappropriate use of antibiotic drugs, has allowed dangerous mutations to form in pathogens.<sup>2</sup> The rise of megacities, globalization, environmental changes, and increased drug resistance has greatly influenced the spread of infectious disease.<sup>2</sup> Even worse, the threat of diseases is growing more quickly than our ability to treat, cure, prevent or control them. Pathogens such as viruses and bacteria have the unparalleled ability to mutate with astonishing speed.<sup>3</sup> New viruses and new strains of known viruses and bacteria emerge constantly, often undetected by public health services.

America's current defense against infectious disease is inadequate, as some scientists explain, "with shortcomings in surveillance, vaccines, testing, and treatment," and the resources in other countries, especially those without access to modern medical care, are even more limited.<sup>2</sup> The deficiencies in medical surveillance allows diseases to rapidly spread and mutate over time and heighten the possibility of a dangerous, unstoppable pathogen to emerge and cause widespread damage.

Recently, many diseases have emerged, acquired antibiotic resistance, and become endemic in new, unforeseen geographic locations. The rising threat of infectious diseases can best be seen through HIV/AIDS, tuberculosis, and malaria, as they demonstrate how globalization, climate change and antibiotic resistance are radically affecting pathogens in a way that has negative consequences for humans. The chance of dangerous epidemics, and even global pandemics, that surpass our ability to treat or prevent is drastically increasing, and we must learn how to intervene and prevent another pandemic as widespread and dangerous as HIV/AIDS from emerging. To do this, we must first acknowledge the recent developments in diseases, best seen through HIV/AIDS, Tuberculosis, and Malaria, and why these changes occurred. Human Immunodeficiency Virus (HIV), is an exemplary recently emerged virus that is intimately bound in globalized structures and processes.<sup>2</sup> Since its emergence as a zoonotic (disease transmitted from animals to humans) infection in colonial West and Central Africa, HIV/AIDS has become a global pandemic with frightening implications for modern medical care and human health.<sup>3</sup> In only two decades, HIV/AIDS has become a global killer, threatening the security, the family structure, and the economic development of developing nations (not just developing).<sup>4</sup> The World Health Organization (WHO) declared HIV as the world's leading infectious killer, as an estimated 36 million people have died from the virus since the first cases were reported in 1981.<sup>4</sup> Today, an estimated 35.3 million people are infected with HIV/AIDS, including 3.34 million children worldwide.<sup>4</sup> Infection rates and prevalence continue to increase rapidly, all at the hands of globalization.

Globalization is "the flow of information, goods, capital and people across political and geographic boundaries."<sup>5</sup> It has given rise to increased global travel and trade, and this efficient and inexpensive transportation has left virtually every corner of the world easily accessible. This global interdependence greatly facilitates the dissemination of HIV infection across national borders. HIV/AIDS has possibly become the world's most serious health and development challenge because of this process alone.

There are several theories regarding the origin and epidemic emergence of HIV/AIDS, but it is most commonly believed that it originated in non-human primates in sub-Saharan Africa in the form of Simian Immunodeficiency Virus (SIV) and was then transferred to humans in the late 19th or early 20th Century. Bushmeat practice, the killing and eating of chimps, is the most likely cause of the zoonosis of HIV/AIDS.<sup>7</sup> The rapid human-to-human spread from the initial infected person is most likely attributed to the changes in population structure, behavior, and possible medical interventions in Africa during the time.<sup>5</sup> European colonial powers began establishing cities, towns, railroads, seaports, and other urban infrastructures in Africa in the 1880's, when the "Scramble for Africa" began.<sup>6</sup> This led to an unprecedented increase in mobility in Africa, and thus allowed the newly emerged virus to spread rapidly among populations.

With newly established urban areas and easily accessible modes of international travel, HIV/AIDS was able to spread across national borders. It is now commonly believed that the infectious disease spread from a metropolitan area in Africa, most likely Kinshasa (Democratic Republic of Congo), to Haiti, to America and around the world.<sup>6</sup> During the 19th century many Haitians were working in the Congo, and the time frame that it is believed HIV emerged in America is around when many Haitians were returning from Africa.<sup>6</sup> In March 2007, international scientists presented data based on a complex genetic analysis of early samples of HIV, showing that the endemic strain of HIV was most likely brought to Haiti from Africa around 1966.<sup>6</sup> HIV would have then slowly spread through the population on the island before being spread to America around 1969 and 1972 by immigrants.<sup>6</sup> The diagrams on the opposite page illustrate the progression of HIV/AIDS from a small epidemic to a global pandemic that continues to haunt the world today.

HIV/AIDS may have never become a global killer if it had emerged in an age without international transportation. HIV/AIDS is the perfect example of how globalized trade and



### Diagram 1

It is commonly believed that that HIV was most likely brought to Haiti from Africa in around 1966.



## Diagram 2

**"Haiti First" Model** — (probability=99.8%) The progression of HIV/AIDS from a small epidemic to a global pandemic that continues to haunt the world today. travel drastically affects the virulence and prevalence of infectious diseases, and how viruses can spread and transform faster than our ability to stop or cure them. As international travel becomes more accessible and common, the possibility of another disease as devastating as HIV/AIDS to surface increases.

Drug resistance has also become one of our most serious health threats, as many bacteria have become, and continue to become, resistant to multiple types and classes of antibiotics.<sup>8</sup> Bacteria are capable of changing in ways that reduce or eliminate the effective-

ness of therapeutic agents.<sup>15</sup> Some bacteria, for example, may have the ability to neutralize or escape the effect of the antibiotic, and "then multiply and replace all the bacteria that were killed off" by antibiotics.<sup>15</sup> In addition, "bacteria that were at one time susceptible to an antibiotic can acquire resistance through mutation of their genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria."<sup>15</sup>

Not only does this loss of effective antibiotics undermine our ability to treat those infected with resistant pathogens, but it also puts vulnerable patients with compromised immune systems in extreme danger.<sup>8</sup> The Center for Disease Control (CDC) states that, "each year in the United States, at least 2 million people acquire serious infections with bacteria

that are resistant to one or more of the antibiotics designed to treat those infections," and "at least 23,000 people die each year as a direct result of these antibiotic-resistant infections."<sup>8</sup> Antibiotic resistance poses a catastrophic threat as antibiotics are extremely important in modern medicine, being the most commonly prescribed drugs used.<sup>8</sup> A loss in effective antibiotics and an increase in drug resistant, powerful pathogens puts humans in an extremely vulnerable and dangerous situation. One disease with newly acquired antimicrobial resistance that demonstrates the danger of this issue is tuberculosis.

Tuberculosis (TB) is caused by the bacteria *Mycobacterium Tuberculosis*, and typically affects the lungs but often spreads through the bloodstream to other organs. TB is transmitted through the air and can be contracted when repeatedly exposed to someone infected. Most infected people don't experience symptoms because TB can live in an inactive form in

#### **Diagram 3**

**Two Faces of TB** — Efforts to tackle drug-susceptible strains have started to cut the global incidence of TB (lbottom), but collapsing health-care systems in the former Soviet bloc have helped drug-resistant strains to emerge and spread.



the body. Those with weak immune systems however, such as the elderly or those with HIV or cancer, become very ill and die if left untreated. This active form of TB causes death of tissue in the organs it infects. Those with the inactive form, also known as latent Tuberculosis, are at risk of the bacteria becoming active and causing disease, so they are treated with antibiotics. According to the World Health Organization, TB "is second only to HIV/AIDS as the greatest killer worldwide," infecting one third of the world's population.<sup>9</sup>

This once widespread disease was virtually wiped out with the help of antibiotics, but recently it has been resurfacing in stronger forms, including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB).<sup>10</sup> These new dangerous strains have created a public health crisis worldwide and left the hundreds of thousands of infected patients practically helpless. MDR-TB is treatable with second-line drugs (therapeutic agents that aren't the drug of choice, or the first normally used to treat a particular condition), however the treatment options are limited, very expensive, and often cause severe adverse effects that can be more damaging than helpful.10 XDR-TB is resistant to even more second-line anti-TB drugs.<sup>10</sup> In 1993, the WHO declared TB a global emergency and it is now the leading cause of death among people with HIV.<sup>9</sup> As seen on the diagram on the previous page, TB infection and mortality rates were decreasing at a promising rate, but now the future is less certain due to the rapid spread of drug resistant strains.

It is evident that most drug resistant TB has arisen in countries with inadequate health care infrastructures and TB programs, such as Russia, where those with the disease haven't been diagnosed or treated. As seen in the diagram, the rate of TB infection worldwide started to decrease after 2000, but now with the resistant strains, the future is less promising. Lapses in health care have allowed TB to become more dangerous, and allow other diseases to do the same. As antibiotics continue to be misused and often overused, the potential for a new, lethal disease even more devastating than TB to emerge greatly increases.

Climate change also plays a large role in the increasing threat of infectious disease, affecting human health and biodiversity worldwide. There are a variety of climactic conditions that ensure the survival and reproduction of organisms. When these conditions are altered, organisms adapt to that new environment, and in the case of Malaria, mosquitoes harboring pathogenic parasites are adapting, putting humans at a greater risk of contracting disease.<sup>11</sup> Climate change can alter the physiology of pathogens as well as allow them to thrive and become endemic in new regions.<sup>11</sup> Worldwide, "increases in heat, precipitation, and humidity" are allowing "tropical and subtropical insects (vectors) to move from regions where infectious diseases thrive into new places.<sup>112</sup> The United States is at increasing risk of becoming home to new diseases, and already has with diseases normally endemic in Africa such as dengue fever and West Nile virus.<sup>12</sup> Malaria is perhaps the best example of a dangerous pathogen that has experienced a change in transmission patterns due to climate change.

Malaria is a serious disease caused by a mosquito-borne parasite called *Plasmodium.*<sup>13</sup> There are five main species of the malaria parasite, all ranging in severity of symptoms.<sup>14</sup> Milder versions of malaria cause chills, high fever, and muscle pain, while more dangerous species of the parasite can cause heart, lung, kidney, or brain damage, coma, lifelong disabilities and even death.<sup>14</sup> There are no existing cures to the disease and treatment

#### **Diagram 4**



Malaria Transmission Occurs Throughout Malaria Transmission Occurs in Some Parts Malaria Transmission is not Known to Occur

options are limited since the parasite is effective in avoiding the detection by the immune system and many parasites are resistant to a number of existing medications.<sup>13</sup> Malaria is carried in the *Anopheles* mosquito, so the parasite is transmitted in tropical and subtropical areas where this species of mosquito can survive and reproduce.<sup>13</sup> Malaria, because of its host, is very sensitive to temperature and climate change. It relies on high temperatures, high humidity, and low altitudes to flourish.<sup>13</sup> Malaria transmission is optimal in the red and yellow colored areas in the graph above.<sup>13</sup>

Already, Malaria transmission is increasing in higher altitude regions in Africa due to changes in climate and its geographic occurrence and intensity in transmission is predicted to change even more in the next 50 years.<sup>14</sup> This causes a serious heath care issue because people living in areas that previously weren't at risk of contracting malaria have a low immunity to the disease and thus more people of all ages are likely to contract it. It is most commonly believed that malaria is moving to high altitude regions such as Sudan, Kenya, Madagascar, South Africa and Angola.<sup>15</sup> Even the United States is at a higher risk of experiencing malaria endemic since the Anopheles mosquitoes live in a multitude of regions so increasing temperatures will allow malaria to thrive and spread.<sup>14</sup> The following diagram predicts the increased risk of malaria transmission by 2020 assuming a global temperature increase of one to two degrees Fahrenheit and no human efforts to prevent or contain the spread of disease.90

It is evident that the threat of the spread of malaria is imminent if no precautions are taken against the infectious disease and if global warming and climate change continue to worsen. As illustrated, North-East America, Russia, and Europe are predicted to be at more that twice at risk of malaria transmission. These are areas where malaria transmission is rare and therefore immunity is low, putting people of all ages at great risk. Climate change coupled with antibiotic resistance and increased international travel makes malaria and many other infectious diseases far more dangerous and devastating than ever before.

It is essential that we start to take our actions into consideration and make strides towards preventing the rising threat of infectious diseases. As seen through HIV/AIDS, malaria, and tuberculosis, globalization, antibiotic resistance and climate changing are drastically changing the world we live in. The future of our health and infectious disease is looking bleak if proactive measures don't start to be taken. Simple actions such as proper antibiotic use, preventative measures towards global warming, and more mindfulness in travel can help save the future from more powerful or new deadly diseases. Governments and health care officials need to be aware and cautionary when treating patients and dealing with antibiotic resistant microbes, and those that have the potential to acquire resistance. The first step towards a better, safer future is spreading awareness about this rising threat that effects families, economies, and the security and welfare of everyone around the world.

#### **Diagram 5**

**Predicted Change in Risk of Malaria** Transmission

#### Amount the Risk Will Multiply





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## Quorum quenching: a novel solution to the rising threat of drug-resistant infection

by Yanai Feldman

## Introduction

ajor US pharmaceutical companies have been ridding themselves of their antibiotic units over the last decade. The world's largest pharmaceutical company, Pfizer Inc., shut down its antibiotic-research facility in Connecticut in 2011 along with Johnson & Johnson. They joined pharma companies like Roche, Bristol-Myers Squibb and Eli Lilly, "leaving only a handful of firms like GlaxoSmithKline, AstraZeneca and Merck & Co. in the game" (Hirschler). The market simply does not demand development of new drugs; patients (or rather insurance companies) might pay upwards of 50,000 dollars for one round of a cancer drug, whereas one round of antibiotic treatment can cost as little as a few hundred dollars (Plumridge). One of the main problems is that research and development can cost hundreds of millions of dollars, and even that won't guarantee that the Food and Drug Administration (FDA) will approve the drug. Even if new antibiotics do manage to make it past clinical trials successfully, companies run quite a risk; bacteria will become resistant to their drugs and cause their obsolescence, thus potentially losing millions to enter a market that does not pay much in the first place.

Due to the drug-resistant bacteria, conventional antibiotics are already becoming ineffective. Many infectious bacteria, like strains of tuberculosis or staphylococcus, have experienced selective pressure to evolve resistance; some antibiotics simply don't work for certain infectious mutants. According to the World Health Organization, "antibiotic-resistant infections now kill around 50,000 people a year in the U.S. and Europe, and that number is rising" (Plumridge). That's in addition to the two million people in the U.S. estimated to contract a "drug-resistant infection, with direct health-care costs of as much as 20 billion dollars" within the next few years (Plumridge). To combat the rising threat of resistant bacteria, new drugs must be developed. But as I've discussed above, research and development of new antibiotics is now unpopular; only one new antibiotic was approved between 2010 and 2012.

Pharma companies and the federal government must inject capital into development of new antimicrobial therapies. Before they do so, however, they must know which therapies to invest in. Researchers at universities around the world have conducted studies and written papers on their own methods and hypotheses of how to fight infectious disease. The methods, molecules and drugs that are closest to clinical trials (already fairly developed) and those that are most likely to be approved for marketing are those that are most worth the gamble.

We know infinitely more about bacteria, their structure, mechanism, evolution, and genetic makeup, than in 1928 when Alexander Fleming discovered the antibiotic properties of the fungi *Penicillium* and penicillin soon thereafter revolutionized medicine. In the 1960s and 70s, a curious microbial intracellular interaction, now called quorum sensing, was noted by Alexander Tomasz in his research on genetic competence, as well as by Hastings in his research on bioluminescence *in Vibrio* species (Bassler and Losick). More recently, Bonnie L. Bassler of Princeton University, who began her work with quorum sensing over a decade ago, has contributed a wealth of insights to the field. She is now the principal representative of quorum sensing and has managed to make it a popular choice of study among those scientists, students, and researches interested in microbiology and biochemistry.

Lucky for us. As it turns out, this field has applications for antimicrobial therapies with novel mechanisms. As the literature shows, quorum sensing has great potential to prevent an insurgence of drug-resistant bacteria.

#### Quorum sensing mechanisms, applications, and significance

Bacteria are generally thought (by the public) to be primitive organisms, incapable of the type of complexity exhibited by "higher-order" life forms, like animals. They are infinitesimal, about the size of the human mitochondria, which snuggly fits into the human cell. We often reference the human ability to communicate linguistically as one of the defining characteristics of our evolutionary complexity. Although not linguistically capable, the lowest order organisms—bacteria—are capable of the sophisticated evolutionary adaptation of communication. Bacteria communicate via a process called quorum sensing. **Quorum sensing** (QS) is the process by which bacteria secrete and detect signaling molecules to coordinate collective behaviors. These signaling molecules, called autoinducers, accumulate according to cell density and, upon reaching a certain predetermined density threshold, bind to receptors and initiate gene expression, which often activates or represses a certain behavior. This cell-to-cell, density-dependent communication mechanism coordinates collective activities that would not otherwise benefit bacteria if individual cells acted alone. QS controls expression of virulence factors, biofilm formation, antibiotic resistance, competence, and other pathogenetic phenotypes, in clinically relevant pathogens.

There are two general types of QS systems. Gram-negative bacteria employ acylhomoserine lactones (AHL) as its signaling molecules, and operate by the Luxl-LuxR mechanism. Luxl is the paradigmatic synthase of autoinducer, while LuxR is the autoinducer's paradigmatic cognate receptor. At low cell density, when AHL concentration is low, intracellular LuxR receptors fail to form a complex with its AHL cognate, and subsequently are degraded. However, at high cell density, AHL diffuses across the membrane, accumulates in the cytoplasm, and binds to its LuxR-type cognate receptor protein. Consequently, the LuxR-AHL complex folds, binds DNA, and activates transcription of the target gene. Sometimes LuxR will fold, bind DNA, and repress gene expression in the absence of DNA, though this is much less common. In the Gram-positive mechanism, signaling molecules (often dubbed as AI-# for autoinducer-#) accumulate in the intercellular space (periplasm) and bind to membrane-bound receptors (rather than intracellular) called two-component sensor histidine kinases. This mechanism is often called the LuxN-type mechanism after the paradigmatic *Vibrio harveyi*, where this mechanism was first discovered and described. At high cell density, detection of the Al by the LuxN-type sensor kinase initiates a change in the downstream phosphorylation activities, which alters gene expression accordingly.

Because bacteria use QS to achieve successful pathogenesis, to infect and weaken through the release of virulence factors, to evade host immunity responses through the production of the biofilm, to initiate competence to improve genetic variation and thus more quickly evolve resistance to whichever dangers are in an environment, to release antibiotic compounds that kill competing bacterial species in the milieu, inhibition of this process will weaken the potency and decrease the likelihood of a successful bacterial infection. The inhibition of QS-related activities through inhibition of the QS mechanism itself is called, **Quorum Quenching** (QQ). QQ of human pathogenic QS processes has been shown to be an indirect method of minimizing the effects of, or preventing altogether, bacterial infection. I emphasize the word *indirect*. Most QS researchers hypothesize that QQ will solve the impending crisis of rising antibiotic-resistance because of the *indirect* process by which QQ interferes with bacterial pathogenesis.

Conventional antibiotics directly block growth. Penicillin prevents the final transpeptidation necessary for the assembly of peptidoglycan, the macromolecule that makes up the bacterial cell wall (Mobley). When treated with penicillin the bacterial cell wall bursts, ultimately leading to the death of the cell. Other, newer antibiotics follow a different mechanism. Tetracyline attaches itself to a spot on the bacterial ribosome, interrupting a key RNA interaction, which prevents full realization of the peptide chain. It thereby inhibits protein synthesis and effectively stops cell growth (Mobley). Sulfamethoxazole, by inhibiting an enzyme that is critical to the production of folic acid—a necessary vitamin—also stops the bacteria from growing and reproducing (Mobley). Though there are varieties of antibiotics, whose mechanisms have only become more complex, they all ultimately do one thing: kill or stop cell growth. Cell growth is an essential function of microbial life; life forms have one purpose: to survive and reproduce. Cell growth is necessary for reproduction, so if it is interfered with, natural selection will work its magic and select those mutant bacteria that are most fit for an environment whose most immediate danger is the conventional antibioticwith its growth interfering sulfonamides, tetracycline, its fluoroquinolones, and penicillin. Bacteria multiply rapidly, so there are countless mutations and possible adaptations arising every few minutes. The process of natural selection happens fairly quickly. To say the least, bacteria do not subscribe to the geological time we normally associate with evolution. Bacterial species have evolved in several short years to become resistant to conventional antibiotics that were recently able to dependably combat infection.

QS, or rather, the signaling molecules and cognate receptors that carry out the process, are what scientists call secondary metabolites. A secondary metabolite is a trait that is not required for the growth, reproduction, or immediate protection, of an individual organism. If one eliminates the secondary metabolite, the organism will have no trouble continuing to grow and reproduce. Overtime, the organism might be at a disadvantage, but what's important is that the elimination or depreciation of the secondary metabolite does not subject the individual to immediate selective pressure. Individuals without the secondary

metabolite are able to pass on their metabolite-less genes to future generations just as the individuals with the secondary metabolite are able to pass down their genes. By interfering with the secondary metabolite signaling molecules and receptor proteins, QQ diminishes the community's ability to infect a host, but it does not affect individual cells' ability to survive and pass on DNA, thus theoretically bypassing the potential selective pressure to circumvent the effects of QQ.

Pharma companies should look to invest in the development of QQ molecules in order to treat infections. The demand for new antibiotics is rising as conventional antibiotic-resistant bacteria become more widespread and dangerous. The risk associated with injecting potentially millions of dollars of capital into developing a drug that might become ineffectual within a few years is eliminated through the use of QQ molecules. Indeed, quorum quenching is a viable treatment for infectious diseases, especially for those that originate from drug-resistant bacteria; many quorum quenching molecules are nearly ready for clinical trials, while at least one is already primed for a clinical study. The focus for the remainder of this paper will be to outline a few specific studies that have identified and isolated drugs that successfully fight infection by inhibiting the QS mechanisms responsible for pathogenesis in many clinically relevant bacteria.

#### Pseudomonas aeruginosa and the mBTL quorum quencher

According to the Centers for Disease Control and Prevention, in 2002, there were an estimated 12,000 cases of a hospital-acquired *P. aeruginosa* infection in which the pathogen was resistant to the antibiotic, Ceftazidime, and an estimated 16,000 cases of hospital-acquired *P. aeruginosa* in which the pathogen was resistant to the antibiotic, Imipenem ("Bad Bugs, No Drugs"). These data are confined to the United States, and are also obviously outdated. Since 2002, resistant bacteria have escaped hospitals and entered the healthy community, and the number of resistant *P. aeruginosa* cases has risen significantly. *P. aeruginosa* is an opportunistic pathogen that mainly infects cystic fibrosis patients, burn victims, and implanted medical devices like intubation tubes and stents. The resulting health care costs, especially in order to replace medical devices, are in the millions of dollars, while the emotional cost of potentially losing a loved one to a fatal nosocomial infection is immeasurable.

QS in *P. aeruginosa* regulates virulence factor production and biofilm formation, which are essential functions of pathogenesis. Virulence factors are agents or traits that allow a microbe to establish itself in a host and cause disease. Toxins, hydrolytic enzymes, and cell surface proteins that facilitate bacterial attachment to the host cell are all examples of virulence factors. A biofilm is an extracellular matrix of polysaccharides, protein, DNA, and water that acts as a protective sac to hold together densely packed bacterial cells. Its functions are to optimize transfer and uptake of DNA in a community for maximization of genetic variation, and to protect biofilm members from attack by the host immune system. A biofilm generally forms on surfaces or air-liquid interfaces, often forming on and clogging medical implants.

In a paper published by the Proceedings of the National Academy of Sciences (PNAS), a coalition between microbiologists of Princeton University and the Howard Hughes Medical Institute in Maryland identified a molecule that has significant QQ capabilities for the opportunistic pathogen, *P. aeruginosa*. The paper, titled, "A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation," written by O'Loughlin et al. (along with top microbiologist, Bassler), presents strong evidence on behalf of the pharmaceutical development of mBTL.

The *P. aeruginosa* QS mechanism contains two LuxI/R-type networks that work in series: LasI/R and RhII/R. LasI produces the AHL, 30C12-HSL, which forms a complex with cognate receptor LasR. The LasR:30C12-HSL complex folds, binds DNA, and activates expression of a few genes, including the *rhIR* gene. RhIR binds to autoinducer C4-HSL produced by RhII, subsequently binding DNA, and activating a second wave of genes.

The team of scientists identified around 30 antagonists in a previous study that will be discussed later. In this study, four of the molecules, which were synthesized and designed based on the chlorolactone structure that had been previously identified as an inhibitor of a separate pathogen, were assayed for their potential to inhibit *P. aeruginosa:* chlorolactone (CL), chloro-thiolactone (CTL), meta-chloro-thiolactone (mCTL), and meta-bromo-thiolactone. Their respective QQ potency was determined by measuring the production of virulence factor, pyocyanin, which is produced via expression of genes activated by the RhIR:C4-HSL complex. When these molecules were mixed with wild-type strain, *P. aeruginosa* PA14, only mBTL and mCTL were able to effectually inhibit production of pyocyanin.

The graph below shows that WT *P. aeruginosa* treated with 100 M of mBTL, mCTL, CTL, and CL, produced a lot less pyocyanin when treated with mCTL and mBTL. Importantly, the growth of WT *P. aeruginosa* treated with mBTL and mCTL was not impaired. This shows that mBTL and mCTL have QQ capabilities, with mBTL having slightly more potency.

mBTL works by acting as an analog of the natural autoinducer. It binds with the cognate receptor LasR or RhIR, taking the place of 30C12-HSL and C4-HSL, respectively. It was previously shown that analogs like mBTL allow the complex to fold and bind to DNA, yet it interferes with the ability of the complex to interact with RNA polymerase, lowering transcriptional activation potential, thus preventing full expression of the target gene.

mBTL's target receptor was determined by treating both *rhl* and *las* null mutants. In the absence of RhII/R, mBTL did not significantly affect gene expression, while in the absence of

0.6 0.6 0.5 0.5 Absorbance (695 mm) Absorbance (695 mm) 0.6 0.4 0.3 0.3 0.2 0.2 0.1 0.1 0.0 0.0 WT PAIA 185H thill 185Hth ŝ cit ncit (BI 10-7

WT PA14

Diagram 1



LasI/R, with RhII/R present, mBTL altered gene expression. This shows that mBTL inhibits P. aeruginosa QS in vivo by antagonizing the QS of the RhIR component.

However, the essential question has yet to be answered: can mBTL actually inhibit virulence in an animal model and prevent killing? The answer is yes. The nematode, *Caenorhabditis elegans*, was infected with the las mutant and the WT PA14 strain. Within 24 hours, 90% and 77% of the worms were killed, respectively. However, when treated with 50 M of mBTL, only 23% of worms treated with WT were killed, while merely 50% of worms treated with the las mutant were killed. According to the researchers, "Together, these results confirm that the relevant in vivo target of mBTL is RhIR and, importantly, that **inhibit-ing RhIR could form the basis of an antibacterial therapeutic strategy."** 

In order to test whether mBTL is capable of protecting mammalian cells from killing by *P. aeruginosa*, human lung A549 cells were infected with the pathogen. All variations of *P. aeruginosa* are capable of killing A549 cells; at a concentration of 100µM, mBTL is not toxic to the human cells. It was shown (refer to graph below) that mBTL prevents killing of A549 lung epithelial cells by the WT and the *las* mutant, but not by the *rhl* and double *rhl las* mutant. This provides further evidence that mBTL functions by inhibiting RhIR. Even though it acts only as a partial antagonist of LasR and RhIR mediated-quorum-sensing, it does not follow that a pure antagonist of both receptors would yield better results. CL, which exhibits



#### Diagram 2

pure antagonism of both receptors, does not reduce production of pyocyanin in vivo, nor does it prevent killing of human lung A549 epithelial cells, whereas partial antagonist mBTL does.

In addition to protecting *C. elegans* and human lung epithelial cells from killing, mBTL was able to reduce the size of *P. aeruginosa* biofilms. Quorum sensing mediated gene expression is necessary for forming the annoying biofilms that are large enough to clog stents and other biomedical devices. Whereas WT strain usually forms biofilms of heights 16-39µm, WT strain in the presence of 100 mBTL reduces that range to 6-14µm.

The study shows significant evidence that there exists a potent quencher of quorum sensing activities in pathogen *P. aeruginosa*. The identified molecule, mBTL, acts as an AHL analog of *P. aeruginosa's* normal autoinducer. mBTL represses genes encoded for virulence factor pyocyanin and prevents killing of *C. elegans* and human lung epithelial cells. In addition, mBTL substantially reduces the height of biofilms formed through *P. aeruginosa* QS. Thus, the study presents a strong case for mBTL not just for its applications as an anti-infective, but also as having applications in industry. mBTL is nearly ready for clinical trials. It is perhaps two short experiments away from being ready. (1) It must be proven that digestive and/or bodily enzymes will not degrade mBTL (which is unlikely because the body does not have potent natural AHL degradation enzymes), and (2) mBTL must be tested on mice with cystic fibrosis that have been infected with *P. aeruginosa*.

(All information and data presented in this section are attributed to O'Louglin et al.)

# *Aeromonas hydrophila* infection in Zebra Fish and the AHL lactonase method

A. hydrophila is a primary, secondary, and opportunistic pathogen found in aqueous environments. Many of its pathogenesis-related traits are controlled by a LuxI/R-type QS circuit, where the receptor is AhyR and the AHL manufacturer/modifier is AhyI. Virulence factor production (of hemolysin, protease, S-layer proteins, DNase, and amylase) and biofilm formation are controlled by the AhyI/R QS system.

Hitherto, I have only discussed the use of AHL analogs as quorum quenchers, but now I will discuss a second class of quenchers: signal degradation enzymes. In a study conducted in 2000, an enzyme was isolated from *Bacillus* sp. strain 240B1 that exhibited QQ potential. It's important to note that many bacterial species have evolved QQ in order to successfully compete against other species that use QS to establish strong niches in communities. The enzyme, called AiiA, was spliced into the genome of plant pathogen *Erwinia carotovora* strain SCG1. The transformed pathogen, which uses QS to regulate the release of virulence factors, was unable to successfully infect potato and tobacco plants because in expressing the quorum quencher AiiA, it effectively reduced the quantity of AHL it produced (Dong et al). AiiA is the representative of about 20 AHL-degrading enzymes that break down the AHL molecule by hydrolyzing its lactone ring. It's called specifically an AHL-lactonase. (There are AHL-acylases, which degrade the AHL by breaking down the acyl chain; lactonases are more specific and thus the most relevant group of signal degradation enzymes.)

In the study by Cao, Y. and Zhou, Z., et. al. published in 2012, an AiiA enzyme was iso-

 $\dot{\mathbf{v}}$ 

lated from *Bacillus* sp. strain Al96, and was orally administered to Zebra fish that had been infected with *A. hydrophila*. This lactonase is special. Where other lactonases of the same family (like AiiA 240B1) are not versatile, AiiA Al96 maintains optimal levels of activity in a wide range of temperatures. Additionally, it is resistant to many digestive enzymes, intestinal juice, and a range of heavy metals that typically inhibit AHL-lactonase activity. Thus it seems that AiiA Al96 would remain active if administered orally, and subsequently absorbed into the bloodstream. Indeed, the orally administered drug was able to withstand all bodily conditions, and succeeded in impairing pathogenesis of *A. hydrophila*, and consequently prevented killing of the Zebrafish.

Zebrafish that were fed the controlled diet (called the CK diet) with the AiiA A196 supplement, and were immersed in a solution of *A. hydrophila* had a significantly lower mortality rate than those Zebrafish that were not given the supplement. In addition, this graph shows the efficacy of another AHL-lactonase of the same family, AiiA B546. AiiA B546 was ineffectual, perhaps due to its lack of versatility, and did nothing to prevent pathogenesis and consequent killing of the Zebrafish.

AiiA Al96 on the other hand is fully capable of existing in a wide range of typically disadvantageous environmental conditions. The top two graphs show that AiiA maintains optimal activity between a pH of 6 and a pH of 9 or 11 depending on the temperature. The bottom two graphs show that AiiA Al96 is capable of maintaining optimal activity between a wide range of temperatures. The bottom left demonstrates optimal performance of enzyme between 0 and 55 degrees Celsius; the bottom left demonstrates that AiiA Al96 is ther-

#### Diagram 3



mostable, even at the seriously high temperature of 70 degrees Celsius.

These researchers concluded that AiiA Al96 and similar AHL-lactonase quorum quenchers present a viable economical solution to the losses incurred due to *A. hydrophila* infection. However, though that is AiiA's most practical immediate application based on the data gathered in this study, the quencher analyzed here has the potential to work for clinically relevant pathogens. The study also showed that AiiA Al96 degrades C4-HSL, C6-HSL, C7-

#### Diagram 3





HSL, C8 HSL, C10-HSL, C14-HSL, 3-oxo-C6-HSL, 3-oxo-C8-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-oxo-C14-HSL, and 3-hydroxy-C8-HSL. AiiA might also be able to quench human pathogen QS activities because many gram-negative bacteria use these AHLs to regulate pathogenesis-related activities. Furthermore, the fact that AiiA Al96 can withstand the adverse temperatures, pHs, and enzymatic activity of an animal's body makes it an easily deliverable antibiotic. AiiA (Al96) acts as an outstanding QQ tool for aquaculture, but it may have implications for control of mammalian pathogens too.

(All information and data presented in this section, unless otherwise noted, are attributed to **Cao and Zhou et. al**)

#### Acinetobacter baumannii and AHL lactonase

Human bacterium *A. baumannii* is an opportunistic pathogen notorious for causing nosocomial infections and being resistant to multiple classes of antibiotics. Like *P. aeruginosa*, it forms biofilms that clog medical devices and help it resist the effects of multiple classes of antibiotics. The bacterium uses the Luxl/R-type QS system to mediate the production of biofilms. Its receptor and synthase, abaR and abal respectively, respond to many signals, but primarily to the autoinducer 3-hydroxy-dodecanoyl-L-46 homoserine lactone (3-OH-C12-HSL). In a study conducted at the National University of Singapore, the researchers isolated a naturally occurring AHL-lactonase from *Geobacillus kaustropilus* (GKL). The researchers then engineered a mutant that produced a lactonase with enhanced catalytic activity and thermostability meaning it degrades the AHL quicker and is able to function over fairly dramatic ranges of temperature.

When the lactonase produced by the GKL mutant was added to a culture of WT *A. baumannii*, the biofilms were significantly reduced in size: in biomass, thickness, and surface area.

The relevant data in this graph—the size of the biofilm produced by the WT pathogen without the engineered lactonase presented on the far left, and the size of the biofilm produced by the WT pathogen in the presence of the enhanced lactonase on the far right—shows that the AHL lactonase was effective in dramatically attenuating the QS processes that control biofilm formation in human pathogen *A. baumanni*.

(All information and data presented in this section are attributed to Chow et al)



#### Diagram 5

#### **Related Studies**

The studies that I have hitherto reviewed concern the pathogens *P. aeruginosa, A. hydrophila,* and *A. baumanni.* To be clear, none of these pathogens are terrible threats to humans. Out of the three, only *P. aeruginosa* is truly clinically relevant. It was determined to be a serious threat, with 6700 multi-drug resistant infections (13% of total *P. aeruginosa*) and 440 deaths in 2013 ("Antibiotic resistance 2013"). Just because these studies do not attenuate QS-controlled pathogenesis in dangerous drug-resistant bacteria does not mean they are irrelevant, or should be dismissed. These studies have successfully isolated molecules—degradation enzymes and AHL analogs—that effectively reduce the relevant effects of QS—that is, virulence factor production and biofilm formation. They also show that these molecules, called quenchers, have the potential to be used to treat a wide variety of bacteria that use similar QS mechanisms. There are many strains of drug-resistant bacteria that have become threats to humanity that do use QS for help in pathogenesis.

We have seen a prevalence of *Streptococcus pnuemoniae* that are resistant to penicillin and erythromycin antibiotic groups. Drug-resistant S. pneumoniae is responsible for 1.2 million infections, 7000 deaths, 19000 excess hospitalizations, and at least 96 million dollars in excess medical costs ("Antibiotic resistance 2013"). The CDC determined that the strain is at a serious threat level. A strain labeled as a serious threat means, according to the CDC, that "this bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow" ("Antibiotic resistance 2013"). S. pneumoniae forms highly organized biofilms in the nasopharynx, lungs, and middle ear mucosa, which helps the bacteria evade attack by antibiotics and the host immune system (Vidal et al). Some gene expression in *S. pneumoniae* is controlled, or partially regulated, by its LuxS QS-system, including genes related to virulence and persistence in the nasopharynx (Vidal et al). In addition, in a recent study it was concluded that the LuxS-controlled QS system is a key regulator of early biofilm formation in a particular strain of S. pneumoniae (Vidal et al). The fact that S. pneumoniae uses QS systems to regulate these behaviors demonstrates that the QQ methods described above might be able to attenuate pathogenesis of S. pneumoniae, thereby avoiding its danger as an antibiotic-resistant pathogen.

Staphylococcus aureus is also of serious concern. Like *P. aeruginosa*, severe *S. aureus* infections occur mostly while hospitalized, and often clog implanted medical devices. Methicillin-resistant *S. aureus* (MRSA) is the most problematic of all drug-resistant strains of *S. aureus*. MRSA causes 80,461 severe infections and 11,285 deaths per year, though it should be mentioned that, fortunately, these rates have dropped about 30% since 2005 ("Antibiotic resistance 2013"). It is widely accepted that a classical gram-positive two-component QS-system controls virulence in *S. aureus* through the gene, *agr. agr* encodes for this particular system's transmembrane sensor histidine kinases, and thus *S. aureus's* QS-system. In a large study on staphylococcal pneumonia, about 20% of the infecting staph organisms lacked the *agr* gene (Traber et al). Though this shows that *agr* might not be essential to infection, those patients who were infected by the *agr*-carrying individuals suffered much worse infections than those patients who carried the strain that lacked *agr* (Traber et al). If there

were a way to inhibit agr-mediated QS in S. aureus, then the frequency of severe staph infections would abate. Fortunately there have been some strides in this direction. A molecule dubbed Solonamide B has been isolated from the marine bacterium, *Photobacterium halotolerans*, and it was recently shown that Solonamide B interferes with the ability of S. aureus's autoinducer to bind to the sensor histidine kinase, AgrC, of the *agr* QS-system (Nielsen et al). Treatment of community-associated MRSA strain, USA300, with Solonamide B, reduced the activity of central virulence factors, alpha-hemolysin and phenol soluble modulins (PSMs) (Nielsen et al). The severity of the USA300 strain is associated with these two virulence factors, and thus quorum-quencher Solonamide B has the potential to weaken the infections of USA300. (Traber et al).

In addition to *S. aureus* and *S. pnuemoniae*, there are a few other QS pathogens that are currently rated as serious antibiotic-resistant threats by the CDC, including Salmonella serotype Typhi, which causes Typhoid fever, and Non-Typhoidal Salmonella. The former is not so prevalent in the US, yet causes 21.7 million infections around the world; the drug-resistant form of the latter causes 100,000 infections per year in the US and costs 365 million dollars annually to deal with ("Antibiotic resistance 2013). Furthermore, vancomycin-resistant Enterococcus is another group of QS pathogens that is becoming increasingly difficult—nearly impossible—to treat with conventional antibiotics ("Antibiotic resistance 2013"). Even more disconcerting are the deaths related to *Clostridium difficile*—a bacterium that causes life-threatening diarrhea—which have increased 400% between 2000 and 2007. This pathogen, which causes 250,000 infections/year, 14000 deaths/year, and one billion dollars in excess medical costs/year, has recently been rated an urgent public health threat by the CDC ("Antibiotic resistance 2013"). Importantly, LuxS-dependent signaling allegedly regulates virulence gene expression in *C. difficile* (Lee et al).

#### **Discussion**

The primary concern with using QQ to treat human infections is that it is too risky of a treatment to invest in. What is to say that QQ will interfere with all the genes associated with infection and pathogenesis? Sure, perhaps QQ will block one or two virulence-related genes, but what's to say that QS is the only process responsible for their expression? What's to say that other, more vital instruments of pathogenesis won't continue on with a successful human infection even without their full QS-capabilities? And lastly, the biggest query of all: how do we know that, even if QQ succeeds in preventing some infection, QQ will not put selective pressure on bacteria to evolve resistance to these new drugs?

The answers to these questions are quite simple actually. Researchers do not propose that QQ should supplant conventional antibiotics; rather, QQ antimicrobial therapies should generally supplement conventional antibiotics and the natural host immune system. QQ interferes with (does not completely eliminate) expression of some genes related to pathogenesis; in some cases it diminishes the strength of a biofilm, in others it weakens attachment of individual bacterium to host cell, and still in others it reduces the amount of toxin produced by a population of invading pathogens. I do not know of one instance in which QQ can entire-

ly eliminate infection by itself. It needs the help of the immune system and/or bactericidal antibiotics. The principle, though, is that we would rely less on conventional antibiotics, and more on the defenses of our own immune system, thereby exercising (and conditioning) the immune system and helping to reverse the upward trend of severe drug-resistant infections. Ideally, treatment would consist of a cocktail of quorum-quenchers and bactericidal antibiotics.

The answer to the last, and most concerning, of the doubts is much more complicated. A handful of papers provide evidence for QQ resistance on behalf of all QQ skeptics, some of which outline lab studies and clinics where bacteria have been shown to evolve resistance to QQ compounds. This evidence, taken at face value, is disturbing for QQ enthusiasts like myself. But like with any assertion, any finding, or any data points, we must lay this evidence under a critical light. Bonnie L Bassler, QS's champion and spearhead, provides a few elo-quent and cogent reasons as to why QQ will be "especially difficult for bacteria to bypass by mutation":

"First, any mutation in the binding pocket of an autoinducer receptor that renders a bacterium immune to an autoinducer antagonist (inhibitor) must not interfere with signaling by the endogenous autoinducer. Such mutations will be extremely rare. Second, even if such a mutation could occur, if collective action is required for a quorum-sensing-controlled behavior (e.g., virulence) to be advantageous, then a single mutant bacterium that is blind to an antagonist does not gain an advantage through the mutation. Although the "resistant" bacterium will indeed switch into quorum-sensing mode at the appropriate cell density, other nearby bacteria that are susceptible to the antagonist will not. Thus, the quorum-sensingcontrolled behavior will not take place. Third, when an individual bacterium develops resistance to an anti-quorum-sensing drug, it does not gain a growth advantage. Rather, in all likelihood, such a mutant will have decreased fitness because it will undertake the energyexpensive quorum-sensing behaviors, but it will not reap the benefits of them because it will be carrying out these tasks in isolation. This quorum-sensing "resistance" scenario is absolutely unlike when resistance to a traditional antibiotic develops and the resistant mutant and its offspring immediately receive a growth advantage. The latter fuels the growth and spread of antibiotic-resistant bacteria, whereas development of immunity to anti-quorum-sensing therapies could, in fact, function to limit resistance." (Swem and Bassler et al).

#### To paraphrase Dr. Bassler and her colleagues:

1. Mutations that change the structure of the binding pocket of the autoinducer receptor that enable the bacterium to become resistant against any AHL analog (antagonist), must not also prevent the receptor from binding with the native autoinducer. Because antagonists are usually analogous to native autoinducers, a mutation that interferes with the binding of the antagonist to the receptor will almost always interfere with the binding of the native autoinducer to the receptor as well. Therefore, this kind of mutation will not allow resistance. 2.3. Even if a mutation in which the receptor becomes immune to the antagonist, but not to the native autoinducer, is possible, there is still no advantage. If one bacterium with this mutation switches to quorum-sensing-mode at the appropriate time, the desired behavior won't take place because the surrounding bacteria won't have switched into quorum-sensing-mode. Therefore, because the advantages of QS depend on a quorum of bacteria responding collectively to autoinducer, one mutant bacterium, partaking in the action, will not gain an advantage, and thus not be selected to survive and reproduce. This mutant bacterium might even be less fit than its non-mutant counterparts because it will be taking on energy-expensive activities with no benefits.

In conclusion: it is counterintuitive, but development of immunity to QQ therapies might in fact limit resistance.

It is clear that antibiotic-resistance is a problem that is only becoming worse. Drugresistant infections are more severe, increasingly prevalent, costly, and relentless. Compound that dangerous predicament with the unwillingness of pharma companies to invest in the development of new, working antibiotics, and thus you have a crisis in the near future. Some QQ compounds and other novel antimicrobial therapies must be clinically tested now. The studies listed above show that the antagonist, mBTL, and variants of the AHL-lactonase, AiiA, are nearly ready for clinical trials. In the words of Bonnie L Bassler and her microbiologist colleagues, "Our results... make a strong case and provide compelling in vivo evidence that an anti-quorum-sensing strategy is a valid alternative to traditional antibiotics for Gramnegative bacteria and that there is merit to pursuing the clinical relevance of such strategies" (Swem et al). Why don't the federal government and these pharma companies heed her word and start doing something to avoid the impending crisis of widespread multidrug-resistant infection?

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## Is Oxytocin all it Seems?

by Rebecca Bender

### Introduction: What is Oxytocin?

ccording to Psych Central, the hormone oxytocin ("quick birth" in Greek) is a mammalian hormone, meaning that it's secreted by the posterior pituitary gland. This hormone acts as a neurotransmitter in the brain. Oxytocin is believed to be released during physical acts, such as hugging, having sex, and touching. Within the brain, the hormone is involved in social skills, such a recognition and bonding. Once the oxytocin is secreted from the pituitary gland, it's unable to enter the brain again. Because of this, the effects of the hormone are found all throughout the body.

The most common name for this hormone is the 'Love and Trust Hormone' though. Though the chemical balances behind the hormone will be discussed later, it is believed that oxytocin accounts for why people behave in certain manners. For example, an oxytocin level could explain why some husbands are more faithful than others, why some people are honest and some cheat, and why some people feel more comfortable in relationships than others. Within our blood and our brain, oxytocin is the chemical that creates trust and love for people anywhere from intimate relationships to politics!

The most common relationship oxytocin deals with though, is between a mother and her child. Oxytocin controls a woman's contractions during labor. The hormone can also be injected in a form known as Pitocin, a synthetic version that can induce delivery. It is also responsible for the calm and attentive focus (or lack of) that mothers have for their children.



One of the fastest ways to increase oxytocin levels is to breast-feed, because it's enhancing a connection between a mother and her child.

Of course, this hormone seems amazing. A hormone that can help reduce social fears, help moms be moms, works as an antidepressant, and release stress seems to good to be true; the best part is, all you have to do is trust someone! This is obviously easier said than done. In a world like ours, I believe always being open and loving is dangerous, and increases the chances of something bad happening. The point of this hormone is not to always be trustful all the time, but rather help maintain the balance between trusting behavior and distrusting behavior.

### Chemicals: What makes Oxytocin, Oxytocin?

Oxytocin is a nonapeptide, meaning that it is a peptide of nine amino acids. Also known as C43H66N12O12S2, the structure is very conserved in placental mammals, though recent tests have shown new structures within New World Primates. Oxytocin is one of two known hormones that are released by the human posterior pituitary gland that acts at a distance, but oxytocin neurons also make other peptides, such as corticotrophin-releasing hormone (a peptide-hormone and neurotransmitter involved in the stress response) and dynorphin that act locally within the body. The magnocellular neurosecretory cells that make the oxytocin hormone are large neuroendocrine neurons, which get excitable and generate action potentials.

As mentioned earlier, the peripheral actions of the hormone tend to release secretion

from the pituitary gland. The ability of the oxytocin neurons to generate action potential depends on the dendritic (pertaining to dendrites) release of oxytocin, which sets up a dendrodendritic communication system.

In diagram 2, the upper portion has five oxytocin neurons lines up next to each other. Neuron #3 works as a pacemaker. It randomly releases the hormone from its dendrites, starting the action potentials, which triggers the release of oxytocin from each nerve ending.

In the middle portion, oxytocin from neuron #3's dendrites' spread to neurons #2 and #4, thus triggering a similar series of actions represented by the green arrows. This feedback now sets up a regenerative mechanism, which allows the neurons to release the hormone in synch.

In the lower portion, the wave of the action spreads from neuron #3 to all its nearby oxytocin neurons.

### Diagram 2



Of course, we are currently discussing the natural oxytocin hormones found in our bodies. Recently, due to studies from Dr. Paul Zak, there are also synthetic forms of oxytocin as well. These drug forms are sold as medications, typically named Pitocin and Syntocinon. Oxytocin can be destroyed, so sometimes it must be administered by an injection or as nasal spray. The hormone has a half-life of about three minutes in human blood. When being administered, oxytocin doesn't enter the brain (unless in rare cases) due to the blood-brain barrier. The blood-brain barrier protects the brain from foreign substances, hormones, and allows the brain to have a constant environment.

#### **Negative Effects of Oxytocin**

Of course, there is always a catch. There are limits to the power of this miraculous hormone oxytocin produced love and trust to just a small group of people, not toward the world. It also can strengthen negative memories of a stressful event to such an extent; the memories remain stressful even years after the event took place. According to an article called, The Darker Side of Oxytocin, "These memories can then add to the fear and anxiety felt in future stressful situations, much like post-traumatic stress disorder (PTSD), making a bad situation worse."

At the University of Haifa in Israel, researchers asked a group of people to play a game (for money) with a fake person Half of the group was given oxytocin spray, the other half a placebo. Three different outcomes could occur; the fake player won more money, the fake player lost more money, or the fake player ended up with the same amount as the actual person. The players previously given oxytocin claimed they had "higher levels of envy and gloating (schadenfreude) during the first two outcomes, but not during the third." The placebo group was much less emotional, not caring about their outcomes, since it was just a game. (Vicki Santillano).

In a new study, conducted by Dr. Karen Bales, Professor and Vice Chair of Psychology at the University of California, Davis, Bales studies oxytocin's long-term effects on Prairie Voles. The Prairie Vole, a small rodent that mates for life, was the perfect choice due to their atypical monogamous behavior (rarely found in other mammals). In the study, the voles were given a dose of nasal oxytocin spray, or a dose of saline spray every day from the day they were weaned to the day they reached sexual maturity. At first, according to the study, the voles on the oxytocin spray increased social behavior in the males, very similar to the effects we see in humans. Once the males got older though, Bales realized that the "Male Prairie Voles which received a dose similar to that being tested in humans, or even a lower dose, did not form pair-bonds normally with their pare-mate. Instead these males chose to associate with a strange female."

Of course, some might claim that this type of behavior is typical for human males, but it is very abnormal for a Prairie Vole to do. Even if their mate were to die, the other vole will stay fully committed, not looking for a new mate. In conclusion, according to the study, "If oxytocin's long-term effect are so different from its short-term impact, this poses great problems for its long-term use for any human condition." (Chronic Intranasal Oxytocin Causes Long-Term Impairments in Partner Preference Formation in Male Prairie Voles).

## The Moral Molecule: Who is Dr. Paul Zak?

Paul J. Zak is a Professor of Economics and the founding Director of the Center for Neuroeconomic Studies at Claremont Graduate University. With degrees from University of Pennsylvania and Harvard, he is also the author of The Moral Molecule, the first book to be written about oxytocin in our everyday lives. It was his lab, who discovered in 2004 that oxytocin had adapted from just childbirth, to a new way to study social structure and behavior. Also known as "Doctor Love" pictured below, he has turned the oxytocin hormone into the "cuddly hormone".



Of course, that molecule is oxytocin. Dr. Zak takes his readers from England, to Papua New Guinea, from weddings to Switzerland, and even his home campus, in Southern California.

### **The Moral Molecule**

In a book written by Dr. Paul Zak, titled The Moral Molecule in May of 2012, Dr. Zak tackles questions, such as "Why so some people give freely while others are cold hearted?, "Why are some husbands more faithful than others", and the ultimate question "Could the key to moral behavior lie within a single molecule?"

Described as "Accessible and electrifying" by his readers, Dr. Zak

has decided to write about the "loving" aspects of the hormone, and how it can single handily, when working properly, turn humans into better versions of themselves.

The Love Doctor also has another claim to fame; oxytocin spray, for just 59 dollars! This self-done oxytocin spray can be bought on Amazon, and is apparently "Fast and convenient. No need to schedule around meals". Apparently the spray will stimulate the effects of the naturally occurring hormone. Some people believe that their bodies need help controlling stress or increasing self-confidence, and they use the spray that's supposed to provide your body with the extra 'boost'.

As previously stated, the long-term effects of the hormone are dangerous. That they can now be administered does not sound safe, nor should be available towards the general public. Especially due to the size of the hormone (very large), the spray's actual outcome hasn't been proven, which makes doctors wonder if it acts as a placebo, since patients do claim they're more confident.

#### The Science Behind Feeling Good While Social Networking

In an article written by April Rudin, for the Huffington Post, Rudin writes about how Dr. Zak claims that Social Networking increases oxytocin levels. This study revolved around networks such as Facebook, MySpace and Twitter over a Blackberry. Dr. Zak claims "These social networking tools simply facilitate and, in some cases, replace the now old-fashion face-to-face conversation." Social Media networks allow the question "What's new with you" to be answered in every possible way, thus giving humans an easier way to share their feelings and trusting people. According to Dr. Zak, the keyboard has become the equivalent of a human touch. Rudin writes, "There is an inborn human desire to communicate with others. Isn't that the most viral definition of social networking?"

When oxytocin is released, the feeling of trust goes up, and concerns decrease. The fact that our brains can't distinguish the difference between legitimate human contact and texting is worry-some. This also brought up some red flags. Oxytocin is known as a healthy hormone, as long as it's produced naturally, but if it's being produced through addicting sites such as Facebook, human contact might not even be needed.

What is the most surprising is that articles about cyber-bullying are published daily. According to DoSomething.org, nearly 43% of kids have been bullied online and 1 in 4 have had it happen more than once. 70% of students report seeing frequent bullying online, but the worst is that 81% of young people think bullying online is easier to get away with than bullying in person. They're not wrong. With over 80% of all students regularly using a cell phone, social networks are an easy way to bully peers. So how is it that people actually feel more confident and trusting online?

#### Hypothesis

Two possible theories came to mind. We'll call them the Blindfold Theory and the New Person Theory.

The Blindfold Theory is a potential way for students to gain confidence over social networking sites. Take Facebook for example. Anyone could post anything they want, whether it is positive or negative about anyone else. If there was a picture of a person they didn't like, the student could post a hurtful comment on it because at no point do they need to see a reaction. This is the Blindfold Theory because the student is blindfolded from any type of reaction. Because there seems to be no apparent 'consequence' the individuals' confidence for not getting caught is higher, thus oxytocin levels are higher. The student isn't looking at someone else's face for a reaction but rather a computer screen.

The second theory is called the New Person theory. For anyone who's seen the show Catfish, the theory is based off of that television show. The term "catfish" is the name for someone who creates fake personal profiles on the Internet. These people pretend to be someone they're not, thus concealing their true identity by using other people's pictures and information. If I were to turn to a person and have a face-to-face conversation, what they see is what they get. The way I look and act will be my

true self. If however I was on Facebook, I could hide my insecurities, and only portray the better parts of my 'self', changing the rest into a new person, or new me.

Both theories do bring a sense of confidence, so clearly the oxytocin levels have been raised. But both theories bring about a large amount of false confidence. Shouldn't the brain subconsciously know that this feeling of satisfaction isn't real? This made me question if the internal hormone actually increased.

## **The Experiment**

My experimental question is to see whether or not Facebook use enhances a teen's self esteem level. I did this by using a control group and an experimental group. I will gathered my subjects (eight per each group, originally) by randomly selecting the upper school directory and choosing eight male and eight females. They were emailed a permission slip with a request to participate in an experiment testing their ability to problem solve. Upon receiving signed permission slips, I invited each subject to start the experiment individually. I will continue to invite students by random selection until I have enough to complete my study.

The controlled group was asked to read a New York Times article about tomatoes online. After reading, "Building a Better Mass-Market Tomato" by Kenneth Chang, a boring article, the subjects will answer questions on an anonymous survey.

The experimental group was asked to log onto Facebook and complete tasks such as viewing a friends profile, commenting on a picture, messaging a friend, and scrolling through their own profile. After, they too were asked to complete a survey.

#### The Survey

For the experiment, two surveys were created, one for the control group and one for the experimental group. Both surveys had to be answered with a number 1-5, 1 being Never, 2 being Occasionally, 3 being Often, 4 being Very Often and 5 being Always.

The Tomato Article survey included "distraction" questions. These were, "I read articles from the New York Times...", "I put tomatoes in my salad/ sandwich...", and "I check what type of tomatoes I eat...".

The Facebook survey included distraction questions such as, "I am logged on to Facebook on my own computer", "I talk to my friends on Facebook" and "I feel comfortable with the way I am portrayed on Facebook.

Of course, there were also the consistent questions. In order to measure oxytocin, I measured each individual's self-confidence levels. First, self-confidence had to be defined. I broke the idea of "self-confidence" into looks, intellect, academics, and public speaking. The questions were, as follows:

- 1. When I look in a mirror I feel great...
- 2. If I don't attend a party, my friends will miss me...
- 3. I am comfortable in a discussion based class
- 4. I care about what I look like in school...
- 5. My grades affect how I feel about myself...

#### Results

FACEBOOK SURVEY	Never	Occasionally	Often	Very Often	Always
When I look in a mirror I feel great		1	2		1
lf I don't attend a party, my friends will miss me		1	1	2	1
l am comfortable in a discussion based class		1	2		2
l care about what I look like in school	2		3		
My grades affect how I feel about myself		2		3	

ARTICLE SURVEY	Never	Occasionally	Often	Very Often	Always
When I look in a mirror I feel great	1	1	1		2
If I don't attend a party, my friends will miss me	2	1		1	
l am comfortable in a discussion based class	1	2	1		1
l care about what I look like in school		1	1		2
My grades affect how I feel about myself		2	1		2

#### Conclusion

According to my results, there doesn't seem to be any major distinctions between facebook and self esteem. The numbers tended to be all over the place, but that could be because only ten people were asked to participate in the survey. With a larger group of maybe fifty, the results might have become more apparent. This pilot experiment confirms my original disbelief that social media truly does raise self-confidence. I think it must have to do with a variety of other factors. Perhaps someone has more self-confidence because they're doing better in school. That person then rewards herself with Facebook. She might feel more confident, but Facebook is an end, rather than the means.

Another possibility is that people are more confident when talking to people, because it makes them feel more secure and wanted. Social Media websites such as Facebook make
it easier to communicate with your friends when you're home. The problem is that Facebook also allows two people who normally wouldn't talk in school to communicate. When one of the individuals realize that in real life, the second individual wont acknowledge them, this confidence is lost, proving that Facebook can't just make you feel better about yourself, because people will also be experiencing a life outside of the computer.

The study of the oxytocin hormone is a new one, and will beyond doubt be studied further. Since this was just a pilot experiment in the future I would like to actually be able to study the hormone in the lab, and study the exact hormone (not just it's affects) in real life situations, such as couples on dates, first time mothers, mothers who are on their second or third child, and even study the different between oxytocin levels of children versus adults.

In the long-term I assume that more experiments will be done about the effects of oxytocin in the long run, and scientists will create better ways to confirm the results of the oxytocin sprays. Who knows, maybe in ten years we will be able to buy an oxytocin spray off the shelf with an 100% chance of giving more confidence during a test or presentation!

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# Attitudes on the Morality and Implications of Prenatal Genetic Testing

by Olivia Cucinotta

#### Introduction: Prenatal Genetic Testing and Geneticization

n May of 1952 in a London laboratory at King's College, biophysicist Rosalind Franklin took the first X-ray diffraction image of DNA. Without the permission of Dr. Franklin, a colleague of hers showed the image to James Watson and Francis Crick, who would use it as a critical evidence for the identification of the double helix structure in DNA. Many look to this image, which would come to be known as Photo 51, and the research that followed it as the starting point of our modern understanding of DNA.

Since then, our understanding of DNA and genetics has skyrocketed as we begin to understand the links between genetics and certain abnormalities. In 1990, the Human Genome Project was founded, and in 2003 it successfully sequenced the entire human genome. Today, we have the technology to screen for thousands of genetic mutations in a single prenatal test, as well as many other tests to look for more specific diseases.

Our knowledge of genetics and genetic diseases has grown and is growing exponentially. We are making more and more innovations about how genes manifest themselves and affect who we are and the characteristics of human life. The more we understand about genetics, the more we must understand its complexities and complications.

In an attempt to begin to understand our own genetics, genomic testing has become a rather common procedure, especially *in utero* genetic testing for expecting mothers. Today, over half of pregnant women choose to have prenatal genetic testing done, ranging from Amniocentesis, (a test of amniotic fluid which can determine high risk for Down Syndrome, Sickle Cell Anemia, Muscular Dystrophy, Tay-Sachs and neurodegenerative and neural tube defect disorders), to MMS (maternal serum screening), which is less comprehensive but less invasive, to CVS (Choronic Villus Sampling) which is done earlier, in the first trimester, and which can screen for hemophilia, Tay-Sachs, and Down Syndrome, but cannot screen for neural tube defects.

With all these advancements in the understanding of genetics and the treatment of genetic conditions has evolved a phenomenon that bioethicists are referring to as geneticization.<sup>1</sup> Geneticization is a term for the ways in which the science and understanding of genetics influences public thought. This can be applied in many ways, from its affect on medical diagnoses, all the way to a more philosophical understanding of the self. While only sixty or so years ago we knew very little about DNA, our rapidly growing understanding and testing has led to a very different concept of what makes us who we are. The question of prenatal genetic testing, of giving the family of a fetus knowledge about its genetics, about who it may grow up to be, puts us in an interesting ethical place. For the first time, we have control over what kind of life we bring into the world; its gender and its ability are things that can, now, inform a decision about terminating a pregnancy. How we deal with this potential, how we navigate this ethical grey zone, and its significance is a subject of confusion and debate.

#### **Literature Review and Background Information**

My research focuses on prenatal genetic testing and its implications. I studied this topic as a specific approach to understanding one facet of geneticization. This area is a relatively understudied one, although in the literature which exists there seems to be a constant point of disagreement: Will prenatal genetic tests and acting based off of that information become the norm as understanding of the science proliferates the mainstream? Or is the nature of this question such that we, as people, will always make the choice based off of our back-ground and circumstance?

The book *Testing Women, Testing the Fetus: The Social Impact of Amniocentesis* by Anthropologist Rayna Rapp argues that more than scientific literacy and access are important when analyzing prenatal genetic testing—that women make this decision based on their background and their culture more than anything else. She argues against "diagnoses as a scientific fact," and that diagnoses (especially prenatal ones) cannot exist independently of their social context.<sup>2</sup>

Rapp published her book thirteen years ago; I'm interested to see how similar or different my conclusions are to hers, as the political climate and access to healthcare is very different now.

Rapp's critics tend to gravitate around the argument that as information about these tests diffuses into the mainstream, the tests will become more and more morally acceptable.<sup>3</sup>

An article tracing trends in attitude of prenatal Genetic testing published in 2008 looking at a 14 year period from 1990 to 2004 essentially agreed. Authors Eleanor Singer and Mick Couper concede there is no "simple causal path from changes in technology to changes in values and beliefs," but we can see increasingly positive attitudes towards genetic testing because information and knowledge of the tests increases as "awareness about the new technology is slowly and gradually diffusing throughout society."<sup>4</sup>

The Journal of Medical Ethics published a study in 2009 outlining how women make the decision to get prenatal genetic testing done. It concluded that women struggle to balance "the interests of the family against that of the fetus in line with their values and their personal circumstances." Ultimately, though, the study concludes that personal circumstances and beliefs are more important than scientific literacy.<sup>5</sup> So although literature on this topic is scarce, it seems to revolve around the central controversy of whether or not our attitudes towards innovations in science, here specifically prenatal genetic testing, become more positive as it becomes both more understood and more available, or if our background and our morality will be the decisive factor.

In my research I attempted to both join and further this conversation. There is, of course, the question of why we find some innovations acceptable and some not, but in this specific context there is also a greater question: What kind of life do we find valuable, what life do we decide is worth keeping, worth investing in?

To gain greater insight about both of these questions I designed a survey slightly different than others on similar subjects. Mainly, I centered my questions not on the decision of whether or not to test but the question of terminating a pregnancy with the information hypothetically learned from the tests.

#### **Methods and Survey Design**

I designed this survey mainly around two questions: Is it personal, ethical reasons stemming from one's background that affect the decision on pregnancy termination, or is it access and information about the tests? I am also very interested in what these responses imply about what we think is important in life (although this is not a question ever fully answered by a survey).

I attempted to answer which markers in one's background, such as religion, socioeconomic status, career, and scientific literacy, affect one's opinion on the morality of prenatal genetic testing and the subsequent decision to keep or terminate a pregnancy. I hoped also expose the accessibility of this test based off of those same background markers, which will have further ethical implications. I did not have a hypothesis; I believe that if I have expectations for my data my questions may become leading and my results will be less valid. Of course I have my own bias, but by not expecting an answer I hoped to make both my questions more empirical and my analysis of the answers more impartial.

To find answers to these questions, my survey begins with asking questions about one's background based off of my belief that religion, socioeconomic status, and scientific literacy may have an affect on the decision to terminate pregnancy. Then, I am interested to see how the access to these tests and abortion clinics affect one's opinion of them. The last questions also are designed to represent extremes in what could be called human abnormalities, cancer and Autism. These questions rely on hypothetical scientific advances, like prenatal tests for a genetic link to cancer, that while possible in the near future are not yet available. Their purpose, however, is to help gain insight through the short answer about what human life is important to us, and why. I began the survey with questions concerning background, and then transitioned to more ethically based ones. They appeared in the order below:

Which Gender do you identify with? What year were you born? Do you have children? Have you, or someone close to you, terminated a pregnancy? What is the highest degree or level of education you have completed? What year did you complete your degree? Please describe your field of work. What is your estimated total household income? What ethnicity do you identify as? How would you describe the religion in which you were raised? How would you describe your current religious affiliation? What is your main news source? What is your interest in science? Would you describe yourself as pro life or pro choice? Please explain. Do you have prior knowledge of prenatal genetic testing? Please elaborate. Do you feel that prenatal genetic testing is available to you? What does the term "high risk pregnancy" mean to you? What does the phrase "normal child" mean to you? If you or a loved one was warned of a high risk pregnancy, would you advise terminating the pregnancy? Please elaborate. As technology advances, if a prenatal genetic test could inform you of a high risk for Autism, would you advise terminating the pregnancy? Please elaborate. As technology advances, if a prenatal genetic test could inform you of a high risk for terminal cancer, would you advise terminating the pregnancy? Please elaborate.

The survey was sent out to approximately 700 Berkeley Carroll teachers, faculty and parents. It was sent with an email, first introducing myself and then explaining the survey's broad goals, assuring the subjects they would remain anonymous and should they choose to take part informing them that their answers would be published, analyzed, and presented. This was reviewed by the IRB and provided the informed consent of my sample group. The email appears on the following page:

Dear All,

For those of you who do not know me, my name is Olivia Cucinotta. I'm a senior at Berkeley Carroll and a student in the Scientific Research and Design Program, which is a three year independent research program, culminating in an original research project.

I'm writing to you today to ask you to be a part of my study. I'm doing a survey looking at the common knowledge of and opinions on prenatal genetic testing and opinions on pregnancy termination. I would be incredibly grateful if you found the time to answer the attached questionnaire, it should take less than ten minutes.

This is an anonymous survey. Please answer all questions honestly and please do not research topics before answering. Some of the questions are personal and challenging. Please know, this is not a test, and all answers will be anonymous at every stage in the research. There is no obligation to participate.

My results will be published by Berkeley Carroll in the third Scientific Research and Design journal, and will be discussed in a presentation at the end of the year. As in the survey, I will not be discussing individuals, only trends, and again you will be anonymous at every stage of research.

If you have any questions, please feel free to contact me or my project advisor.

Please be aware that by submitting the survey you are giving your consent for your answers to be analyzed and published anonymously.

I would like to thank you in advance for taking the time to help me with this project.

Sincerely,

Olivia Cucinotta

The group of people related to Berkeley Carroll is a sample of both the BC community, and the majority of the responders were white, did not have a religious affiliation, were liberal, and many had higher education. Most were also involved with education. The implications of this sample group will be discussed in the analysis of the results.

I used survey monkey to collect my data anonymously and as a tool to organize and interpret my data. Some of the language from my survey comes from the NAIS Diversity Conference Brochure which lists racial affinity groups.

I realize that these sorts of questions, especially the short answer questions, are difficult to analyze, and any conclusion I draw is of course open to multitudes of other interpretations. However, I feel that leaving space for elaboration is important. This is a study on

How would you racially describe yourself? (Question 9)

	Black, African Herit	tage,					
	Latino/a Heritage, H	lispanic					
	East Asian Heritage, Souteastern						
	First Nations Herita	ge,					
	White, European H	eritage,					
	Greater Middle Eastern Heritage,						
	Multiracial Heritage, Multiracial						
	Trans-racialy Adopted Heritage,						
0%	20%	40%	60%	80%	100%		

#### **Diagram 2**

What gender are you? (Question 1)

	Male				
	Female				
	Gender Neutral				
0%	20%	40%	60%	80%	100%

human ethics, which requires qualitative research. It will be complex but I think it will also allow for complexities, as morality rarely boils down to a yes or no.

Out of my sample of over 700 people, 96 responded. 76% of responders were female (19% were male and 3% identified as gender neutral), over half had earned a Master's degree, over half were involved in education, 81% of responders identified as white.

The responses to demographic questions demonstrated a rather un diverse sample of the general population. Many questions had very nearly unanimous answers: 96% of responders identified as pro choice, 71% felt informed about prenatal genetic testing and only 3% believed prenatal genetic testing was not available to them (although 37% were unsure). There was slightly more diversity in religion and income, although still apparent trends.

I could find no direct correlation between demographic information and answers to the questions on ethics and pregnancy termination. However, trends in the short answer responses suggest that it is individual experience that informs thoughts on terminating a pregnancy and, perhaps, it is also individual experience which determines what life we find valuable.

The answers to the questions related to pregnancy termination yielded much more

How would you describe your current religious affiliation? (*Question 11*)



diverse answers than the demographic questions. The most common answer for each of these questions was "unsure," but generally there was disagreement between those who said "yes" and those who said "no." The question on terminating a high risk pregnancy had 60% respond unsure, 20% respond yes and 19% respond no. The question on terminating a high risk for cancer (one of the hypothetical question, rather than one which is immediately possible), had 50% unsure, 26% yes, and 24% no. The nature of cancer seemed to seems, then, to be a risk on life less worth taking. Autism, on the other hand, was generally more valued. 40% of people said they would not terminate a pregnancy if they knew the child had a high risk for pregnancy. 47% of people, however, were unsure, and 13% of people said they would. The sort answers, discussed later, illuminate what exactly it is that this sample value that informed the answers discussed.

An analysis of language in addition to statistics, however, shows a pattern of answers related to both personal stories and the common disclaimer of "I can't speak for others." This discussion of circumstance leads me to the conclusion that my sample group generally believes the decision to terminate a pregnancy to be a very personal choice, not one with universally mandated by a belief system. 79% of my responders have completed at least a Bachelor's degree, 97% follow the news and 90% describe an interest in science and keep

Do you feel prenatal genetic testing is available to you or your family? (*Question 16*)



#### Diagram 5

Would you describe yourself as Pro Life or Pro Choice? (Question 14)

	Pro Life				
	Pro Choice				
0%	20%	40%	60%	80%	100%

#### Diagram 6

If you or a loved one was warned of a high risk pregnancy, would you advise terminating the pregnancy? (*Question 19*)



up with innovations. This seems to be an informed sampling, and those included in the aforementioned majority especially tend to offer more personal or speculative answers. Responder 78 answered, in response to a question about terminating a high risk pregnancy, along with selecting unsure: "This is such a personal situation to consider. There are many specifics that may help me determine what I would do." Many answers echoed this idea—that it is a personal choice and the situation is very important. Responder 23 had a background in special education, she specified she would not terminate an pregnancy with a high risk of Autism, but specified "I feel that knowing ahead of time that there is a high risk for Autism would allow someone to find early intervention starting immediately." Similarly, some grounded their uncertainty in experience.

My second question was: what kind of life to we value? The responses to a question asking what the phrase "normal child" means to the subject, yielded many responses. The

As technology advances, if a prenatal genetic test could inform you of a high risk for Terminal Cancer, would you advise terminating the pregnancy? (*Question 20*)



#### Diagram 8

As technology advances, if a prenatal genetic test could inform you of a high risk for Autism, would you advise terminating the pregnancy? (*Question 21*)



most common by far was a simple, clinical response. "Ten fingers, ten toes" was a common phrase, as well as the idea that a child would develop according to typical standards. Some responders took a more personal approach: "Two hands, two legs, two eyes, conforming to the general proportions of Da Vinci's Man (just kidding). I want the baby to cry when he/she/it first comes into the world. That's a normal child." the same person, in response to the question about terminating a pregnancy with a high Autism risk responded "OBVIOUSLY NOT. An autistic child conforms to my idea of a 'normal child.'" Others, like the 12th responder, took issue with the connotations of the term: "Well. Everyone says it means healthy. I guess from a scientific standpoint I'd agree just semantically: does your child fall within the norms? Okay, he's normal. But I hate the connotations—that normal is good, that everyone has to want to be normal." Still others, even semantically, disowned the term all together: "I think that is a weird phrase I would never use."

Responder 12's answers, though, are emblematic in that we see what she values as life informing her decision. By disagreeing with the idea "that normal is good, that everyone has to want to be normal," we see what she values in life and this seems to inform her answers to other questions. For autism, she specifies "People don't understand that "autism" can mean a lot of different things and most of them are just another way of having a life" and on the subject of testing and pregnancy termination generally, "I'd say that there are so many variables in any life that it's usually a mistake to think that a test for a condition will

give you enough information to act on—unless you know for certain that the mother or child will suffer a lot or die immediately." We see, here responder 12's definition of what is valuable in life informing her other answers.

#### Conclusion

The goal of my research was at first to discuss the question I found to be most prominent in the literature: what informs our decision about prenatal genetic testing and the decision of whether or not to terminate a pregnancy based off of that information? Is a decision based on personal experience, background, morals, or access? Or is it a matter of understanding and scientific literacy? Is there any agreement?

The results from the survey demonstrate a lack of diversity which makes it difficult to answer the above question. Any results I have apply only to a sample of the Berkeley Carroll community, and generalizations about this topic cannot be applied to every person.

However, within this sample we can understand that it seems personal experience shapes a decision on prenatal genetic testing and its implications. While this is limited and while we cannot use these results to generalize about people, the implications here are that personal experience may tend to be more important than understanding or access. That despite the fact that my sample group was informed, and most felt they had access to testing, there was little agreement on the decision to terminate. The choices about testing, and about a decision to terminate pregnancy were, for this group, not dictated by a dominant moral principle, but rather were circumstantial. Same goes for what sort of life was valued enough to cary out a pregnancy. We learn that within the boundaries of the implications of this study, our own experiences have a greater affect on our opinions than science's proliferation into society.

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## Antibiotics and Superbugs: The Knowledge of Antibiotic Resistance in the General Public

by Rebecca Ennis

#### Abstract

uring my time in the Science Research and Design Program, I have been studying antibiotics and antibiotic resistance. The use of antibiotics has completely changed the way that bacterial infections are treated and the way that our society views illnesses. For example, "an estimated 90 to 180 million kilograms of antibiotics are used yearly," according to Carlos F. Amábile-Cuevas, author of *New Antibiotics and New Resistance* (Amábile-Cuevas, 2003). But since penicillin started being mass-produced in the 1940's, antibiotic resistance has become increasingly problematic ("Closer to Understanding, Averting Drug Resistance," 2012). New reports of antibiotic strains of bacteria are in the news almost every day, and the Centers of Disease Control and Prevention has recently highlighted antibiotic resistance as one of the major threats to public health ("Antibiotic Resistance: A Growing Concern," 2013).

However, most journal articles and books I have read argue that the public is better informed about antibiotics and the risks of using them incorrectly or unnecessarily than they had been in the past (Levy & Fischetti, 2003). While much of the literature I read stressed the tremendous strides taken to combat the misuse of antibiotics and the success of attempts to better inform both patients and doctors about the dangers of antibiotic resistance, the number of new highly resistant strains of bacteria is rising yearly and more antibiotics are quickly becoming obsolete. I felt that there was a large disconnect between what these articles were saying and what the current statistics about the use of antibiotics indicated. To better analyze this dichotomy, I created a survey to gather information about how people use antibiotics and to test what they actually know about their proper and improper use.

#### Background

Antibiotics work to kill, or at least impair, bacterial cells, but do not affect other cells. Bactericidal antibiotics, like penicillin and cephalosporin, kill cells (Kofke-Egger, Heather, Udow-Phillips & Marianne, 2011). Examples of bacteriostatic antibiotics which work by inhibiting bacteria's ability to reproduce are streptomycin and rifampicin (Day, 2001). The use of antibiotics is crucial in modern medicine, especially with such illnesses as staph infections or tuberculosis. Multi-drug resistance is the term given to bacteria that are no longer affected by more than one type of antibiotic. These are also referred to as superbugs. Typically, a bacterium must become resistant to at least three to four different antibiotics to be classified as multi-drug resistant (Amábile-Cuevas, 2003). The combination of the misuse of antibiotics (especially in the last few decades), and the lack of new antibiotics being created has led to the growing problem of antibiotic resistance, especially regarding multi-drug resistance. The term "misuse" can refer to using the incorrect antibiotic, using an antibiotic unnecessarily, not completing the dosage, or overusing antibiotics.

My focus has been on how bacteria become superbugs, and ways to either combat strains of multi-drug resistant bacteria, or alternate ways to treat it. Even though 40% of antibiotics used in the United States are for animals and this use in factory farms is central to the growing emergence of antibiotic resistance (since many broad-spectrum antibiotics are used preventively and to make livestock grow faster at larger commercial farms, this overexposure promotes resistance [Day, 2001]), I have mostly focused on antibiotic use in humans. I have looked at strains of tuberculosis (a bacterial infection that affects the lungs, also referred to as 'TB'), and bacteria that fall under the Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Staphylococcus aureus* (VRSA), or Vancomycin-Resistant Enterococci (VRE) umbrellas. These can refer to common staph infections, or diseases like malaria.

#### Survey

The survey tested what the general public knows about antibiotics, and whether their use of antibiotics is consistent with that knowledge. After the Berkeley Carroll Institutional Review Board (IRB) approved the survey, I distributed it largely in an online format, with around ten distributed in a paper format. I distributed the survey mostly in the Berkeley Carroll School community to students and faculty, and to the faculty of the Bronx Guild High School. All participants had to be 13 years of age or older, as I felt that younger children would not be able to accurately recount their antibiotic use, or might be unaware of it. I also predicted that young children might not have a very active role in their own medical decisions, which would negate their involvement in the study. While asking the participants' age and gender, the survey was anonymous, and did not ask for any information that would have allowed me to connect a survey to a participant. However, I did ask if the participant is in a medical or health services field to account for a predicted greater knowledge about the survey topic, as it could skew the data about how informed the average person is.

In the consent form that participants received, the nature of the survey was detailed, including the fact that it was anonymous. If they agreed to be in the study, they were asked to answer all questions in the survey fully and truthfully to the best of their ability/memory, to refrain from consulting any outside resources to answer the questions, and to not discuss the questions with others who had not taken the survey. The survey was twenty-eight questions long, with the majority multiple-choice. It took participants an average of three to five minutes to complete the survey.

#### Data

My survey is reproduced at the end of this paper for reference. The survey was completed by forty-seven participants. I originally planned to make three separate consolidations of the data, one of the entire population that took the survey, the second with only the information of those who said they were in a medical or health services field, and the third with only the information of those who said they were not in a medical or health services field. The three groupings would have allowed me to see the data of the population as a whole, and also see how the information of those in a medical or health services field affected the data, especially in respect to questions #18-23. However, only one of the participants said that she/he was in a medical or health services field, so I consolidated the data.

Thirty of the participants were female, and fifteen were male. Only one person indicated that she/he had personal or religious beliefs that discourage the use of antibiotics. Fourteen participants were teenagers, nineteen were in the 20-40 range, and twelve were 50 or older. In response to how the use and understanding of antibiotics changed in the participant's lifetime, the majority said that it had not changed or that they were unsure if it did, while fourteen said it did, but with only nine people giving examples of how it changed.

All but two people said that they knew what antibiotics were. Almost all the answers involved the words "bacteria" or "infection," and twenty-seven people gave simple answers categorizing them as "medicine" or "drugs." Eleven people gave more detailed answers that often had scientific explanations involved. Only six people gave answers that did not involve any elements of a correct definition of antibiotics or that defined them as fighting "viruses." In all the answers, language like "kill" or "fight" was frequently used.

Twenty-seven people reported having taken antibiotics in the last year, while nineteen had not, and only one person was unsure if they had. Out of those who had taken antibiotics, nine had taken it only once, fourteen had taken them 2-3 times, and four had taken them 4-5 times. The most common illnesses for using antibiotics were sinus, urinary, and ear infections, strep throat, bronchitis, or preventative measures taken in response to dental or oral surgery work.

Twenty-six people reported having been prescribed antibiotics in the last year, while twenty had not been prescribed any, and only one person was unsure if she/he was. Twentyfive of these people filled their prescriptions, while two people were unsure if they had. Twenty-two people took the entire prescription course, while two people did not, and three people were unsure if they had. The two people who stopped taking their prescription both said that they felt better before finishing the entire prescription.

Roughly half said that they had read an article or watched a news report about antibiotics in the past year. Out of those who said yes, television, newspapers, websites/internet, and scientific journals accounted for 83% of the media. From those who answered yes, 55% reported that the media had to do with antibiotic resistance or "super" something, 25% of the media was about antibiotic use, and 20% was about specific news stories or "unknown."

Only five participants had ever wanted to be prescribed an antibiotic, but were advised not to by a physician, healthcare provider, or pharmacist. Half of these people reported that they had a respiratory infection at the time, and the other half reported a sore throat. All were told that antibiotics would either be unhelpful in their situation, or were unnecessary due to the nature of their illnesses. Nine people reported that a physician, healthcare provider, or pharmacist recommended that they take an antibiotic, but that they did not agree/want to. Out of these people, six said that they either try to avoid taking antibiotics unless absolutely necessary or that they believed that the doctor was prescribing the antibiotics for something other than a bacterial infection. The other three people were concerned with side effects, the detrimental effects of long-term use, and taking the prescribed antibiotic with other medications.

# Results for the section testing knowledge of and proper use of antibiotics



On average, 77.78% of the people answered each question right.





#### Uncertainty

The main source of uncertainty in my study came from the small population size of my survey results. It is only possible to make conjectures based on the amount of information the results of my survey provided. A larger sample would by definition allow for more variance in age, education, job, socio-economic status, and other factors that could affect a participant's responses.

Another source of error also had to do with the survey population. All participants worked/went to school and/or lived in New York (or had lived there at some point in their lives). The majority of the population also worked or went to school at Berkeley Carroll or the Bronx Guild High School. This restricts how pertinent these results are in assessing the antibiotic knowledge of the general population. It gets closer at assessing the knowledge of students and faculty members in New York City high schools.

#### Conclusion

As a group, the participants in my study were relatively well-informed about the proper use of antibiotics, and had an idea of what antibiotic resistance was. For the majority of the participants, their self-reported knowledge of antibiotics (from the questions in the first part of the survey) matched their results in the true-or-false section of the survey. Overall, the participants in this study got many of the questions correct, and had a vague to firm understanding of what antibiotic resistance was. However, very few individuals got all of the true-or-false questions correct and/or gave substantial definitions for an antibiotic. One of the basic facts about antibiotics (that they are to be used for bacterial infections, and not viruses) was only known by 64% of the survey group. In conclusion, my results support the claim that the popu-

lation does have a high level of knowledge of antibiotics and antibiotic resistance (as supported by most literature on antibiotics published in the last decade), but that most individuals still have a rather shaky understanding of antibiotics and their proper usage.

#### **Reproduction of Survey**

- 1. Do you know what antibiotics are? □ Yes □ No
- 2. If you answered yes to the previous question, please give as complete a definition as possible:
- Do you have personal or religious beliefs that discourage the use of antibiotics?
   Yes No
- Have you taken antibiotics in the last year? (including the use of topical antibiotics)
   Yes □ No □ I don't know
- 5. If you answered yes to the previous question, how many separate times over the last year have you taken antibiotics?
  1 time 2-3 times 4-5 times more than 5 times
- 6. If you answered yes to the previous question, what illnesses and/or symptoms were these antibiotics used to treat?
- 7. Have you been prescribed antibiotics in the last year? ☐ Yes ☐ No ☐ I don't know
- 8. If you answered yes to the previous question, were the prescriptions filled?
   Yes □ No □ I don't know
- If you answered yes to the previous question, did you take the entire prescription?
   Yes No I don't know
- 10. If you answered no to the previous question, why did you not take the entire prescription?
  - $\Box$  allergic or adverse reaction  $\Box$  incorrect prescription
  - ☐ felt better before finishing the full prescription ☐ other:
- 11. Have you ever wanted to be prescribed an antibiotic, but were advised not to by a physician, healthcare provider, or pharmacist?
  Yes No
- 12. If you answered yes to the previous question,a) what symptoms did you want treated?b) why were you advised not to?
- 13. Has a physician, healthcare provider, or pharmacist ever recommended that you take an antibiotic, but you did not agree/want to?
  Yes No

14. If you answered yes to the previous question,
a) what symptoms were you experiencing?
b) why did you not want to take the antibiotic?
c) why were you advised to?

- 15. Have you read an article or watched a news report about antibiotics or antibiotic resistance in the last year?
  ☐ Yes ☐ No
- 16. If you answered yes to the previous question,
  a) what form of media was it? (check all that apply)
  television and radio newspaper website/internet
  scientific journal other
- 17. b) what was the information about?
- 18. Has your use of antibiotics changed in your lifetime, and has your understanding of the purpose and use of antibiotics, and the meaning of antibiotic resistance changed?
- 19. Taking an antibiotic for the flu is a good way to feel better. □ True □ False □ I don't know
- 20. Strep throat needs to be treated with antibiotics. □ True □ False □ I don't know
- 21. Antibiotics cannot treat viral infections. □ True □ False □ I don't know
- 22. If you have an unused prescription or partially finished prescription, you should save it and use it if you ever experience similar symptoms.
  True False I don't know
- 23. If you feel better midway through taking a prescription and your symptoms have gone away, you should stop taking it.
  True False I don't know
- 24. Antibiotic resistance can stem from taking antibiotics frequently. □ True □ False □ I don't know
- 25. What is your age?
- 26. Gender?
- 27. Are you in a medical/health services field? □ Yes □ No
- 28. If you answered yes to the previous question, what is your position?

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## Do Binaural Beats Enhance Performance?

By David Colon

#### Abstract

his research paper contains the results of an experiment testing whether or not alpha binaural beats enhance students' performance on a "chess test". The hypothesis was that binaural beats would aid the student in achieving a higher score and thus provide evidence that binaural beats are relatively effective in combatting stress. The results do not support this hypothesis since there was no significant evidence of improved results on the chess test in the presence of the binaural beats. On the other hand, students showed a significant difference (p-value=.002896) in their score the second time taking the test compared to their first time taking the test indicating that practice, rather than binaural beats, improves results. The binaural beats might have even been a hindrance for the subjects as multiple subjects said that the noise distracted them.

#### Introduction

Throughout the past decade scientists are doing more research on the brain to better understand how it works. This curiosity along with better equipment has resulted in new avenues of neurology that are now being explored. One of these new areas of research concerns brainwaves and its connection to humans' daily activities. To understand what these brainwaves are you must first know a little more about how the brain works.

The brain is made up of billions of neurons. Each day these neurons are continuously active sending and receiving electrical signals so that the brain can effectively and efficiently process information. Due to this, scientists have figured out that there are electrical patterns in the brain from these neurons and have been able to pick up these patterns using an electroencephalogram, or EEG, which records electrical activity in the brain. Brainwaves are these natural electrical patterns in the brain. There are four main types of brainwaves: beta, alpha, theta, and delta waves, and each type is associated with certain activities. The beta rhythm occurs when humans are active throughout the day, such as when people are playing sports, walking, or doing a project in a team. The alpha rhythm is generally associated with relaxation and meditation. The theta rhythm happens when someone is in light sleep, also called REM sleep. Finally, the delta wave is associated with deep sleep, when your body and brain are least active and are resting. Each of these brainwaves occurs at a certain frequen-

cy, which is basically how active your brain is. In the beta range, the frequency is higher, 13-39 Hz, than the delta range, <4 Hz, because your neurons are more active sending signals than while when you are in deep sleep, which is when the brain is nearly at rest. The alpha range is from 8-13 Hz and the theta range is from 4-8 Hz. This study focuses more on beta and alpha brainwaves through the use of brainwave entrainment.

Brainwave entrainment is the process in which scientists are able to cause someone's brainwaves to shift to a certain frequency. While there are many types of brainwave entrainment, this experiment concerns auditory entrainment (AE), which is entrainment through sound, such as through the use of monaural beats, isochronic tones, and binaural beats. As mentioned above, this experiment uses binaural beats.

Binaural beats are beats that are able to induce brainwave entrainment both safely and effectively. They are generally a novel idea and as of now only work through the use of headphones. Each headphone produces a different frequency into each ear and the difference between those two frequencies is the frequency to which someone's brain will be entrained. For example, if one headphone has a frequency of 150 Hz and the other headphone a frequency of 160 Hz, then the brain will be entrained to a frequency of 10 Hz, the difference between the two frequencies. Since 10 Hz is in the alpha range (8-13 Hz) the subject will start to relax.

This paper capitalizes on this new wealth of information by creating an experiment to test whether or not these binaural beats are actually effective. The experiment determines whether or not the beats work by seeing if students who know how to play chess can score higher on a timed "chess test" when listening to alpha binaural beats than when those same students listen to nothing. This follows the path of similar tests being done to test the effectiveness of binaural beats, such as the test of beta binaural beats on people with ADHD to help improve their focus.

The reason this experiment uses alpha binaural beats is because of the effects they will produce. As mentioned before, the alpha range puts people into a relaxed state. Students will take the "chess test" after school so they will presumably be tired or stressed out. The alpha binaural beats will allow students to relax again and better concentrate thus helping student's attention span and focus. Since chess requires a certain amount of mental skill the hypothesis of the study is that students who do not listen to the alpha binaural beats will perform worse on the "chess test" than when listening to alpha binaural beats. If the hypothesis had been supported by the data this experiment could be taken as promising evidence in favor of expanding the use of binaural beats to the point where they could be seen as a valid therapeutic procedure for those with test anxiety or for people under extremely stressful situations.

#### Method

#### **CHESS TEST**

The test involves participants solving as many "chess problems" as possible in 20 minutes. A chess problem will be a situation in chess in which you can checkmate someone in a certain amount of moves. Gameknot is a website that gives out these chess problems as defined above. They also have ratings to show the difficulty of each chess problem. For this experiment there were only be one-star difficulty problems to try and avoid any bias for skill. There were more problems on the test than are possible to solve so no subject completes the entire test before time expires.

#### SESSIONS

Participants will come in for two sessions. In the first session half of them took the first "chess test" while listening to binaural beats while the other half listened to nothing. The students came in for a second session where they did the second "chess test". The group of students that listened to nothing the first session listened to the binaural beats for the second session. The group of students that listened to the binaural beats the first session did not listen to anything the second session.

The experiment used students who know how to play chess already. Whether or not the subjects know how to play the game will be determined by their knowledge of the rules of the game and how the pieces move. If they know the basic rules of the game then they know how to play chess.

Participants were asked to fill out a survey after each test concerning how they thought they did on the test and the noises that they heard during the test. The students listened to the same binaural beats.

#### **Results**

#### Diagram 1

Subject	Number Correct With Binaural Beats	Number Correct Without Binaural Beats
1	4	6
2	0	3
3	9	7
4	8	6
Average	5.25	5.5

#### Subjects Listening to Binaural Beats vs. Nothing

# Subjects Listening to Binaural Beats Compared to When Listening to Nothing



Diagram 3

#### When Subjects Took the Test the First and Second Time

Subject	Number Correct the First Time Taking the Test	Number Correct the Second Time Taking the Test
1	4	6
2	0	3
3	7	9
4	6	8
Average	4.25	6.5

#### Diagram 4

#### First Time Compared to the Second Time They Took It



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#### Discussions

#### DATA ANALYSIS

The results of the data overwhelmingly show that there is no statistical evidence that binaural beats improve results on the chess test. The average number of puzzles correct (5.25 for the subjects when listening to binaural beats, 5.5 for the subjects when listening to nothing) clearly show that the binaural beats did not enhance students' ability. Both Subject 1 and Subject 2 both performed worse when listening to the binaural beats while Subjects 3 and 4 both did better. Results were analyzed by way of a correlated two tailed t-test, which is a comparison of means (assisted by the website vassarstats.net). The p-value, which shows the probability that your data could not have happened by chance, should be less than 0.05 to suggest a significant result. The t-test results in a p-value of 0.861439. Indeed, in the questionnaire answered by the subjects after they took the test showed that most of them thought the binaural beat noise was "distracting" or "annoying". This means the binaural beats could have possibly even negatively affected the students when they were taking the test.

In fact, when looking at the data it seems that there was actually a correlation between the first attempt at the "chess test" and the second attempt. The subjects definitely performed better the second time they took the test since they all solved more puzzles than when they first took it. When doing a t-test taking comparing the number of puzzles solved from the first time the subjects took the test and the second time there is actually a significant increase (p=.002896). This is interesting as it seems that the first time the students were getting accustomed to doing this "chess test", while during the second session they all felt a lot more comfortable and were able to complete more of the problems.

#### **EXPERIMENTAL ANALYSIS**

When looking back at the study there are clearly a number of things that could be improved to make the study more reliable and accurate. A bigger sample size is definitely necessary to make any type of correlation with the binaural beats. This study only had four people and they were all males around 15 or 16, but having a group of over 15 with both genders and different age groups seems like it would yield more conclusive data. Another flaw in this experiment is that not all the kids in the experiment had the same skill when it came to chess so people who were better could have solved more skewing the data. For a future test, getting kids who all have the same chess rating could help make the data more reliable. Or another test could be run in future experiments instead of a "chess test" so that skill level of the subjects does not play a result in the data. Since the data did show that people got used to taking the "chess test" by the second time and performed better, future experiments could let students take one practice "chess test" and then follow up with a second and third session where they already know how to take the test. This will mean that the data will be more accurate as people have already adjusted to taking the "chess test" and higher scores will be based off the binaural beats. Another potential flaw in this study is that there is not enough incentive to do as well as you can on this test, which is necessary to create stress. The binaural beats are suppose to ease that stress, but if there is no incentive the subjects will not care if they do not perform to the best of their ability. This pilot test is a preliminary test that shows the potential to show conclusive data with more resources used as well as tweaks to the design of the experiment.

#### ACKNOWLEDGEMENTS

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# Battling Red Tides: The Potential Effects of *Artemia Salina* Age on Algae Population Growth

By Caleb Gordon

A Red tide Harmful Algal Bloom off the Coast of La Jolla in California

#### Introduction

Igae are confusing, and harder to kill than you would expect. Evolutionary biologists have trouble classifying algae. They best define them as an aquatic group of planktonic, oxygenating, photosynthetic organisms (Andersen 1992). In other words, all algae drift by the water's surface, produce oxygen as they respire, and photosynthesize for food. Incredibly diverse, algae are polyphyletic, meaning that they don't fit neatly into any one taxonomic group, because the term "alga" predates effective phylogenetic classification (Andersen 1992).

Algae have inhabited this planet for billions of years—since the first cyanobacteria oxygenated our atmosphere—and remain key ecological players today ("About Algae" 2014). Algae absorb atmospheric carbon dioxide, oxygenate the planet, and form the basis of *all* marine food webs (Gilbert et al. 2005; "About Algae" 2014; Andersen 1992). Algae are thus pivotal parts of all aquatic habitats.

However, algae may also become *invasive* to their environment. Populations of algae that vitiate their surrounding habitats are called Harmful Algal Blooms, or HABs. Algae can become harmful in three main ways: they may produce toxins, possess harmful morphological features like spines or quills, or proliferate so much that their sheer biomass leads them to block sunlight from subaquatic vegetation and/or cause water anoxia (Gilbert et al. 2005). If a population of algae exhibits any or all of these characteristics, it is classified as a Harmful Algal Bloom. HABs are often referred to as "red tides," because many HABs caused by red dinoflagellate algae appear as red stripes or pools in the water, but HABs can come in a wide spectrum of colors, depending on the species (Gilbert et al. 2005).

These harmful algae wreak havoc upon the world. With their spines or other deadly physical features, HABs cripple fish and other macroscopic organisms (Gilbert et al. 2005). In their unchecked proliferation, they deoxygenate the water and degrade its nutrient content



A magnified image of Volvox Green Algae Cells

(Gilbert et al. 2005). With their toxins, they kill millions of fish, and thousands of birds and marine mammals every year (Bibak & Hosseini 2013; Gilbert et al. 2005; Moore et al. 2008). Their variety of cytotoxins, neurotoxins, dermatoxins and so on cause a plethora of morphological and developmental problems in multiple fish species, but their effects aren't limited to the sea (Gobler et al. 2008). These toxins bioaccumulate up the marine food chain, and reach us through our seafood, sickening over 60,000 people every year and killing an average of about 900 (Gilbert et al. 2005; Moore et al. 2008). If the destruction of

vertebrate and human life weren't intrinsically enough, these direct effects of HABs have a variety of awful environmental and economic consequences (Marcoval et al. 2013). In addition to devastating aquatic habitats and marine food chains across the globe, the deleterious impacts of HABs cause about \$82 million dollars of damage annually in the United States alone, and may cause hundreds of millions around the world—when the medical insurance costs, reaped tourism profits and food stocks, and other expenses are taken into account (Wines 2013; Bibak & Hosseini 2013; *Economic Impacts of Harmful Algal Blooms (HABs) 2012*).

To make matters worse, the severity and range of HAB proliferation has been growing worldwide for decades (Moore et al. 2008; Gilbert et al. 2005). Many ecologists have attributed this trend to a variety of unfortunate human activities: nutrient runoff from aquatic and terrestrial farms, human overconsumption of natural grazers, the human-facilitated dissemination of invasive algal species (through ballast water and other dispersal mechanisms), global climate change and general habitat degradation all serve to augment the threat of HABs across the globe, so that the crisis only worsens (Moore et al. 2008; Baskin 2012; Smith 2013).

**Recreation and Tourism Impacts** 

**Coastal Monitoring & Management** 

Of course, scientists, organizations and governments have developed various ways of combating HAB proliferation, but these actions have had little overarching success. HAB control methods include mechanical, chemical, and biological con-

Commercial Fisheries Impact	\$38 million per year
Public Health Costs of Illness	\$37 million per year

\$4 million per year

\$3 million per year

Economic effects of HAB's in the U.S. are at least \$82 million per year.

*2005 dollars, Hoagland and Scatasta (2006)	). Based	on s	ubst of	outbreaks in	1987-20	00.
(From NCCOS)						

trol (Bibak & Hosseini 2013; Park et al. 2012).

Mechanical control includes the use of abiotic factors such as filtering systems, other infrastructure or manual skimming to remove HABs from certain areas (Bibak & Hosseini 2013). Unfortunately, mechanical control is incredibly expensive in the long run, and can have deleterious impacts on the surrounding environment (Bibak & Hosseini 2013; Park et al. 2012).

Chemical control is the use of chemical agents to mitigate blooms (Bibak & Hosseini 2013). While at times effective, these compounds are non-specific and thus hazardous to the surrounding environment, and must be constantly reapplied, so that they're not only harmful but also costly (Sigee 1999; Park et al. 2012).

Biological control agents include



Acartia tonsa adult (above); Artemia salina adult (below)

organisms that consume or kill harmful algae (Bibak & Hosseini 2013). These generally include pathogens like viruses, bacteria and parasites, and macroscopic grazers like copepods that consume algae (Sigee 1999).

As I studied these three different methods in tandem, biological control seemed to me the most promising, as it has fewer reported negative externalities and a higher potential long term payoff. Because of algae's evolutionary resilience, most harmful populations tend to evolve too quickly in response to microscopic biological agents for these parasites to stymie blooms (Andersen 1992; Gilbert et al. 2005; Sigee 1999). As a result, I've chosen to focus on *macroscopic* predators of algae; organisms that consume algae for food are called grazers. Macroscopic grazers provide a selective pressure against which I had speculated harmful algae couldn't as effectively adapt. I've searched since then for an *ideal* grazer that could theoretically consume all harmful algal species, undeterred.

Different studies have reported the potential of a variety of different grazers, from tilapia fish to heterotrophic protozoa (Bibak & Hosseini 2013; Campbell 2012), but from what I've read, the most promising grazers seem to be branchiopods and copepods. Copepods are tiny crustaceans, typically only a few millimeters in length (Walter & Boxshall 2014). They include the 13,000 species comprising the taxonomic subclass Copepoda (Boxshall & Defaye 2008).

One copepod grazer that showed promise was the calanoid *Acartia tonsa*, which The National Centers for Coastal Ocean Science reported was immune to many of the deadly HAB cytotoxins that deter other potential grazers ("Grazers Avoid Toxic Algae" 2012).

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However, one study conducted by Dr. Xiaodong Jiang, a professor at the School of Life Sciences at East China Normal University, found that *A. tonsa* populations ultimately reach 100% mortality when faced with greater concentrations of the harmful dinoflagellate, *Cochlodinium polykrikoides* (Jiang et al. 2009). Among the deadliest of harmful algae, *Cochlodinium polykrikoides* (Jiang et al. 2009). Among the deadliest of harmful algae, *Cochlodinium polykrikoides* is a motile twin-tailed, chain-forming, predatory mixotroph that produces a variety of harmful ichthyotoxins and neurotoxins (Jiang et al. 2009). *C. polykrikoides* thrives across Asian and North American Coasts, and has spread into Long Island, New York within the last decade (Jiang et al. 2009; Gobler et al. 2013). Despite the formidability of this red tide alga, *A. tonsa* can effectively graze *C. polykrikoides* at lower cell densities, and thus serves to prevent some blooms from emerging (Jiang et al. 2009). However, Dr. Jiang found that, at higher concentrations, the toxins of *C. polykrikoides* populations lowered copepod ingestion rates of *C. polykrikoides* and inhibited copepod growth and fecundity (Jiang et al. 2009). At bloom densities, the algae caused 100% mortality of its *A. tonsa* grazers (Jiang et al. 2009). From these findings, it's evident that *A. tonsa* falls short as a grazer.

However, a follow-up experiment conducted by researcher Dr. Alejandra Marcoval (a prolific researcher at the Departamento de Ciencieas Marinas at the Universidad Nacional de Mar del Plata) and Dr. Christopher Gobler (a professor at the School of Marine and Atmospheric Sciences at Stony Brook University) found a new potential grazer: the branchiopod, *Artemia salina*. Commonly known as brine shrimp, *A. salina* are copepod-like organisms whose wide salinity and temperature ranges (see Dumitrascu 2011) suggest that they could be practical potential grazers (Marcoval et al. 2013). Of course, the real test is how effectively they consume harmful algae at higher cell densities. In a set of experiments, Dr. Marcoval and Dr. Gobler compared the algicidal efficacies of *Acartia tonsa* and *Artemia Salina* on three strands of harmful algae, including *Cochlodinium Polykrikoides*. The researchers tested the

grazers against different concentrations of the 3 harmful algae species, and estimated clearance rates and grazer mortality over a period of 24 hours (Marcoval et al. 2013). The researchers ultimately found that A. salina was a better grazer than A. tonsa. A. salina could still graze at very high algae concentrations, while A. tonsa grazing slowed and then stopped (and mortality rose) as concentrations increased (Marcoval et al. 2013). The researchers propose that A. salina should be strongly considered as a biological control method

#### Graph 1

A graphical analysis of grazing efficacy. This graph shows how quickly A. tonsa and A. salina consumed C. polykrikoides at different cell densities. Clearance rates (mean ± SD).



against HAB's in the future (Marcoval et al. 2013). As it's cheap, easy to hatch, tolerant of various environmental conditions and capable against multiple strains of harmful algae, A. salina now seems to have the greatest algicidal potential of any grazer I've read about so far. Thus, I knew that I would assess the algicidal efficacy of A. salina. From this research I planned to conduct an experiment that would test the effect of branchiopod age on C. polykrikoides population growth, to see whether A. salina size and development plays a role in its grazing efficacy. However, I never could have begun without any experience in a real lab setting.

#### Graph 2

This graph shows the percentage of A. tonsa and A. salina that died off when exposed to different densities.

From Marcoval et al. 2013, p.239.

Mortality (%) of Arcartia tonsa and Artemia salina estimated over 24 hrs when fed Cochiodinium polykrikoides, clone CP1.



🗖 A. Salina 📃 A. Tonsa

In June 2013, I interned at Dr. Gobler's lab in Stonybrook, South Hampton, Monday through Friday from the 17th to the 28th, from 10:00 to 5:00. I came into the lab expecting to be the honorary Gobler dishwasher, but thanks to an incredibly welcoming laboratory crew, I had the opportunity to observe and/or take part in a variety of projects, both in and out of the lab. While there was, admittedly, a lot of beaker cleaning, material transportation and clam poop-scooping, I helped collect, count, analyze, transport, inoculate and establish various different cultures (of different HAB species as well as scallop larvae and T-ISO). I also observed two lab staff meetings, went on three boat expeditions, assorted and organized various chemical samples, carried out important preparatory procedures. Perhaps most importantly, I had the great opportunity of helping doctorate student Alexander Mintz build his own special algal culture tank for his experiment about the nitrogen consumption of Ulva, a generally

Mortality (%)

harmless green alga. I learned how he gathered and prepared the algae, and how he would quantify and measure his results.

In addition to helping Alex Mintz prepare for his nitrogen consumption experiment, I helped doctorate student Andy Griffith conduct an exposure of C. polykrikoides to scallop larvae. In six different buckets, we inserted specific volumes of scallop larvae, T-ISO (to serve as food for the larvae), and C. polykrikoides. Andy would later assess the impact of the HAB on larvae growth and fecundity. This taught



The Gobler Lab

me how exposures (using multiple different species of organisms) work.

These were only two of many projects that provided great insights into how to inoculate, analyze, maintain and expose algal cultures. I just describe these in detail because they most influenced how I'd like to conduct my own experiment.

I've stayed in contact with Mr. Griffith and Dr. Gobler, and with their help and that of many others working at the lab, I devised my own experiment, which I began this past January at the Berkeley Carroll School 181 Lincoln campus.



The apparatus Alex Mintz designed for his experiment on Ulva nitrogen consumption.

The experiment sought to test the algicidal efficacy of *A. salina* of different ages, to see if the grazers are better equipped at certain stages in their life cycle, or if relative size has an impact on their algae consumption (since older grazers are also larger and longer). The experiment recently failed, and while I have started again, I will not finish it in time to publish the results in this journal. I hope to present my findings at the third annual Scientific Research and Design symposium this April.

In this paper I will discuss my attempt thus far at an experiment on *A. salina* grazing efficacy, provide an explanation of why it went wrong, propose the ideal protocol for my experiment, and do a preliminary analysis on some potential results and their implications.

#### **Materials/Methods**

First, I will explain the general design of my experiment. (Note that I discuss the basic experimental procedure in the future tense because I haven't yet begun it. So far I have set up an apparatus and prepared starter cultures, but the cultures aren't yet ready to be exposed to one another.) To test the effect of *Artemia salina* age on algae consumption, I plan to expose

*A. salina* of different ages to *C. polykrikoides* cultures and calculate how much each *A. salina* age group hinders algae population growth. I will inoculate twelve total *C. polykrikoides* cultures, comprised of four different groups, each with three replicate cultures (to minimize random error). Group A (including three cultures) will serve as a control, and be left to grow without being exposed to *A. salina*. Group B will be exposed to 2-day old *Artemia*, Group C to 4-day old *A. salina*. To sum up, I will have one



The set-up of Andy Griffith's grazer exposure experiment.

control group (Group A) and three experimental groups (Groups B, C and D). Each group will have three replicates so that I'll have a total of twelve *C. polykrikoides* cultures. I will inoculate and maintain all 12 cultures in 50 mL of 32.5 ppt saline GSe media solution contained in 100 mL Earl & Meyer Flasks.

I will grow three different cultures of A. salina (one 2-day old, one 6-day old, and one 8-day old) and expose 12 A. salina from each group to their respective C. polykrikoides culture group at the same time. The algae cultures should ideally all be at cell densities of 1000 cells/mL during the exposure, to mimic near-bloom densities. From here on, over a period of 48 hours, I will calculate and compare algae population growth in each group. To do this, I will do a cell count for every algae culture, using a standard light microscope and a Sedgwick Rafter counting slide. I will count cells from three different 1 mL samples of each culture flask, and average my findings to obtain an average cell concentration per milliliter for each sample. I will then multiply this cell concentration by the total volume of the culture to obtain a reliable value for the cell density of each culture. After this, I will average the cell densities of the replicates of each group, to find the average cell density of each culture group. I'll perform this cell counting procedure every 3 hours for the first 12 hours, and then every 8 hours for the last 12. I'm finding cell density because it will let me assess algae population growth. As a population of algae grows within a fixed volume of water, its cell density (the physical number of algae cells per milliliter) increases. For example, if in 3 hours the cell density of a cultured population of algae doubled, this would indicate that its population doubled in size, and relative cell density thus indicates relative population growth. In short, by conducting cell counts every few hours, I'll be able to compare the average population growth of each C. polykrikoides group over time.

During cell counts, I will also keep tracks of any chains formed among algae cells. *C. polykrikoides* cells have been known to form chains, and (as I'll discuss in my conclusion) chain formation can impact the performance of certain grazers, and make them less capable of consuming algae cells. Thus, I will record the number and size of *C. polykrikoides* chains in each culture during each cell count, and obtain an average for each group.

In this experiment, I'm trying to figure out whether or not grazers of different ages consume algae at different efficacies. A control method's 'algicidal efficacy' refers to how well it kills algae. I'll use algae population growth as a sort of proxy for how effectively grazers consume the algae. If one age set of grazers is especially effective, the group of algae it's exposed to won't exhibit as much population growth as a group exposed to a less effective age set. Grazers are a selective pressure, a density-dependent limiting factor on algae population growth, so less effective grazing means more algae growth, and more effective grazing means less algae growth. Group A, the control culture of algae, should exhibit the more population growth than the experimental cultures, since its population won't face the selective pressure of grazing at all.

So, in short, to test the algicidal efficacy of differently-aged *Artemia* on *C. polykrikoides*, I'm exposing 3 different age sets of *Artemia* to different groups of algae cultures. By counting cells every few hours, I'll calculate the relative population growth of each algae group, and compare them to see which populations grew the most and which

grew the least. Any discrepancies in population growth among the experimental cultures might have significant implications, which I'll discuss in my Conclusion/Analysis section.

Artemia salina and Cochlodinium polykrikoides cultures require a lot to be successfully grown and maintained. Ideally, I would replicate most of the culture conditions followed by Dr. Marcoval and Dr. Gobler in their experiment comparing the algicidal capabilities of *A. salina* and *A. tonsa.* The algae cultures



Artemia salina swimming

would be vigorously oxygenated, maintained at a constant  $21^{\circ}$  C, and evenly exposed to fluorescent lights with an intensity of ~100 µmol quanta m-2/s for a 12 – 12 hour light-dark cycle (Marcoval et al. 2013). In addition, all culture beakers would be autoclaved (and thus sterilized), and I would add 1 mL of antibiotics to each flask, before they were inoculated with culture.

With regards to the *A. salina* cultures, they would ideally be fed a volume of T-ISO (microalgae) equivalent to the biovolume of *C. polykrikoides* at a cell density of 2000 cells/mL every few hours, though I have yet to verify precisely how often to feed them to achieve the best results. A day before being exposed to algae, the *A. salina* would be weaned off of T-ISO, so that each brine shrimp culture would be ready for a new food source. Before exposure, each *A. salina* culture will be filtered through a .2 micron sieve, to remove any T-ISO remaining within the culture, and 12 *Artemia* will be exposed to each algae culture flask. To keep conditions constant for the *Artemia* populations, they should ideally be maintained in the same lighting, temperature, and salinity conditions as the algae, prior to exposure. These would be the ideal conditions for my experiment, but I'll now explain what I've done so far.

On Monday, December 30th, I went to the Gobler Lab to pick up algae and T-ISO cultures. I purchased aquarium tubing, splitters and a pump from Petco. The school purchased a Sedgwick Rafter for conducting cell counts.

I grew experimental and control cultures of algae from a *Cochlodinium polykrikoides* starter culture inoculated on Sunday, December 29th at the Gobler Lab in Stony Brook, South Hampton. The culture was initially contained in a sealed plastic container. I obtained this culture and drove it to my house on Monday, December 30th. The next day at school, I transferred this culture to a 1000 mL Erlenmeyer flask. The culture itself was comprised of 240 mL of GSe Media and 60 mL of culture.

The entire experiment is taking place in the Chemistry Lab at Berkeley Carroll. All other culture flasks in this experiment were cleansed with ethanol and distilled water three times thoroughly before they were inoculated with cultures. All culture flasks (*A. salina* and algae alike) are being rigorously oxygenated by an aquarium bubbling apparatus involving an

oxygen pump and plastic tubing from Petco and several 2 ml serologic pipettes. We have not yet obtained the necessary grow light for the algae, but hope to soon. So far the culture set obtains about 12 hours of light from incandescent bulbs about 9 feet above them, which isn't sufficient.

Some algae began to grow the first week following the inoculation of the starter culture, but perhaps because of temperature fluctuations or lack of sufficient light, the starter culture seems more or less barren now. The Chemistry Lab fluctuates somewhat sporadically between 20° and 24°C, so the algae aren't being grown at a constant 21°C. In addition, they don't experience an ideal 12-12 hour light-dark cycle. The lights in the Chemistry Lab currently go on at around 8:30 AM, and likely go off at any time from 4:30 to 9:30 PM, depending on the day. Likely due to these shortcomings, the culture remains pretty desolate. A single dark brown blotch (about 5 cm in diameter) has formed near the center of the flask, but I have yet to confirm whether this is *C. polykrikoides* or some invasive bacterial colony.

The school ordered Brine Shrimp (*Artemia salina*) shell free EZ eggs from Brine Shrimp Direct. I created the first *Artemia* culture on January 29th with a fifth of one scoop of shell-free eggs. These shrimp were hatched in a 50 mL of 32.5 ppt saline solution in a 100 mL Erlenmeyer flask. They didn't seem to hatch within 24 hours as they were supposed to (likely because they were hatched under my experimental conditions, which are hyper-saline compared to their ideal hatching conditions, and because they didn't have enough light). These brine shrimp seemed dead, but on February 5th, 7 days after the *A. salina* batch was prepared, it became evident that the brine shrimp were actually thriving. Since I had presumed they were dead and not fed them, they must have gone cannibal to survive, eating either their dead kin, who failed to hatch, or their smaller living kin. This presents a problem because it essentially exposes them to no external food source, and may make them less inclined to eat algae (and perhaps more inclined to eat one another) once they're exposed to algae.

I inoculated a second culture of *Artemia* on February 5th, with about 2 mL of eggs, but as I have yet to verify the precise frequency at which I should feed them, I believe that both of my two prevailing *A. salina* cultures have been exposed to too many confounding variables to use in my experiment.

Because of these errors with the brine shrimp and *C. polykrikoides* cultures, I haven't been able to begin my experimental exposures. The algae starter culture that I obtained from the Gobler Lab hasn't yet grown enough (lacking the light and stable temperature) for me to use it to inoculate 12 new *C. polykrikoides* cultures. And, of course, my brine shrimp thus far can't be exposed as they've been raised as cannibals, and this unfortunate turn of events would have them skew results if used in the experiment. In addition, for my first two *A. salina* starter cultures, I hatched a huge and uncounted number of eggs which prevented me from calculating how much to feed them. I want to keep the amount of food the *Artemia* receive constant throughout their lifespan, to prevent a different availability of food from somehow skewing results.

Because my previous *A. salina* cultures didn't work out, I will inoculate new cultures, each with exactly 36 eggs, and give the culture exactly three times the amount of food that

the 12 brine shrimp exposed to the algae would have at their disposal. I will inoculate the first of these new cultures on Tuesday, March 4th.

I will expose these new grazer starter cultures, and the *C. polykrikoides* starter culture to a grow lamp that the Scientific Research and Design program has recently ordered. It won't deliver exactly 100 µmol quanta m-2/s, but it should provide all of the energy needed for the algae to grow quickly. This light will be hooked up to a timer, and provide the 12-12



The initial set up for my C. polykrikoides starter culture.

hour light-dark cycle used in Dr. Marcoval's experiment.

Again, though preparations continue, the experiment itself has yet to officially commence, so the results could not be printed in this journal. Regardless, the following section discusses the basic results possible and what their causes and implications might be.

#### **Potential Results and Analysis**

The results of this experiment could demonstrate one of three major trends: a negative correlation between grazer age and algae population growth, a positive correlation, or no correlation at all. I'll now discuss what each of these trends could suggest, what the implications of each result would be, and what potential confounding variables might have skewed them.

A negative correlation between *A. salina* age and algae population growth would suggest that older, more developed *A. salina* graze *C. polykrikoides* more effectively. The results would show a general negative trend only if *C. polykrikoides* populations grow most in Group A (exposed to no *A. salina*), second most in Group B (exposed to 2-day old *A. salina*), third most in Group C (exposed to 4-day old *A. salina*), and least in Group D (exposed to 8-day *A. salina*). In this case, the older the *A. salina* it was exposed to, the more rapidly the *C. polykrikoides* was grazed.

A negative correlation could have multiple causes. It could, for one, mirror the positive correlation between *A. salina* age and body length, or jaw size. This would fall in line with the Size-Efficiency Hypothesis, which researchers John Brooks and Stanley Dodson proposed in 1965. The hypothesis argued that zooplankton grazers (including branchiopods like *A. salina*) compete for suspended consumable matter (ranging from 1 to 15  $\mu$ ), and that "Larger zooplankters do so more efficiently and can also take larger particles," subsequently outcompeting any smaller rivals (Brooks & Dodson 1965, p. 31). A negative correlation between *A. salina* age and *C. polykrikoides* population growth could thus fit the Size-Efficiency Hypothesis: the older *A. salina* were larger, with larger jaws and larger appetites, and thus grazed the algae more efficiently than their younger counterparts did.

A negative correlation could also be attributed to the varied responses of different age groups to *C. polykrikoides* chain formation. An experiment conducted by Dr. Gobler and others in 2010 found that *C. polykrikoides* had developed an additional, behavioral advantage against the copepod grazer *Acartia tonsa*, known as 'chain formation' (Jiang, Lonsdale & Gobler 2010). In response to *A. tonsa* exudates, *C. polykrikoides* cells formed an abnormal frequency of



A 4-cell chain of Cochlodinium polykrikoides

chains. In other words, when they detected grazers, the algae cells started linking togetherand, as a result, grazer consumption declined. The researchers speculated that chain formation creates a "prey-predator size mismatch" in which, in chained form, the algal prey can greatly exceed the A. tonsa's ideal food size (Jiang, Lonsdale & Gobler 2010, p. 460). Fewer algae were consumed because of this predator-prey mismatch (Jiang, Lonsdale & Gobler 2010). This makes chain formation a behavioral defense against grazing at all cell densities, which might then hinder the biological control of algae populations that haven't yet reached bloom density (Jiang, Lonsdale & Gobler 2010). No experiments have yet tested whether C. polykrikoides cells form chains in the presence of A. salina, but it seems plausible that C. polykrikoides could quickly evolve to do so against A. salina as it did against its local predator, A. tonsa. If chain formation is prevalent among culture populations, it could explain any negative correlation between branchiopod age and algae growth. Larger branchiopods should theoretically consume larger prey, according to the Size-Efficiency Hypothesis, and older brine shrimp are larger (Brooks & Dodson 1965); thus, the older shrimp should be less phased by the predator-prey mismatch imposed by chain formation and subsequently consume C. *polykrikoides* at higher rates, leading to less algae population growth.

The negative correlation might have less to do with *A. salina* size, however, than the morphological differences that come with development. *A. salina* undergo a variety of subtle morphological changes as they grow. To highlight one key change, juvenile brine shrimp (nauplii) have a single eye, called a photoreceptor, while adults have three eyes (Dumitrascu 2011), and this—among other differences—can cause *A. salina* of different stages in their development to perform differently as grazers. For example, due to their single photoreceptor, *A. salina* nauplii are phototactic—swimming toward light—while adults are not (Dumitrascu 2011). A negative correlation might indicate that *A. salina* nauplii are morphologically and [as a result] behaviorally less equipped to graze *C. polykrikoides* than their older counterparts. The morphological and behavioral disparities between different *A. salina* age groups could have an impact on algicidal efficacy equal to or greater than that of grazer body length.

A negative correlation between branchiopod age and C. polykrikoides population
growth seems to me the most probable result, considering the benefits that come with increased phytoplankton size according to the Size-Efficiency Hypothesis, as well as the benefits that come with an adult physiology. For example, phototaxis limits nauplii to move in certain patterns, while adults cover more ground as they lack that behavior. This is, of course, not taking into account potential confounding variables, which I will address at the end of this section.

A *positive* correlation between *A. salina* age and *C. polykrikoides* population growth would suggest that branchiopod grazing efficacy decreases with age. In a consistent negative trend, *C. polykrikoides* populations would still grow most in Group A (exposed to no *A. salina*), but second most in Group D (exposed to 8-day old *A. salina*), third most in Group C (exposed to 4-day old *A. salina*), and least in Group B (exposed to 2-day old *A. salina*). That is, younger grazers would eat *C. polykrikoides* more quickly than older grazers. A few things could account for this.

Older *A. salina* might require more energy and oxygen than younger *A. salina*, and thus encounter a greater shortage of required energy or nutrients. One study conducted by researcher Dr. Vidal on zooplankton physioecology supports this possibility to some degree. Dr. Vidal found that, at times, zooplankton net production efficiency—the amount of consumed matter converted into usable energy—decreased with body weight (Vidal 1980).



The Stages of Brine Shrimp Development

That is, larger zooplankton are less efficient in their use of energy, and thus require a much greater amount of food to sustain themselves. However, this might more likely yield a negative correlation, stimulating the older brine shrimp to eat more voraciously. That said, it could also lead the brine shrimp to consume one another, or die of some nutrition deficit, so that older shrimp would perform worse as grazers. This seems highly unlikely, though, considering *A. salina's* resilience as a species. For example, a greater demand for oxygen from larger branchiopods shouldn't impact results, as adult *Artemia* have been known to survive bouts of severe water hypoxia (Dumitrascu 2011).

A more likely explanation for a positive correlation would revolve around the morphological differences of *A. salina* at different stages of development. As I explained earlier, *A. salina* change considerably as they develop, and these morphological changes trigger behavioral changes that could easily impact their grazing efficacies. Just as these developments could improve grazer performance and cause a negative correlation, they could also hinder grazer performance in some way and thus create a positive correlation. To stick with a previous example, phototaxis might actually cause nauplii to graze more effectively than adults, by leading them to the greatest source of light—which would also likely contain the densest concentration of *C. polykrikoides* cells. It's a stretch, of course, but it's possible. Such morphological variations among grazer age groups could potentially cause a positive relationship between grazer age and algae population growth.

However, I find a positive correlation highly unlikely. The Size-Efficiency Hypothesis suggests that larger grazers are more effective predators, and the morphological developments that come with branchiopod age seem more likely to increase consumption rates than decrease them. In addition, a positive correlation would likely require that chain formation not be prevalent. As larger grazers can consume greater units of food, *C. polykrikoides* chain formation would obstruct consumption much less in an older group than in a younger one, and thus help to trigger a negative correlation, as explained above.

A partial positive or negative correlation—for example, a positive or negative correlation for just two of the three experimental groups—might imply that certain *A. salina* grazing benefits and hindrances outweigh one another differently at different ages. It might also merely be the work of extraneous variables. In addition, depending on the results, a partial positive or negative correlation might actually evince no correlation.

Lastly, there's the possibility that the results will show no correlation between *A. salina* age and *C. polykrikoides* population growth. In this case, results would be all over the place, and no population growth trend would emerge across age groups. Algae populations would probably still grow most in Group A, but there would be no statistically significant different in algae population growth among Groups B, C and D. Or, perhaps one or two of the experimental groups would show an especially elevated or mitigated population growth that doesn't follow a positive or negative trend.

First and foremost, this could show that age, body length and morphological development just have little to no impact on grazing efficacy, at least for *A. salina*. I find this unlikely, considering the significant changes in branchiopod behavior and energy usage that stem from age. That said, I concede that it could be difficult to identify a distinct correlation of any kind in my experiment, since the age range across my experimental groups isn't especially comprehensive, and the experiment itself will be short. Ideally, I would expose *C. polykrikoides* cultures to 2-day old, 4day old, 16-day, 32-day old and 64day old batches of *A. salina*, to get a fuller age range. It may be that 4-day old and 8-day old branchiopods are too similar, or lie within the same stage of development. If the ages I've



A magnified image of Diatoms, a type of algae.

selected don't translate to significant variations in morphology or body length then this could explain the lack of correlation.

However, it may also be that confounding variables precluded any potential distinct correlation. Even under the ideal conditions I listed above, bacteria could invade and possibly proliferate within my culture flasks or the tubes of my oxygenating apparatus. If bacteria colonies became invasive in the cultures of one group, say Group B, for example, this additional growth might skew results. It might hinder algal growth by stealing nutrients from the water, or being otherwise harmful to *C. polykrikoides* cells. Conversely, it might support algal growth by somehow harming *A. salina*, perhaps claiming nutrients vital to their survival, or becoming pathogenic or otherwise deleterious in some way. For the most part, I could probably remove extraneous bacterial growth in the future by autoclaving all culture flasks prior to inoculation, rinsing them with mild antibiotics, and sealing flask tops securely with Parafilm wrap.

Another confounding variable could be *A. salina* cannibalism. Though it seems highly unlikely, the *A. salina* of certain cultures might resort to cannibalism, particularly in the older cultures, if the food they're fed before I expose them to the algae doesn't satiate their appetites. Cannibalism is common among many crustaceans, and larger branchiopods have been known to devour their smaller counterparts when food is scarce (Thorp & Rogers 2010; Yúfera, Rodriguez & Lubián 1984). In addition, the first batch of brine shrimp that I hatched continue to thrive even though I have never fed them, and where several once swam now only three large brine shrimp remain. This may indicate that *A. salina* resort to cannibalism in the absence of sufficient food, or (less likely) that the remaining brine shrimp merely outcompeted their smaller competitors for food. Regardless, as unlikely as it is, if the *A. salina* of certain cultures resorted to cannibalism, it could skew results by reducing their consumption of algae. If cannibalism proved a major issue, one could reduce its prevalence by providing more food prior to exposure, and exposing *A. salina* to greater densities of *C. polykrikoides* to maintain an equivalent volume of food provided to the brine shrimp before and after exposure.

If a correlation does in fact emerge, and branchiopod age does turn out to impact grazing efficacy, it may help conservation efforts in assessing the dynamic between a grazer population and a local bloom. If, for example, certain environmental conditions or human actions incidentally killed off *A. salina* eggs but not adults, or perhaps adults but not eggs or nauplii, that could have a severe impact on how efficiently the *A. salina* population manages the local bloom in the short run.

In their study on the comparative algicidal efficacies of *A. salina* and *A. tonsa*, Dr. Marcoval and the others argue that *A. salina* should be employed as a grazer against *C. polykrikoides*, and various other harmful blooms (Marcoval et al. 2013). While harmful in a variety of ways, *C. polykrikoides* doesn't actually produce toxins lethal to humans (Marcoval et al. 2013). The researchers caution against using *A. salina* to mitigate blooms that produce saxitoxins, or other exudates that are harmful to humans, as it may cause these toxins to bioaccumulate up the food chain (Marcoval et al. 2013).

*A. salina* also isn't invincible: Dr. Marcoval and the others cite previous studies that show how the toxins of certain HAB species (including *P. parvum, Prorocentrum lima,* and several strains of *Alexandrium*) do harm *A. salina*. In other words, *A. salina* aren't immune to all HAB toxins.

Regardless, the researchers argue that *A. salina* holds significant potential as a biological control against HABs, since it is so easy to grow, "is known to be a nutritious source of food for multiple marine organisms," can tolerate a wide range of conditions, and consumes *C. polykrikoides* more effectively than perhaps any other grazer currently under study (Marcoval et al. 2013, p. 242). *A. salina* trumps any other grazer l've researched in its ability to consume *C. polykrikoides* undeterred, and shows promise against various other HAB species for the aforementioned reasons. It may, in fact, be as close as we can currently come to the ideal biological control against HABs.

However, we still shouldn't overestimate *A. salina*, even against *C. polykrikoides* and other algal blooms that it seems bound to vanquish. *Artemia salina* have been around for a mere 100 million years (Dumitrascu 2011), while algae have been around for nearly 3 billion ("About Algae" 2014). In particular, dinoflagellates like *C. polykrikoides* have been around for several hundred million (Macrae et al. 1996). Algae are incredibly diverse, and their rapid single-celled reproduction allows them to potentially reproduce at a greater rate than any macroscopic grazer. In terms of its 'evolutionary wisdom', zeal and potential, algae outmatch *A. salina* as a collective. Whether through natural selection or mutation, morphological or behavioral adaptation, algae may likely always remain one step ahead of macroscopic control agents like *Artemia salina*, regardless of how large, aggressive and resilient they are. Exposing zealous branchiopods or copepods to algal blooms might not have the overarching success we would hope for. We're pitting a squadron of Goliaths against an army of Davids, and we all know how that story ends.

That said, no one is advocating for a means to exterminate algae, but merely to prevent and mitigate harmful blooms. *A. salina* seems among the best candidates for an all-purpose macroscopic biological algicide. In addition, a better understanding of how branchiopod age affects grazing efficacy might inform conservation efforts by broadening our understanding of grazing dynamics for grazers at different stages of development. Hopefully this can help us better understand the intricacies of *Artemia salina* grazing as the search continues for something to battle the red tides.

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# Investigating the Relationship Between Sleep and Academic Performance

By Andrew Colon

# Abstract

e, humans, sleep every day of our lives. Sleep must be essential to homo sapiens' processes because it has not been filtered out over time. This article reports the findings of an observational study originally organized to answer the question: "How does the amount of sleep affect quality of schoolwork?" In this study, "quality of schoolwork" is measured by students' overall grades. The results not only shed light on the relation of sleep vs. grades, but also *time spent on homework (effort) vs. grades, study participation frequency (commitment) vs. grades, and study participation frequency (commitment) vs. sleep.* The study suggested that there was no correlation between any of the variables except possibly for the link between participation frequency and overall grades.

# Sleep: All That It Is Cracked Up To Be

Humans sleep one third of their entire lives. We zone out of consciousness the same way our species always has for hundreds of millions of years. Although the cognitive benefits of sleep and detriments of sleep deprivation have emerged in recent decades, we have always intuitively known sleep is good. But why? Why has the most intelligent and complex species on earth not evolved from its major Achilles heel? If we think to our species' most primitive days, sleep meant a dissolving of all senses that left early humans susceptible to predators. "It has to have a basic evolutionary function. Otherwise it would have been eliminated," says Maiken Nedergaard, leading Danish biologist and professor at University of Rochester. Before we speculate about other benefits of sleep, we must look at what the field already knows about the subject.

We know that sleep is key in the processing and retaining of memories and the upkeep of neuronal connections according to the journal article, "The Expense of Sleep". Studies have also shown that a lack of sleep weakens the immune system, slows down cognitive function and physical responses to stimuli, and causes mood volatility ("Effects of sleep deprivation on performance: A meta-analysis"). When the body is awake, information is encoded in the hippocampus, the central component of short-term and long-term memory. When in deep sleep, otherwise known as slow wave sleep, the encoded memory trace is replayed by the hippocampus over and over again. The movement of information between the

neocortex and hippocampus repeat over each sleep cycle. The brain needs sleep to remain healthy and perform all of its bodily functions effectively. However, this paper looks directly into the effects of sleep on grades. It does not take into account biological reasons for the outcome(s) of the study such as insomnia or anxiety.

After going over ample research that supports the correlation between sleep and cognitive health, I wanted to test my research practically. How does the amount of sleep affect quality of students' schoolwork? I hypothesized, if students sleep more, then they will perform better, which would translate into better grades than their peers who did not sleep as much. I predicted this result for two main reasons. First, I figured that students who slept more would attain more slow wave sleep, thus helping their memory consolidation. This would be beneficial to remember material learned through notes or homework in order to do well on tests and quizzes, which would translate to better grades than their tired counterparts. Second, I thought more sleep would lead to a larger attention span with greater concentration due to their rested cognitive capabilities.

I also recorded *amount of time spent on homework* and *frequency of participation* of students who consented to the observational study. The data collected from each individual's *time spent on homework* would be analyzed to see if this variable had any effect that could separate two people that slept the same amount, but performed differently academically. According to bestselling author, Malcolm Gladwell, in his book **Outliers**, it has been found that it is possible to "compare the educational achievement of one country with another's," simply by ranking the national averages of pre-test questions answered by students on the TIMSS (science and math) standardized test. Countries whose students answered more of the pre-test questions performed better on the test 100% of the time. This shows that countries with students that could concentrate more on the tedious pre-test questionnaire do better at solving science and math problems. I wanted to measure if the frequency of participation per subject in the observational study held the same outcome; that is, do the students who cooperated with the study perform better in the classroom?

## Method

I collected my data through 24 subjects' surveys that were completed Tuesdays through Fridays for eight weeks. Weekends and Mondays were not included because the data would be misleading due to students free choice what day(s) they would do their homework that weekend. Just because a student didn't do their homework Saturday does not mean they did not have homework, yet that student would still mark down "I did no homework today" on the survey.

The group of subjects was comprised of boys and girls, with diversity from a few different ethnicities. However, some aspects of the observational study were uniform. All students were from the same grade (freshmen) and were taught the same biology curriculum by the same biology teacher. This eliminated the possibilities of differing grading policies and different aspects of the biology courses being highlighted more than others. Each student shared the same amount of time on each topic of each unit.

#### **Initial Survey Data**

Days in Study	Average Sleep	Overall Grade	Average Homework Time
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# **Calculated Survey Data**

Time Stamp	Participant	How much sleep did you get last night
If you took a nap(s)	How much did you	How much time
yesterday, how long	sleep in total rounded to	did it take to complete
was it for?	the nearest .5 hour?	Bio Homework?

#### \*\*Anonymity\*\*

One important logistic of the observational study to highlight is the safeguard of students' anonymity. I wanted subjects to feel comfortable and even confident that the information they provided would be completely harmless to their academic life at Berkeley Carroll. If there was distrust in subjects, this could cause a misrepresentation of data to fit what students thought their teachers wanted to see, when in truth the data was kept strictly confidential during the study and presented in a responsible manner to the community. A double-blind procedure was set in place to protect students' information.

Finally, a permission slip was sent out and returned by each participant that ensured data given was truthful and parents were aware of their minor's participation in the observational study.

# **Results**

Grades are measured on a number scale where 10 is equivalent to an A, the highest grade possible. 0 is equivalent to an F in the class.

## Graph 1

#### Amount of Sleep per Night Affect on Overall Grade

This graph debunks my hypothesis. I predicted that as sleep increased, overall grades would improve in students. However, the data depicted suggests that the hypothesis is incorrect. There is no correlation between grades and sleep. In the graph, there is no clear trend line. As we can see, some kids with 7-9 hours of sleep reached the top of the graph (10), meaning their overall grades were A's. But, we can also see that in that 7-9 hours



slept range, there are numerous students who scored considerably less, with one student even scoring a (1) D in the class. This data comparison has an  $R^2$  value of .032 which means 3.2% of the data correlate.

# Graph 2

#### **Time Spent on Homework Affect on Overall Grade**

Although the data relationship portrayed in this graph was not a relationship I actively searched for, by the end of the study I was able to organize the information to how students' amount of time spent on homework affects their performance in the class. This graph demonstrates the indeterminate relationship between data sets, thus informing that time spent on homework does not necessarily affect students' overall grade in a class. Through this graph we see



in the 1.5-2 increment on the X-axis a large disparity. Four data points show the highest performance possible (10) from 1.5-2 hours of homework time and four data points land in the dissatisfactory range of 5 or less within that same homework time range. This data comparison has an R<sup>A</sup>2 value of .063 which means 6.3% of the data correlate.

#### Graph 3

#### Days in Study vs. Average Sleep

This represents another relationship between data sets that are not parallel. No trend is apparent in the graph. I would have hypothesized this outcome of inconsistency because there is no practical reason these two would be related. This data comparison has an R<sup>A</sup>2 value of 0.02755 which means 2.755% of the data correlate.

\*\*\* Related to Malcolm Gladwell's study on effort and performance in STEM fields.



#### **Graph 4**

#### Number of Days in Study vs. Overall Grade

This graph depicts the most telling relationship between two data sets this observational study produced. Malcolm Gladwell popularized a study done that correlated the number of pre-test questions students answered, to their grades, in his book **Outliers**. Gladwell confirmed that the students who answered more pre-test questions, performed better. He attributed this finding to students' work ethic



demonstrated by their meticulousness before the test.

Gladwell states, "Countries whose students are willing to concentrate and sit still long enough and focus on answering every single questions in an endless questionnaire are the same countries whose students do the best job at solving [STEM] problems". This assessment could be related to the observational study that this paper analyzes especially because it focuses on the effects of commitment in the STEM fields, such as Biology (the class involved with this study). The number of days that students contributed to the observational study in relation to performance (overall grade), does in fact indicate some correlation. This graph is most conclusive out of the rest and agrees with Gladwell. This data comparison has an R^2 value of .117 which means 11.7% of the data correlate.

# **Uncertainty Analysis**

One major error in the study is the difficulty of the course work that was not accounted for. Although students may be in the same grade, they do not perform the same because some subjects are more difficult for numerous reasons. Some students do not to perform as well in certain subjects because of classmate distractions, material that lacks their interest, or an actual cognitive incapability to understand the course, among other reasons. In these cases, it would not matter how much an individual sleeps, spends on homework, or contributes to a study, that student will have trouble performing.

# **Future Studies**

One future endeavor I am really interested in is seeing the difference between males and females while maintaining this observational studies procedure. Do different genders approach homework differently? Do different genders study differently?

Another future endeavor I am considering is measuring how a school's culture affects students' approach to homework. Berkeley Carroll's free periods might promote a stigma around schoolwork that schools without free periods inhibit.

I also wonder if states or cities with curfews (Omaha, NE) have teenage residents who are cognitively healthier than teenagers from bigger states (Chicago, IL / New York City, NY). It will be interesting to see if teenagers that grow up in the suburbs go to sleep earlier than teenagers in the city. Would their cognitive health transcend to the grade book?

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# WE LOOK WITH FAVOR ON ALL FORMS OF LEARNING, BUT WITH PARTICULAR GRACE WE ENCOURAGE PHILOSOPHICAL STUDIES, ESPECIALLY THOSE WHICH BY ACTUAL EXPERIMENTS ATTEMPT EITHER TO SHAPE OUT A NEW PHILOSOPHY OR TO PERFECT THE OLD."

#### KING CHARLES

from the 1661 Charter for the formation of the Royal Academy of Science; the proceedings of which are the oldest journal in existence