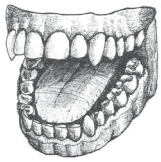
P.H.A.G.E.S.

Symbol	Notes
	Predation
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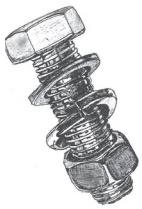
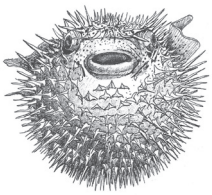
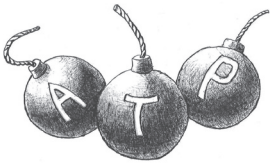
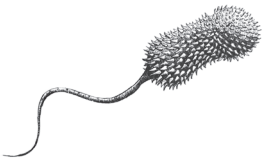
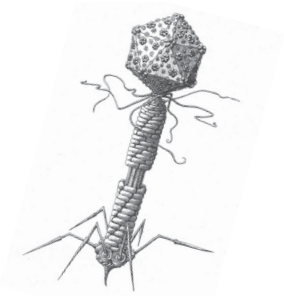
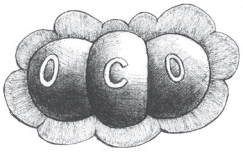
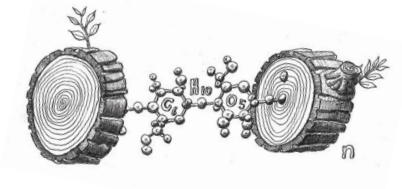
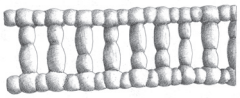
	Assembly
	Governors (GEMS) E = Energy M = Matter S = Space
	Expansion
	Selection

Other Acronyms and Key Terms

Anabolic	Metabolisms that build biomass
Bacteriophage	Viruses of bacteria; a.k.a., bacterial viruses, and phage
BAM	Bacteriophage Adherence to Mucus Immunity
Catabolic	Metabolisms that make ATP
CAZymes	Carbohydrate-Active Enzymes
CF	Cystic Fibrosis; a human genetic disease that is characterized by thicker mucus, which is colonized by viruses and bacteria
CFRR	Cystic Fibrosis Rapid Response
DIVA	Dinner Is Very Available microbes
Goldilocks Line	Hypothetical line where metabolism switches from predominantly anabolic (biomass-building) to catabolic (ATP-generation); usually driven by ratio of sugar to oxygen
HGT	Horizontal Gene Transfer
morons	Genes added to viral genomes via illegitimate recombination.
Microbes	Organisms that can only be seen using a microscope, usually single-cells; the most common microbes are viruses, Bacteria, Archaea, and protists (single-celled Eukarya); often viruses are referred to separately because of their special status as life forms, but not necessarily organisms
ORF	Open Reading Frames are roughly equivalent to a gene.
ORFan	An ORF with no similarity to other known ORFs.
Proviruses	A virus living inside a cell, usually integrated into a chromosome
NCD	Non-Communicable Diseases like a stroke or Parkinson's. Obesity is typically thought of as a NCD, but there may be a communicable aspect involving the microbiome.
SCFA	Short Chain Fatty Acid; made by fermenting bacteria
WEIRD	Western, Educated, Industrialized, Rich, and Democratic diet

Icons for Flow Charts

Icon	Notes
	ATP
	bacteria
	bacteriophage, viruses that infect bacteria, phage
	carbon dioxide
	cellulose
	coral
	DNA

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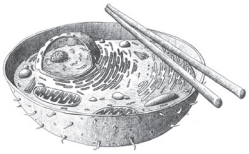	eukaryotic cell
	fire
	glucose
	humans

Icons for Flow Charts

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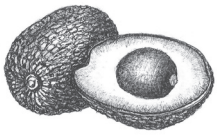
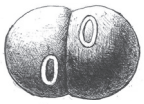
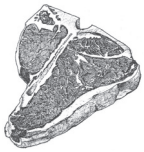
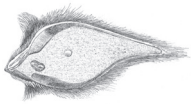
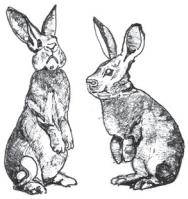
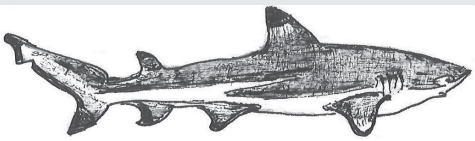
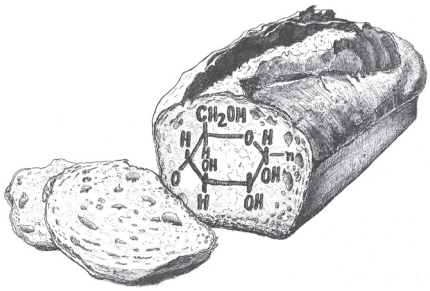
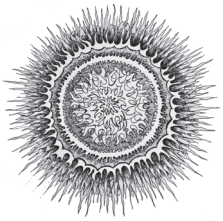
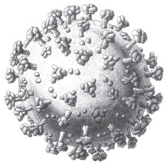
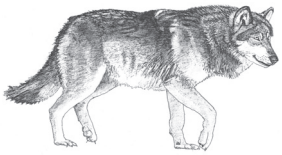
	lipids
	NADPH
	oxygen
	protein
	protist
	rabbits
	RNA
	seaweed, including fleshy macroalgae and turf algae

Icons for Flow Charts

Icon	Notes
	sharks
	starch
	sun
	virus that infects eukaryotic cells
	water
	wolves

P.H.A.G.E.S.

Forest Rohwer, PhD
and
Breeann Kirby, MSc

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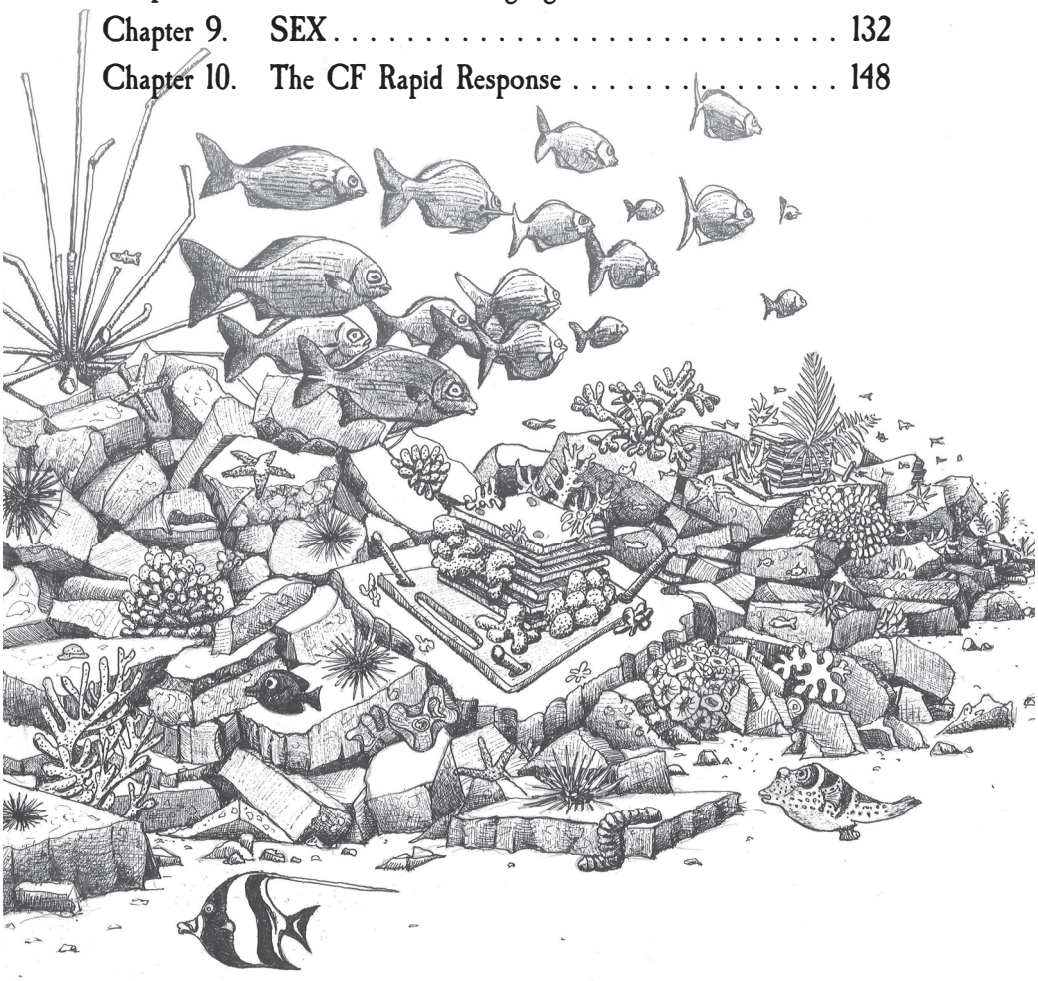
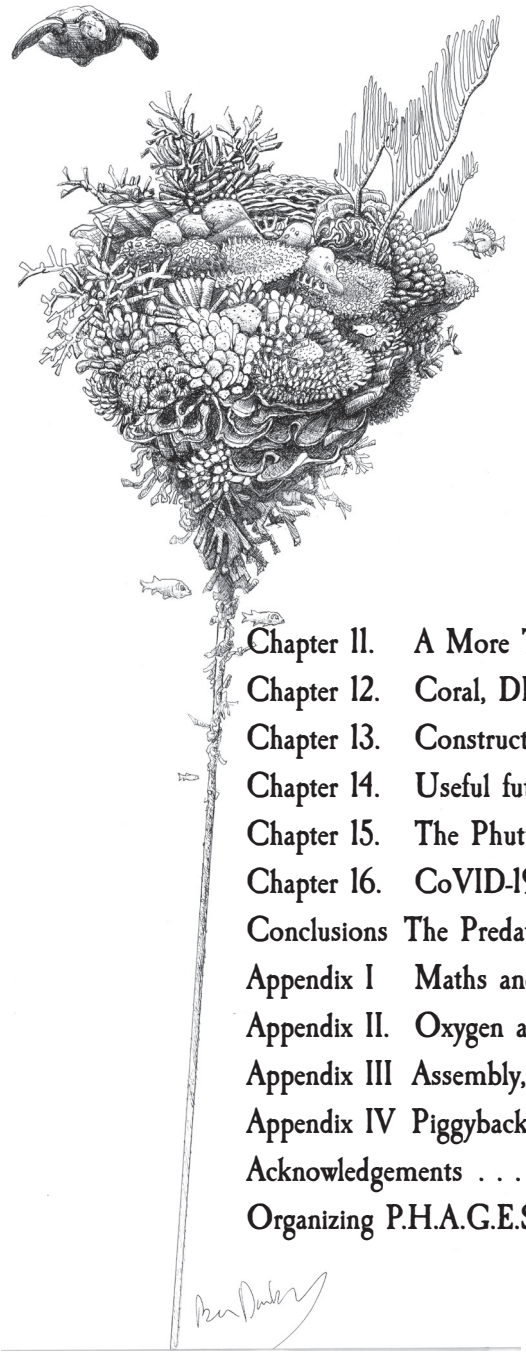




For Mike Furlan

For Bee and Citrine

Acknowledgements

Foreword

P.H.A.G.E.S. was originally proposed as a popular science book tenatively titled *The Predators Within* (TPW). TPW was to focus on phage and the human microbiome. As Breeann, Leah Pantea (illustrations), and I worked on TPW, it bacame clear that the science was still too nascent for a true synthesis. I decided that the best way forward, much to Breeann's chagrin, was to create a synthesis of microbial ecology that would be the basis for TPW. P.H.A.G.E.S. is the result.

P.H.A.G.E.S. and the Goldilocks Line are my personal way of thinking about ecosystems. This approach has served me well and hopefully will help other readers. Aspiring biologists, as well as interested non-specialists, may find P.H.A.G.E.S. useful for understanding the fascinating complexity of nature. The target audiences for P.H.A.G.E.S. are my lab members and immediate colleagues who range from undergraduates to full professors in science, math, and engineering. The goal is to get this disparate group using the same framework for studying ecosystems with particular emphasis on how viruses are dominant players. My hope is to turn the mostly qualitative P.H.A.G.E.S. into a quantitative science. This will probably take another 20 years and P.H.A.G.E.S. will continually update.

Breeann is busily turning P.H.A.G.E.S. into *The Predators Within*.

Sincerely,
Forest Rohwer,
March 2021

Introduction

The story arc for P.H.A.G.E.S. is relatively straight-forward. All living things must navigate a landscape where energy is used to move matter in space. These are the **G**overnors. Simultaneously, all living things must avoid **P**redation while predating, they must interact with other living things to form Assemblies, replicate themselves through **E**xpansion, and generally do a better job than other living things, a process called **S**election. Where each living system starts is **H**istory. To understand and manipulate living systems, we must simultaneously consider all of these P.H.A.G.E.S. processes.

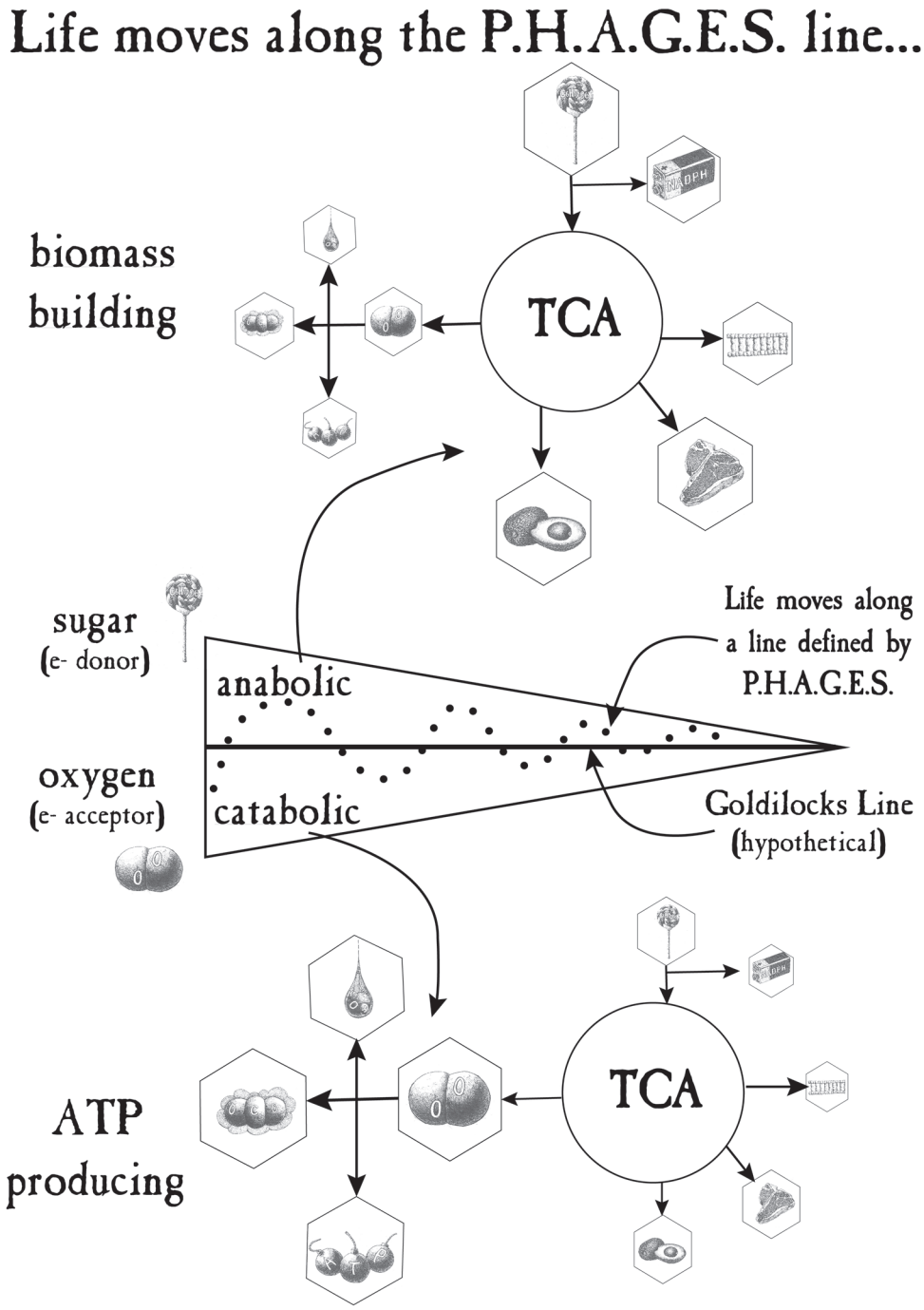
Luckily, the complex, everchanging landscape of P.H.A.G.E.S. is divided into two major regimes by the Goldilocks Line. On one side of this line, living organisms build more biomass. On the other side of the Goldilocks Line, organisms harvest energy to do things like hunt and mate. By determining on which side of the Goldilocks Line a system is living, much of the complexity of P.H.A.G.E.S is reduced.

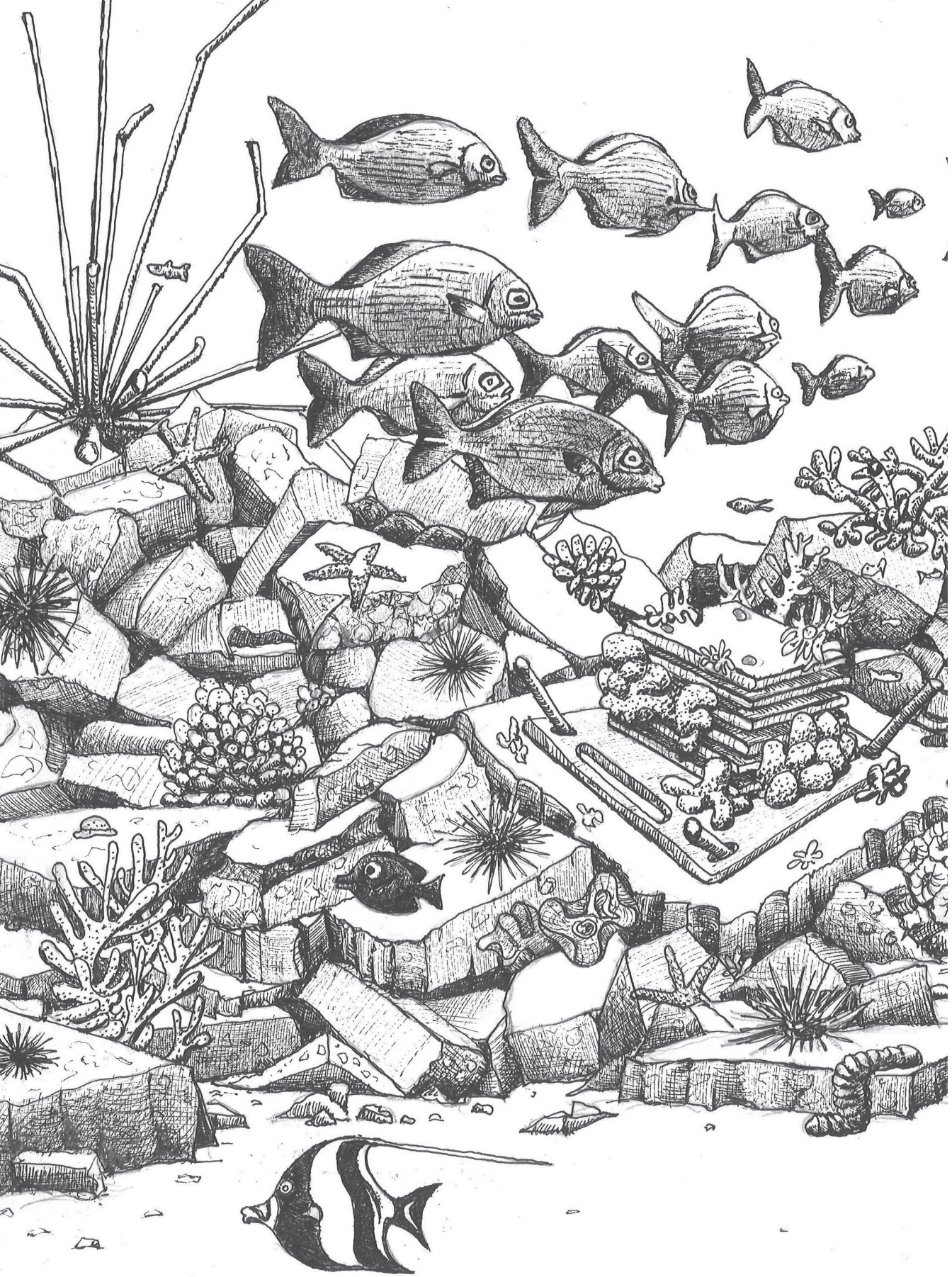
Organisms keep a written record of how to navigate the P.H.A.G.E.S. dimensions in their DNA. Humans are now able to read and manipulate these DNA-encoded stories, but we are newcomers. Viruses have been manipulating P.H.A.G.E.S. for billions of years. Our very survival depends on listening to and learning from the viruses, the most successful life forms on the planet.

Switching from a macro-organism and cell-centric thinking to a virocentric point-of-view is difficult. The Goldilocks Line and P.H.A.G.E.S. are our attempt to guide others into this world.

Goldilocks Line

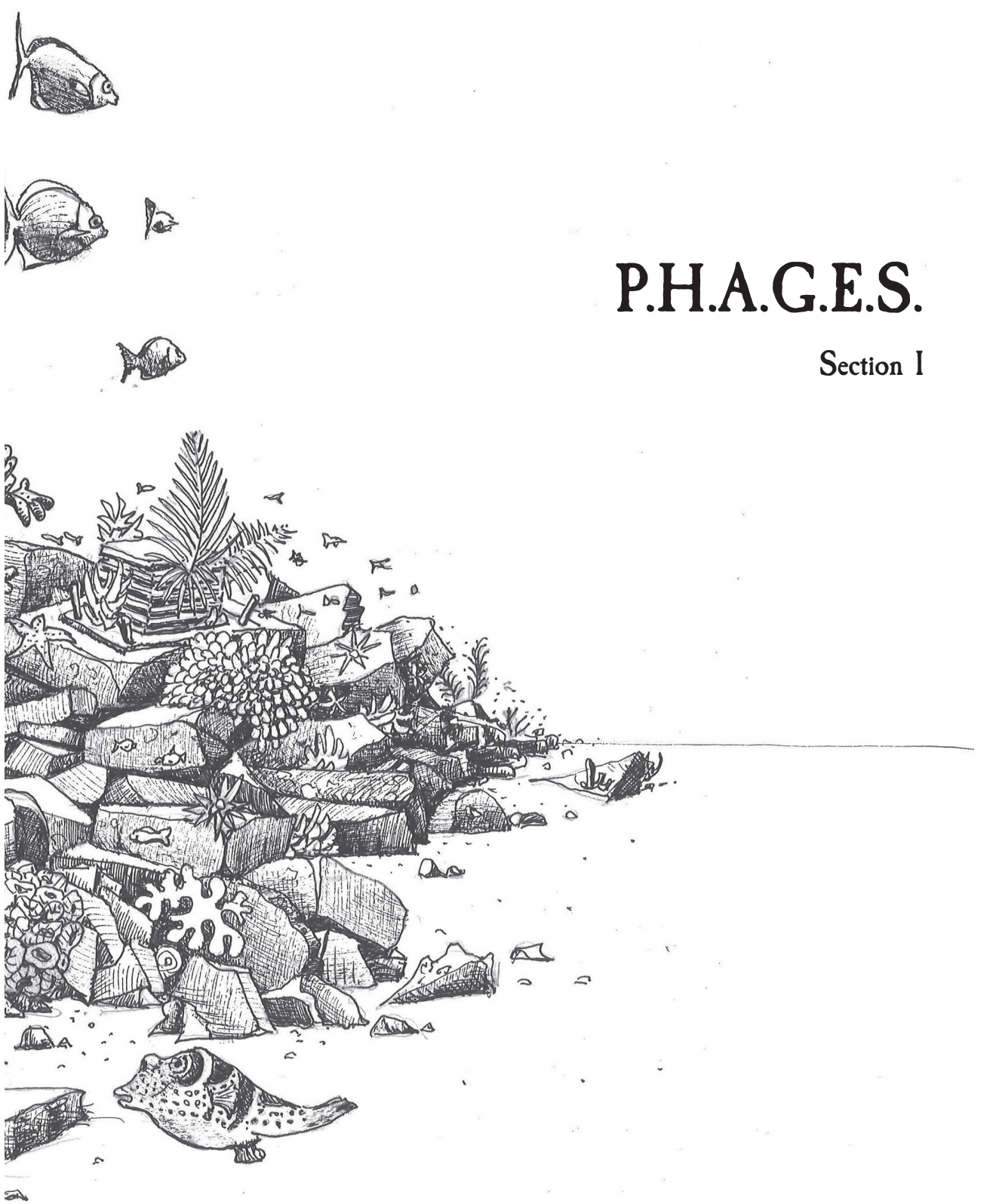
1) All of life must constantly navigate a theoretical space divided by the Goldilocks Line.
2) The Goldilocks Line defines the boundary where high-energy electron donors can move to electron acceptors. Practically, we can think of the Goldilocks Line as the ratio between sugar and oxygen.
3) If there is excess sugar compared to the oxygen, then the sugar is used to build biomass. This is *anabolic* metabolism.
4) If there is less sugar than oxygen, the sugar is used to produce ATP. This is catabolic metabolism.
5) The sugar and oxygen are never perfectly balanced. Therefore, the ratio is never exactly equal to 1. This hypothetical switching spot between anabolic and catabolic metabolisms is called the Goldilocks Line.


Life moves along the P.H.A.G.E.S. line...
biomass building
TCA
sugar
(e- donor)
anabolic
Life moves along a line defined by P.H.A.G.E.S.
oxygen
(e- acceptor)
catabolic
Goldilocks Line
(hypothetical)
ATP producing
TCA
& back-n-forth across the Goldilocks Line



P.H.A.G.E.S.

Section I



The Enemy of My Enemy

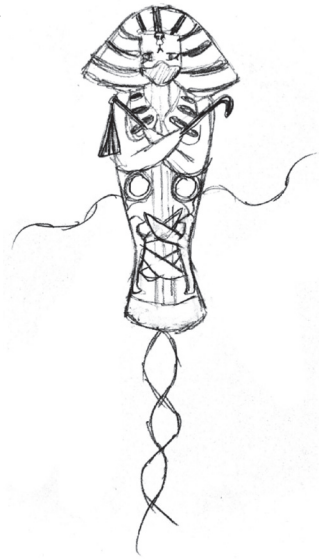
It's Monday morning and the coffee was just beginning to kick-in. I was headed to the gym to suffer under the reign of Justin the Evil Trainer with my longtime partner and fellow bacteriophage fanatic, Anca Segall (also a professor of Biology at SDSU). I noticed that there were about 15 voicemails from Texas. Ry Young, the Old Man of the bacteriophage world, was trying to get hold of me. I asked Anca if she knew what was going on. She mumbled something about "UCSD", "bacteriophage therapy", "dire" and finally, "go away". Anca is not a morning person. So, I called Ry. Anything to avoid Justin and the gym.

"There is a patient dying at UCSD hospital. The FDA has approved a bacteriophage therapy protocol and we sent some bacteriophage to their pharmacy. However, there is too much endotoxin (leftover pieces of bacteria) in the preps and we need you to clean them up. Today!"

Rule 1: Never check your voicemail on Monday morning.

Acinetobacter baumannii *is a seriously nasty bacterium that has quickly risen to the top of Most Unwanted List in hospitals worldwide. It was a huge problem in wounds suffered during the Iraq War. Because of its notoriety, bacteriophage that kill* A. baumannii *had been isolated by a number of different lab groups.*

By all accounts, Tom Patterson is a fun and interesting person, a Professor of Psychiatry at UCSD with 400+ academic publications to his credit who helps people suffering from chronic diseases like dementia. In November of 2015, he and his wife had taken a trip to Egypt, where Tom developed gallstones in his pancreas. At some unknown point he acquired A. baumannii. *After treatment in the local hospital, Tom was flown out to Germany and then back home to San Diego, and placed in the ICU at the UCSD hospital.*



At this point conventional treatments were working and Tom started to recover. Then one of the internal drains slipped and the A. baumannii *leaked into his blood (i.e., sepsis). Every time the doctors beat the bacteria back with antibiotics, some more would escape the abscesses, and Tom's health would decline. The* A. baumannii *was resistant to every antibiotic available for treatment. By the time I first saw him, this formerly energetic man was in and out of a coma, and in lung, heart and kidney failure. The doctors gave him less than a week to live.*

However, Tom had two amazing people advocating for him. His wife Steffanie Strathdee and his doctor Robert "Chip" Schooley. Steffanie is a well-known infectious disease epidemiologist. She had realized early on that bacteriophage might be the key to saving Tom's life, and Chip was willing to try. Together they rounded up A. baumannii-*killing bacteriophages from companies and research labs around the country. Most of these bacteriophages ended up in Ry's Texas A&M lab so that they could be propagated. Another set came from the US Naval Medical Research Center of the Biological Defense Research Directorate (yes, they are really named that).*

Chip had also processed the necessary paperwork with the US Food and Drug Administration (FDA) that would allow using the bacteriophages as a treatment of last resort. And this was a key turning point: the FDA responded favorably with a specific protocol. Up to this point, the FDA had been wary about bacteriophage therapy. However, they were going out on the proverbial limb and giving us an unprecedented opportunity to show that bacteriophage therapy could work. The problem, of course, was that we had never used bacteriophages in this manner and there was only a couple of days to go.

Having escaped the gym, I started calling anyone that might have good ideas. Research doctors reassured me that the residual endotoxin would probably not kill Tom; he had so much of it in his system already from the sepsis that it was not a major concern. Luckily, Jeremy Barr, a post-doc in my lab, had been working on a protocol to clean up bacteriophages for his studies of BAM Immunity.[1] A quick survey of the literature showed that Jeremy's methods might be the best way to rapidly clean up the bacteriophages for Tom's treatment. Supplies were ordered and $1,000s of expedited shipping bills later we had bacteriophage preps that met the FDA's guidelines.

The first bacteriophage treatment was administered. Days later the Navy bacteriophages arrived and were added to the treatment regime. And Tom woke up.

Bacteriophages, the little- known viruses that kill bacteria, seemed to have saved Tom's life. Using bacterial viruses to kill bacteria that kill humans seems like a great idea. So why aren't we using these viruses to kill bacteria in hospitals and at home? To answer this question, we need to weave a story involving antibiotics, Stalin's henchman, the rise of modern biology, and some complications arising from how viruses kill cells.

---

1 Bacteriophage Adherence to Mucus (BAM) immunity will be discussed later in this book. Briefly, BAM immunity works because some bacteriophage hold onto mucus and kill bacteria that try to get through the mucus to attack the underlying animal tissue.

Chapter 1.
The Good Killers?

Viruses can save your life. This statement may surprise you because we most often hear about viruses that cause diseases like CoVID-19, Ebola, AIDS, or the Flu. In actuality, viruses are life forms of vast contradictions. Some cause food poisoning and others prevent it. Some viruses help your immune system while others destroy it.

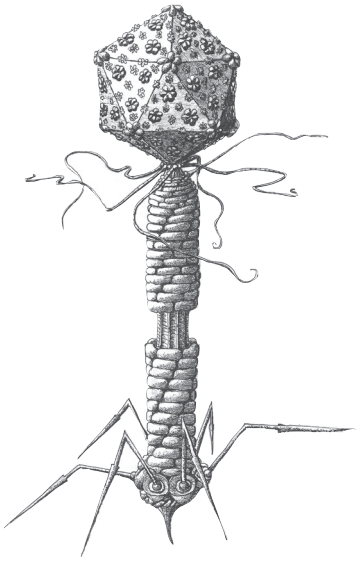


Figure 1.1. Viruses that Infect Bacteria: Also known as bacteriophages, the head of these viruses is called a capsid. This is where the viral genome is stored. The tail acts as a syringe to inject DNA into bacterial cells. The long-wispy tail fibers help the bacteriophage find its prey.

Viruses are everywhere. Every breath of air has thousands of viruses. We swim in lakes and oceans, which have millions of viruses per milliliter. And every day we eat trillions of viruses. The viruses that are living in, and on your body right now outnumber your human cells by at least 10 times. Even the DNA of your cells is more viral-like than human.

From childhood we are taught to protect ourselves against this vast viral universe. However, most of the viruses don't harm humans. Most viruses are **p**redators of bacteria. These viruses

are called *bacteriophages (a.k.a., bacterial viruses),* and most look something like an alien spaceship. Bacteriophages thrive where their bacterial prey lives, so there are billions of them living in animal guts, including humans.

These viruses hunt by attaching to bacterial cells and then injecting their viral genes. In turn, these genes hijack the cellular processes and turn the bacteria into a virus-making factory. Eventually, the newly created viruses will exit the cell, often by bursting out in a process called *lysis*, leaving behind the former bacteria as a dead derelict hulk. This viral horde then begins the hunt for new hosts.

Since the discovery of bacterial viruses over a century ago, doctors and others have tried to use bacterial viruses to save lives. One such life was that of Dr. Tom Patterson. After a relatively minor hospitalization while on vacation in Egypt, Tom was infected with the bacteria *Acinetobacter baumannii*. This soil bacteria had traded in some of its free-living abilities to become an *opportunistic pathogen* in wounds.[1] Opportunistic pathogens, as the name suggests, only become dangerous when they have the opportunity. For example, when a person's immune system is weak from illness or the normal microbiome is disrupted by antibiotics or other stressors. A combination of these two allowed the opportunistic pathogen *A. baumannii* to invade Tom's body and slowly start killing him.

A. baumannii has the genes to resist many different types of antibiotics as well as survive for almost a month on things like bedrails in hospitals. The Center for Disease Control (CDC) estimated about 10% of bloodstream infections are related to *A. baumannii* and incidences of infection are on the rise.[2] Infection in burn victims is even more problematic. One

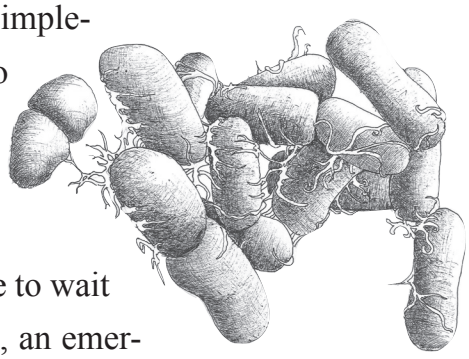
1 Peleg, Anton Y., Harald Seifert, and David L. Paterson. "*Acinetobacter baumannii*: emergence of a successful pathogen." Clinical Microbiology Reviews 21.3 (2008): 538-582.

2 Centers for Disease Control and Prevention (CDC) "A*cinetobacter baumannii* infections among patients at military medical facilities treating injured US service members, 2002-2004." MMWR. Morbidity And Mortality Weekly Report 53.45 (2004): 1063.

study showed that in a military burn unit, *A. baumannii* was the most common infection and more than half of the strains isolated harbored multidrug resistance genes, causing the mortality rates of those infected to be more than double that of burn victims who were not.[3]

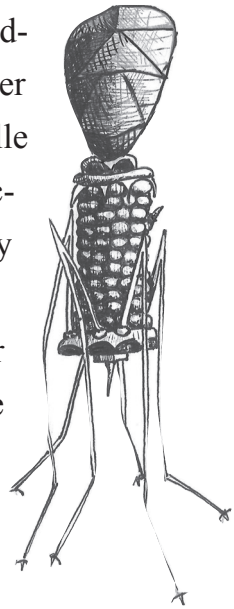
With the virulent *A. baumannii* population expanding in his body, Tom Patterson was in deadly trouble. Even with aggressive antibiotic treatment Tom kept getting sicker and sicker, often becoming confused and paranoid as his body began to fail and the infection started affecting his brain. When he finally flew home, the fluid from various infection sites puffed his body like a balloon. In San Diego, Tom was moved in and out of the intensive care unit (ICU) over the next three months. Every time it seemed like *A. baumannii* was defeated, the bacteria would rally and resist the administered antibiotics. Eventually, Patterson deteriorated so much that any hope of his leaving the ICU alive disappeared. All the clinical signs suggested that he wouldn't survive to the end of the month.

Dr. Steffanie Strathdee, Tom's wife, and his medical doctor Robert "Chip" Schooley, petitioned the Food and Drug Administration (FDA) for an eIND that would let them use bacteriophages to kill the *A. baumannii*. IND stands for Investigational New Drug. The application process for an IND is time-consuming because it can be difficult to predict just how a new drug will work. Thus, the FDA implemented safeguards on human testing to avoid harming people with treatments that show promise in animal models but haven't been tested in humans yet. However, Patterson didn't have the time to wait through the regular protocol. Therefore, an emer-



3 McConnell, Michael J., Luis Actis, and Jerónimo Pachón. "*Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models." FEMS Microbiology Reviews 37.2 (2013): 130-155.

gency IND, an eIND, that bypassed many of the normal safety protocols was needed. Why did Steffanie and Chip gamble that bacteriophages could save Tom's life? Because they knew that there was a 100-year history of using bacterial viruses to kill pathogens. A treatment regime called *bacteriophage therapy*.[4]



Bacteriophages were first discovered in 1915 by Frederick Twort. He went off to fight in World War I and never followed up on his discovery. Two years later, Félix d'Herelle coined the term bacteriophage, which means "eaters of Bacteria", and spent the rest of his career researching these tiny **p**redators of bacteria.

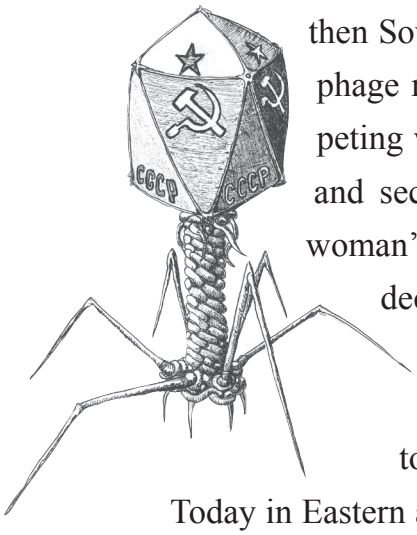
While working as a volunteer in Paris at the Pasteur Institute, d'Herelle noted that the eaters of bacteria could be filtered from the fecal slurry taken from patients recovering from dysentery. The bacteriophage killed the pathogenic microbes responsible for the illness. D'Herelle's speculations about bacteriophage, while perceptive, were not something he could fully support. The first microscope able to directly see bacteriophages would not be invented until the end of the 1930s. And other methods, like DNA sequencing, would not be figured out until the 1970s.

This lack of knowledge didn't deter d'Herelle. He began to filter more of this bacteria-eating juice. First, he ingested some of the concoction himself to "scientifically" prove its harmlessness. Then he enlisted a few other doctors to swallow shots of filtered poop. Finally, d'Herelle administered his filtered crap, which contained the bacteriophage, to patients sick with dysentery. Though not 100%, the recovery rate was high enough in

4 Often bacteriophages are called phage for short. For clarity, we will always use bacteriophage or bacterial viruses to refer to these viruses and reserve P.H.A.G.E.S. as an acronym explained later.

those treated with the bacteriophage mixture to convince many people that the bacterial viruses were working. These bacterial viruses were hailed as "nature's G-men". They even got a Pulitzer Prize with Sinclair Lewis' *Arrowsmith*, the unconventional scientist that uses phage therapy to treat a disease on a fictional Caribbean island.[5] Major pharmaceutical companies began working with bacteriophages and doctors around the world prescribed these bacterial viruses as treatments for infections.

In the mid-1930s, d'Herelle partnered with George Eliava from the then Soviet Republic of Georgia to start a large bacteriophage research facility. Unfortunately, Eliava was competing with Lavrentiy Beria, chief of the Soviet security and secret police apparatus under Joseph Stalin, for a woman's affection. This wasn't a good idea; Eliava was declared an enemy of the people and executed. Still the institute and research continued, and the Eliava Institute of Bacteriophage remains active to this day.[6]



Today in Eastern and some parts of Western Europe, bacteriophage therapy is relatively common. In Poland, bacteriophage therapy is considered an experimental treatment option that is prescribed after traditional therapies have been exhausted. In France and Belgium, some medical practitioners use bacteriophage therapy in very specific and difficult-to-treat cases of bacterial infection. In the Republic of Georgia, mixtures of bacteriophages are sold at the local pharmacy to treat gut disorders, skin infections, and many other ailments of a bacterial nature.

But if bacteriophage therapy is so common in parts of the world,

5 Lewis, Sinclair. "Arrowsmith". 1925. New York: Signet.

6 For the whole sordid story with affairs, bodyguards, tyrants, and executions read Anna Kuchment's "The Forgotten Cure" and Summers' "The strange history of phage therapy." Bacteriophage 2.2 (2012): 130-133.

why would Steffanie and Chip need an eIND to treat Tom's infection in the USA? Even though more than a century of research shows that bacteriophage therapy has more positive than negative effects, it remains a fringe medical practice. There are three main factors that contributed to bacteriophage therapy's obscurity: 1) The discovery and success of antibiotics, 2) The way bacteriophage therapy has been practiced, and 3) The difficulty in predicting and patenting the outcomes of bacteriophage therapy. Much of this book is going to explore how we are starting to better understand viral ecology to effectively use bacteriophages, as well as other ecology approaches, to manipulate human and environmental health.

Despite its promising start, since the mid-1930s, bacteriophage therapy in humans was practically abandoned in the U.S. and most of the world because Fleming and others discovered antibiotics. Patients treated with antibiotics usually rapidly recover. The healing power of antibiotics is reproducible from study to study, patient to patient. Bacteriophage therapy's effectiveness varies from laboratory to laboratory and between patients. Thus, antibiotics emerged as the clear winner for the routine treatment of bacterial infections.

The second reason for the decline of bacteriophage therapy was clinical and research malpractice. Most bacteriophage therapy studies are not well documented, and generally only the successes are reported.[7] During bacteriophage therapy's early years, many doctors interested in curing their patients did things that made it unclear if bacteriophages were killing the harmful bacteria or other factors were involved. For example, some doctors used bacteriophage therapy to treat wounds that would normally heal on their own. Many practitioners added antibiotics to their bacteriophage mixture. In these cases, patient recovery could not be ascribed directly to the bacteriophages. Even though the FDA did try to put research guidelines

7 Tom Patterson's story is anecdotal and would probably not have made it into the medical literature if Tom had died.

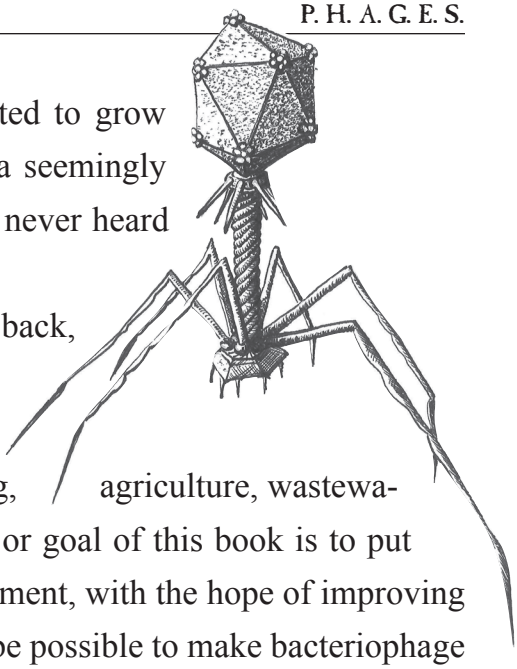
on bacteriophage therapy research and development, its public popularity often made doctors forgo the rigors of scientific method.[8] All of this forced the American Medical Association (AMA), in the 1940s, to declare officially against bacteriophage therapy as a viable treatment for bacterial infection. These less-than-perfect practices continued in Eastern Europe even as bacteriophage therapy was abandoned in the West. Bacteriophage therapy's reputation was not helped by the fact that it was popular east of the Iron Curtain. The Cold War and communist-capitalist mistrust led to further declines in bacteriophage therapy research in the West.

Another hurdle to widespread adoption of bacteriophage therapy lies in that bacteriophages are biological entities that change over time. Worse, they don't always just kill their target bacteria, sometimes they join up with the bacteria to make a more dangerous pathogen. This means that a bacteriophage that works in one patient may not work in another. A lot of work needs to go into matching the correct bacteriophage with its prey bacterium, which is expensive and almost impossible to patent. These concerns make bacteriophage therapy unattractive to investors.

For all these reasons, bacteriophages became the tool of microbiologists rather than a medical doctor's dream cure. And as scientific tools, bacteriophages have thrived. Scientists used bacteriophages to figure out how DNA encodes life's language. Bacteriophage are the key tools for manipulating the DNA. These bacterial viruses, and their products, have created one of the great economies of the modern world called biotechnology, or biotech, for short.[9] In 2017, biotech was estimated to be

8 Sinclair Lewis' novel, published in 1925, about Martin Arrowsmith did not help people's impression of bacteriophage. Dr. Arrowsmith abandons his scientific principles, and uses bacteriophages to save everyone from the plague. He is a hero, but a traitor to himself and science.

9 Cairns, John, Gunther Siegmund Stent, and James D. Watson. Phage and the origins of molecular biology. Cold Spring Harbor Laboratory Press, 2007.



worth over $400 billion[10] and is projected to grow to ~$800 billion by 2025. Not bad for a seemingly obscure life form that most people have never heard of.

Bacteriophage therapy is also back, driven by the growing problem of antibiotic resistance. Bacteriophages are being used to control bacteria in food processing, agriculture, wastewater, and increasingly in the clinic. A major goal of this book is to put bacteriophage in context of their environment, with the hope of improving bacteriophage therapy. In fact, it should be possible to make bacteriophage therapy even better than antibiotics.

Bacteriophage therapy is an option in extreme cases like Tom's, but it is not routine or even organized. Steffanie and Chip knew they had to find the right bacteriophages to kill the *A. baumannii* growing inside Tom. They asked Ry Young at Texas A&M and Theron Hamilton at the Biological Defense Researcher Directorate of the Naval Medical Research Center to check their stocks of frozen bacterial viruses - Texas Bacteriophage and Navy Bacteriophage, respectively. Out of the thousands of bacterial viruses resurrected from little tubes that filled boxes in the freezers, only a few from each lab killed the specific *strains*[11] of *A. baumannii* that were attacking Tom.[12]

10 Transparency Market Research market intelligence report on the global Biotechnology Market titled, "Biotechnology Market by Application (Biopharmacy, Bioservices, Bioagri, Bioindustrial), by Technology (Fermentation, Tissue Regeneration, PCR, Nanobiotechnology, DNA Sequencing & Others) - Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2010 - 2017."

11 The naming of viruses and microbes is always a little tricky. Officially, there is a taxonomy with genus-species designations like *Acinetobacter baumannii*. A strain refers to variants of the same species. In practice, knowing the genus-species give a doctor or microbiologist some general information about the virus or microbe. It is at the strain level, however, where differences between life and death are played out. In the case of bacteriophage therapy, the strain of the pathogen determines both how deadly the organism is, as well as how well the bacteriophage can kill it.

12 In most viral and microbial infections, exponential **e**xpansion and **S**election lead to multiple strains in the person's body. This complicates bacteriophage therapy.

This was only the start. Growing up a whole bunch of bacteriophages is technically difficult, especially when the host is a deadly pathogen that can kill the bacteriophage farmer. And once you have the bacteriophages, it is important to get rid of the pathogen and the pieces of the pathogen. It would not be a good idea to re-infect the patient with the pathogen or the toxins that the pathogen make (i.e., the cause of the disease itself).

It was at the cleanup step that I was reluctantly dragged into bacteriophage therapy. The Texas bacteriophages were not pure enough and were shipped to my lab at San Diego State University, where we had a protocol to purify the bacteriophages to meet the FDA guidelines. These guidelines were reasonable: 1) the bacteriophages must be purified of residual toxins that could kill Tom, and 2) there would be enough bacteriophages to stand a chance of being effective. We had experience in this area and were able to clean up the bacteriophages sufficiently.

With the FDA-approved cocktail, Tom's medical team injected the bacteriophages into various abscesses that were the concentrated sites of the *A. baumannii* infection. Tom did not improve, but the bacteriophages did not seem to be making him sicker. Two days later, the Navy bacteriophage cocktail was injected into Tom's bloodstream. Two more days of the combined treatment and Tom became conscious for the first time in a week, recognizing his daughter who was sitting by his bed. A day after regaining consciousness, he began to decline once again. Chip and colleagues discontinued the bacteriophage therapy for a day while they figured out if he was reacting negatively to the bacteriophages; then they started it again when it seemed the bacteriophages were not responsible.[13] After this, Tom's recovery was slow, with some setbacks, but he did recover. The bacteriophages appeared to weaken the *A. baumannii* population to such an extent that the bacteria became susceptible to antibiotics. The co-treatment of bacteriophages and

13 This is a common occurrence; the cellular remains of the killed bacteria make the patients feel worst for a couple of days.

antibiotics eventually cleared Tom of the *A. baumannii* infection.

Bacteriophage therapy practitioners try to use obligate *virulent bacteriophages*. These bacteriophages infect and immediately turn the host cells into viral factories. Once the new virions have been constructed, the host cell is broken open to release the progeny. This is lysis.[14] The other broad class of bacteriophages often don't immediately turn their hosts into virus-making factories. Because of this more temperate lifecycle, they are unimaginatively called *temperate bacteriophages*. Instead temperate bacteriophages tend to hang out in the bacterial cell until conditions change. In practice it is relatively hard to guarantee that a bacteriophage is virulent or temperate. Despite more than 100 years of research, we are still pretty bad at predicting just how bacteriophages and bacteria will get along.

It's easy to look back at the widespread use of antibiotics and the unstandardized research on bacteriophages in health care and figure out where challenges arose. In the early 1900s, there weren't many scientific tools to study bacteriophages; they were essentially invisible agents that sometimes cured a disease and sometimes did not. Now, the necessary technologies to understand how bacterial viruses behave are rapidly being invented. To routinely use bacteriophages to kill acute infections like *A. baumannii,* we need to understand how these viruses fit into the ever-changing story of biology. It isn't as simple as prescribing some bacteriophage cocktail; we need to understand how bacteriophages interact with all the elements that make up the animal body, including the viruses and microbes already in residence.

14 Brüssow, Harald. "What is needed for phage therapy to become a reality in Western medicine?." Virology 434.2 (2012): 138-142.

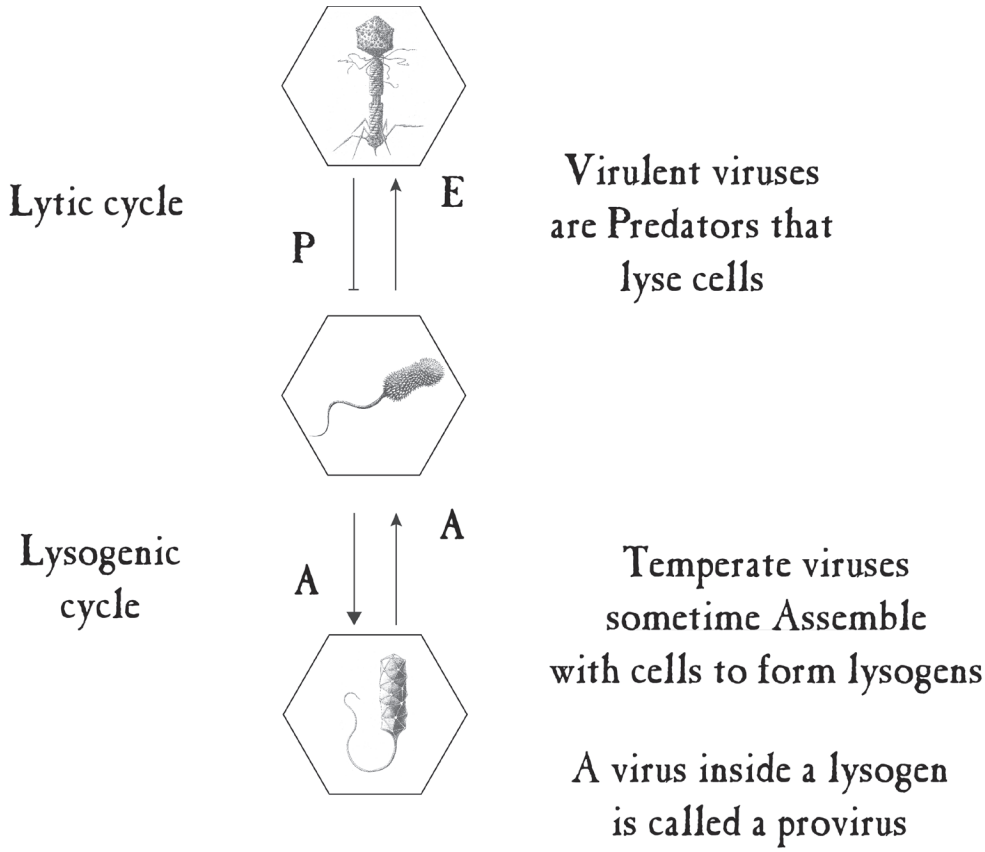


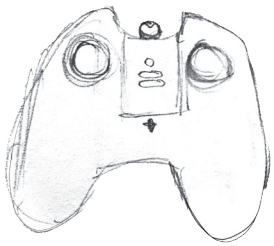
Figure 1.2. Virulent and Temperate Viruses Figure. Viruses float around in water or air until they bump into a host cell. The virus then injects its genome and takes over the cell, and makes an average of 25 new viruses. After the new viruses are built, the host cell is blown open so that these baby viruses spread out and infect new cells. This is called the lytic lifecycle. Most viruses can also enter into an uneasy truce with their host cell after infection. These temperate viruses hang out in the cell while times are good. While in this *detente* mode, the viruses are called proviruses. When the host cell starts to weaken, the proviruses react and enter the lytic life cycle.

Throughout this book, things like viruses, cells, molecules, and animals will be represented by word and/or icons inside hexagons. These things will be connected to each other by arrows that indicate processes, particularly P.H.A.G.E.S. processes. The goal of these figures is to provide a bridge between the qualitative, written descriptions that are best for communicating biological complexity to more quantitative mathematical models. These models are necessary to deal with the high-dimensional spaces of biological phenomena. More detailed descriptions of these schematics will be provided as we progress through the book. There is also a cheat sheet in the front of the book.

Flying Fucks

The Flying Fuck arrived as a Christmas present. It was about 6 inches across, with two propellers to lift it off the ground and a radio controller.

"Dad, can I fly The Fuck?", asked my eight-year old daughter, Willow.



"Only if you can recite the most important sentence in the English language."

A slight eye roll, learned or inherited from her mother, and then, "Fuck You, You Fucking Fuck." I love educating the children.

Mike Furlan, the lab's manager, took over flight training and soon Willow was dive-bombing undergraduate students on the campus mall with the Flying Fuck. As we watched, the conversation turned to how drones might be useful for our research. We really didn't have a great reason, but it sounded like great fun to have a drone. So, a couple of days later, a GoPro-carrying drone appeared, with the vague idea that it would be used to map coral reefs in the middle of the Pacific.

The test field was the grassy quad in the middle of the campus. Mike set the drone on the sidewalk and slowly lifted it off the ground with the remote controller until it hovered slightly overhead. After the successful liftoff, he fully engaged the forward button. The drone zipped off horizontally at dive-bomber speed. The students causally walking and staring at their phones became highly motivated to engage in the real world and scramble out of the way. The drone skipped across the sidewalk, scattering backpacks, books and coffee cups. After successfully clearing the path free of students, the drone

came to a rest by slamming into a light pole.

"Fuck," Mike said. "Great reactions by the students though." Turns out that one of the propellers was installed upside down.

Drones and other electronic gadgetry are excellent examples of hacking. Basically, a new product is built by putting things together and making them work. You don't build a drone by inventing a computer controller, helicopter and camera. Instead you take pre-existing versions of each, modify them a little, and assemble them together. This is exactly what biology does, except the hardware is cells, running on software encoded by DNA.

*The easiest way to build a living system is to hack together two or more pre-existing biological entities in an **a**ssembly. The most common assemblies are between viruses and cells. Different types of virally infected cells assembled together create organisms. Various organisms thrown together create ecosystems. By hacking together different life forms, life creates an infinite number of competing organisms and ecosystems. And like our technological systems, the main hackers are viruses.*

Chapter 2. Healthy?

Viruses, bacteria, and other microscopic life forms are extremely common on and in our bodies.[1] These micro-inhabitants contribute to our metabolism, immune responses, mental health, and much more. The close associations between viruses, microbes, and our cells are not unique to humans. Every plant and animal on Earth are *wholobionts*, the organismal equivalent to an ecosystem.[2] Since humans are complex walking ecosystems, our microscopic inhabitants are important for our health. This complexity makes it hard to understand health and disease.

Humans are very good at thinking about simple cause-and-effect relationships. It is relatively intuitive that someone, like Tom Patterson, infected with a pathogen can be cured by treating with bacteriophages that kill the *Acinetobacter baumannii*. We are not so good at thinking about what

1 Biology is a filled with terms that have both broad and narrow meanings that can be confusing. In general, we've tried to keep the terminology as simple as possible. However, there are some words that we just need. Microbes are anything that we can only see with a microscope. This includes single cellular life forms like bacteria and protists. Viruses are non-cellular, microscopic life forms that infect cells.

2 The term wholobiont will be used in this book to avoid confusion with a number of problematic theories and models that use "holobiont". There is a reasonably sordid history around the term holobiont and holism. In 2002, Nancy Knowlton and I proposed the coral holobiont as a term to describe a coral colony. The intention was to remind people that the colony included viruses, Bacteria, Archaea, fungi, algae, as well as the coral animal. This ecological assemblage is essential to acclimatization of the coral colony to changing environmental conditions. In the original version of this paper, the term wholobiont was proposed. However, the editor asked that we change this to holobiont. This was unfortunate because Dr. Lynn Margulis had used the term to describe evolutionary units like the cell and mitochondria. This has led to a lot of confusion. We'll come back to this later.

happens in complicated, interacting ecosystems that change over time with lots of viruses, bacteria, and human cells. In fact, it is fair to say humans are pretty horrible at predicting how these wholobionts will react to changes.

To get a mental image of how complex the human wholobiont really is, let's go back to Junior High geometry and think about dimensions. Everyone is comfortable with a three-dimensional world. For example, the location of a steak on the dinner table is easily described with three spatial coordinates (traditionally x, y, and z).

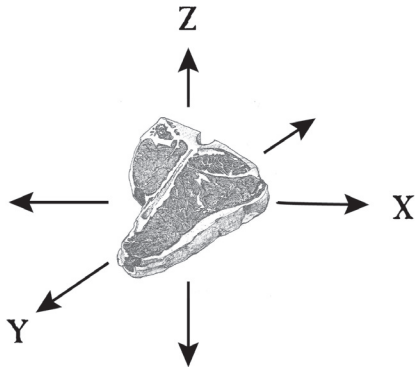


Figure 2.1. Three Dimensions of a Steak. Normal space is defined by points on the three traditional axes of the Cartesian coordinate system.

It is also useful to describe the steak's nutritional values in three-dimensions. Yes, nutrition has dimensions just like the regular world. The energy dimension of the steak is measured in Calories and the matter of the steak has a macro-nutrient dimension that includes fats, carbohydrates, and proteins. Still another dimension describes the micro-nutrients including iron and vitamin B12. So, like x-y-z space, nutrition can be described in a three coordinate dimensions that describe how "healthy" a food is: Calories, macro-nutrients, and micro-nutrients.[3]

3 Nutritionists are used to these three dimensions of food. Other scientists might not like these dimensions because they are not completely independent; the macro- and micro-nutrients are both building blocks and contain energy. A physicist, for example, might try to describe these three dimensions in terms of just matter and energy. In the end, the descriptions of the dimensions are dependent on the questions being asked.

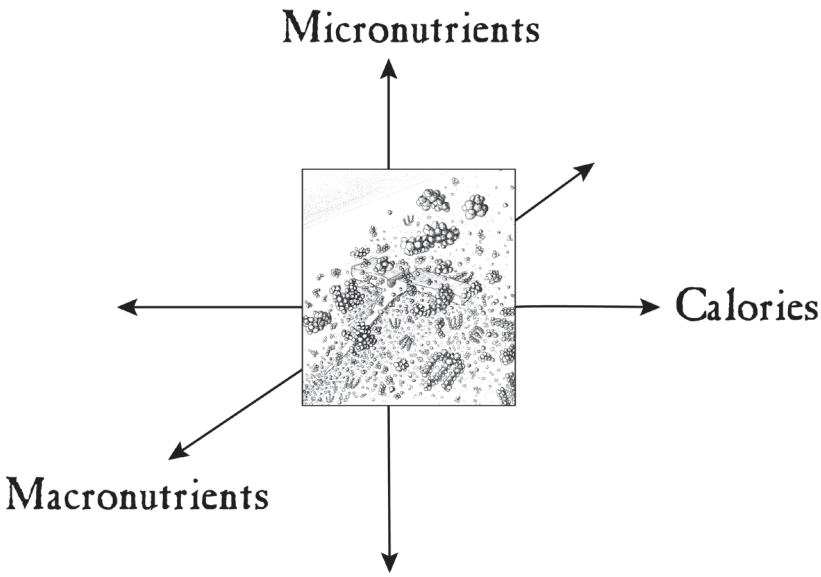


Figure 2.2. Three Dimensions of a Steak's Nutritional Value. Non-traditional axes like Calories, macro-nutrients, and micronutrients are also used to describe the properties of objects.

Now sit down at that table, cut off a piece of steak, chew, and swallow. Imagine tracing the steak in both the space and the nutrient dimensions. As you mentally unpack the steak along these 6 dimensions, start thinking about all of these things that are changing along yet another dimension: time. You can't do it right? This is the challenge. To make informed decisions about what food to eat for our health, it is necessary to account for all of these dimensions.[4]

4 Dimensions can even be something based on ethics. For example, was the cow raised on grassland or in a feedlot.

Figure 2.3. Dimensional reduction of a steak. The different dimensions of a steak are manifested in the person that eats it. The **h**istory of an individual human wholobiont produces different outcomes.

If just eating a steak is so complicated that our brain shuts down, then is it even worth trying to understand plethora of dimensions describing the human wholobiont with the goal of achieving health? The answer is a definitive "yes", but we need some modern scientific tools and some thinking tools, both of which will be presented in the subsequent chapters.

Not Healthy

All of the relevant dimensions make many biological questions extremely complicated, but there is hope. Humans are adept at reducing many dimensions into stereotypes. For example, steak is nutritious, salads are not.[5] These stereotypes are useful but also frequently misleading. For example, many people consider salads healthy, which may be true if your problem is over-nutrition (e.g., a modern American). If you are starving in West Africa, then a salad is not very healthy compared to a steak. Your definition of healthy is dependent on current circumstances.

5 And, *a la The Simpsons*, you most certainly don't make friends with salad.

Even though we all want to be healthy and spend hundreds of billions of dollars each year towards this goal, describing the dimensions of healthy is bordering on impossible. In fact, it is far easier to describe "not-healthy". Someone with food poisoning is not-healthy. A person with the flu or cancer or type 2 diabetes or all three is definitely not-healthy.

The ease of labeling something not-healthy leads to simple, cause-and-effect thinking; the presence of a virus or bacteria in the body is a disease. The disease can be cured by killing the invader. The same thinking means that healthy is the absence of an invader. And this is what most people, including many doctors, have been taught; the healthy human self is essentially sterile and set apart from the viral and microbial worlds. These are "germs" we are taught to fear as children. If our germ-free, healthy self is occupied by something other than human cells, then we are infected, not-healthy, and contaminated with pathogens and parasites. The wholobiont view directly challenges this paradigm.

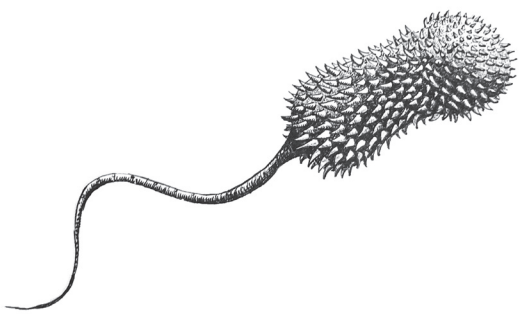
Humans, other animals, and plants aren't pristine, inviolate entities. The viral and microbial elements of wholobionts contribute to the health of every macro-organism on the planet. Without resident viruses and microbes, digestive and immune systems do not develop correctly, making wholobionts less robust and resistant to outside pathogens.[6] It even looks like being too vigilant against germs leads to the increase in allergies and other autoimmune diseases.[7]

Wholobionts are also important in the context of non-communicable diseases (NCDs) which don't have a specific pathogen as the cause. The World Health Organization calls the rise of NCDs like type 2 diabetes, metabolic syndrome, high blood pressure, and irritable bowel disease, to

6 Gensollen, Thomas, et al. "How colonization by microbiota in early life shapes the immune system." Science 352.6285 (2016): 539-544.

7 Bach, Jean-François. "Six questions about the hygiene hypothesis." Cellular Immunology 233.2 (2005): 158-161.

name only a few, a global "slow-motion catastrophe". NCDs are rapidly increasing across cultural, age, and socioeconomic demographics. These chronic diseases are the frontline in the war to improve human health. To address the NCDs, it is important to consider the complex interactions of our human wholobionts. Simple cause-and-effect thinking leads people to blame not-healthy on about everything: urbanization, sugar, fat, chemical exposure, *et cetera*. In truth, NCDs and other health challenges have millions of dimensions and are not reducible to singular causes and cures. Human wholobionts are walking ecosystems and therefore human health requires an understanding of ecology, particularly microbial ecology.



Fire Control

Lab outings are good for morale. There's nothing like watching grad students and post-docs carry heavy things up and down mountain trails to make everyone happy. This particular lab outing was to sample the viruses in the desert. And we had lots of exceptionally heavy sampling tools that needed to be moved through gullies in the unrelenting sun. It was exhausting to watch, but you could see morale starting to rise just like steam from perspiring brows.

Mike and I were tired out after drinking coffee and supervising. "Go ahead and set everything up, and do the sampling. We're going shooting."

Being from Texas and Idaho, respectively, Mike and I both recognized that it was important to exercise our Second Amendment rights whenever possible, especially when there was choice between killing aluminum cans and physical work.

Several hundreds of rounds of ammunition later, Mike and I returned to watch the grad students and post-docs carrying everything back down to the car, pack up the samples, and then unpack everything for camping. As the sun started to set, it was time to start the fire.

"Just hand me the matches and we can all get warm."

"Who brought the matches?" After much searching it was clear that the incompetent grad students and post-docs had forgotten to bring anything to light the fire with. "Do I have to do everything?" I grumbled.

"Maybe we can use this as a teaching experience, where the labbies learn to make a fire by rubbing sticks together." This

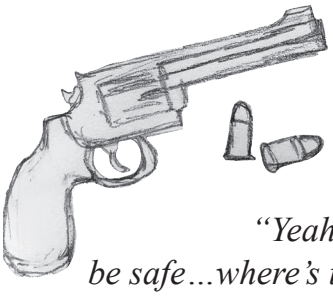
seemed like a fine chance to provide some mentorship to the younger generation. Things were getting a little desperate as the temperature started to plummet below 72°F; San Diegans start to freeze at 68°F. Mike and I, well experienced in the viciousness of the SoCal winter, broke out the whiskey to keep the blood pumping.

Various stick rubbing exercises were tried. Mike and I provided guidance by snickering and giggling. It's important for leaders to maintain their composure in stressful times. One misguided youth suggested just driving back to the little town 10 minutes down the road and buying some matches. Sweat started rolling off exhausted foreheads. At least they were getting warm. Not a puff of smoke, however.

The temperature continued to plummet into the mid-60s.

Luckily the second pint of whiskey killed off some of the weaker brain cells that had been slowing down our thinking. Hell, we had firearms. The operative word being "fire". All we needed was to harness the power of our right to bear arms and everything would be fine. What could go wrong? This was probably the exact sort of situation the Founding Fathers envisioned when amending the Constitution.

"Maybe we should get rid of the bullet?". Always thinking ahead, Mike used his teeth to pry the lead projectile off a .22-caliber cartridge, which was duly loaded into a Colt revolver.



"We only have about 600 rounds left, so we better be sure this works."

"Yeah, it would be best to use an accelerant just to be safe…where's the gas for the generator?" Gas was liberally sloshed onto the firewood. "Do you think that's enough?" This very question has been asked by men throughout the Ages and we already knew the answer was an emphatic "No!". More gas was sloshed into the fire pit.

Grad students and post-docs rapidly retreated from the immediate area. It was almost like they didn't trust us with alcohol, gas, and a gun. They didn't realize our formative years in two of the more enlightened states had trained us for such eventualities.

A little more gas, a nip of whiskey, and everything was ready. The revolver was cocked. Mike assumed the proper gunfighter's position, learned from Gunsmoke, and BAM! Flames jumped 20 feet into the air. Mike fell backwards over a camp chair, smoking slightly. Aahh the sweet smell of success and scorched eyebrows.

After stomping out the bigger embers and counting the shrapnel holes in Mike's shirt, we sat back down to enjoy our hard-won warmth. Craven labbies slowly emerge from the darkness. More whiskey was poured. Someone mentioned that they really should have brought food.

"That's what the beer is for. Kids nowadays just don't know anything about camping. Anyone want a cigar?", Mike asked.

He casually reached into his pack and pulled out a cigar case.

And his lighter.

Life is a controlled fire that needs fuel, oxygen, and a spark. The sparks are cells struggling to get enough fuel and/or oxygen. A main rule of life is that there is either too much or too little fuel for the amount of oxygen. We are calling the hypothetical line where there is too much oxygen and then then not enough oxygen the Goldilocks Line. Identifying the Goldilocks Line defines the stage where the rest of life plays its games, including competition to obtain limiting resource and between ***p****redators and their prey. In turn, these competitions lead to* ***s****election, the driver of evolution.*

Chapter 3.
There is No Goldilocks

Microbial ecologists consider dimensions ranging from biological to geological to chemical; *biogeochemistry* in the shorthand. The human wholobiont is a wonderful example of biogeochemistry. The biology includes viruses, microbes, human cells, fungi, parasites, and many other living creatures. All these organisms interact through chemistry; and this chemistry is ultimately determined by even larger geological processes that include nuclear fusion in the Sun and weathering of the Earth. Getting a mental handle on biogeochemical complexity can be challenging. To help, we are introducing the Goldilocks Line and acronym P.H.A.G.E.S.

Goldilocks Line

Life is a fire. Literally. Life needs energy and most of the energy comes from burning things like wood. The most familiar biological fire consists of sugar as the fuel, oxygen from fresh air, and living creatures as the sparks. Burning the sugar releases energy that the organisms need. The quickest way to understand an ecosystem is to look at the fuel and oxygen feeding the biological fire triangle. Every ecosystem will either have: 1) more fuel than oxygen, or 2) less fuel than oxygen. The oxygen and fuel will never be absolutely balanced. Therefore, there are no Goldilocks conditions of "just right" for wholobionts or ecosystems. Living systems move across this imaginary Goldilocks Line defined by the relative amounts of sugar and oxygen.

Let's illustrate the Goldilocks Line with a beer fermentation. To make a tasty IPA, a brewer puts a whole bunch of sugar (the fuel) plus some yeast cells (the sparks) into a container that can be sealed shut. Almost immediately the yeast starts to grow and uses up any residual oxygen in the container. The beer ecosystem (sugar, water, and yeast) is severely oxygen-depleted because it is sealed off from the atmosphere. As a result, the yeast starts to make alcohol, which is a way for the cells to burn the sugar without oxygen. This is a smoldering fire. A human placed in the beer ecosystem would immediately die from suffocation. The human wholobiont always needs to be in oxygen-rich ecosystems.

The Goldilocks Line arises from the fundamental relationship between *respiration* and *photosynthesis*. Respiration is the process of burning sugar and oxygen to release energy to do things like build more living tissue, have sex, and kill prey. Respiration produces carbon dioxide and water, which are used by plants in photosynthesis. Photosynthesis is the reverse reaction of respiration. To perform photosynthesis, the plant cells absorb energy from sunlight and use that energy to produce sugar and oxygen. This cycling between photosynthesis and respiration continues *ad infinitum* with sunlight providing the outside energy source.

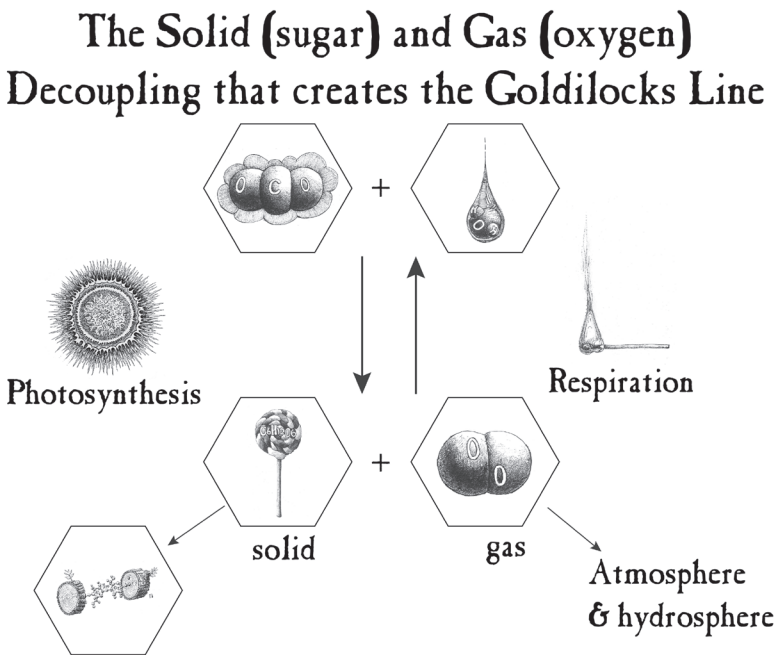


Figure 3.1 Photosynthesis and Respiration. Cyanobacteria, algae, and plants are photosynthesizers that use short-wave, light energy to make sugar and oxygen from carbon dioxide and water. The sugar is then used to construct other complex molecules like wood. Cells ignite the sugar and oxygen to produce carbon dioxide and water in catabolic processes that release energy. The oxygen is usually whisked away by winds or water, while the sugar is retained. This decoupling between solid sugar and gaseous oxygen creates the Goldilocks Line.

The Goldilocks Line occurs because the sugar and oxygen produced by photosynthesis have different physical properties. The sugar is a solid and stays with the plant. The oxygen is a gas and is released into the surrounding atmosphere or water. If the plant happens to be barley, then a lot of the sugar is stored in the grain seed while the gaseous oxygen escapes. Putting all of that sugar into the brewer's closed container means that there is not enough oxygen to convert all the sugar back to carbon dioxide and water. Instead, ethanol is produced. Add some hops and you have an IPA. A bunch of sugar without oxygen leads to *anabolic metabolisms*.

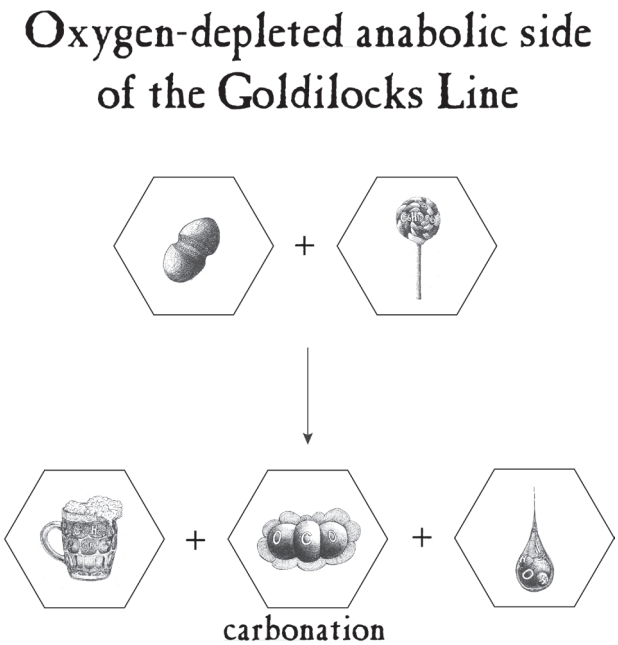


Figure 3.2. The Goldilocks Line and Beer. In a beer vat, yeast uses sugar to produce alcohol, water, and carbon dioxide.

To reiterate, all ecosystems have either too much or too little oxygen. This occurs because the products of photosynthesis, sugar and oxygen, are a solid and gas, respectively. Oxygen drifts away from the sugar leading to the Goldilocks Line. This is the key process in life and identifying whether an ecosystem is oxygen-rich or oxygen-limited is the first step in being a microbial ecologist.

The second set of rules is a whole lot more complicated...sorry. To make these other processes easier to remember, we are introducing the acronym P.H.A.G.E.S.[1]

1 Through the book, the acronym P.H.A.G.E.S. will be capitalized and stands for **P**redation, **H**istory, **A**ssembly, **G**overnors (matter, energy, space), **E**xpansion, and **S**election. Hopefully, P.H.A.G.E.S. won't be confused with phages, the bacterial viruses.

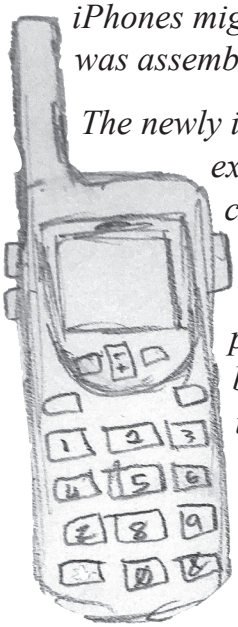
Natural Bureaucracies

"We need an import permit to get the export permit?"

"Yes. But we actually don't need an import permit to bring the samples into the US. Therefore, the Fish and Game won't give us one."

My head started to hurt, "Which means we can't get the export permit that we do need."

"Correct. It's totally FUBAR." As lab Praetor Urbanus, Mike was responsible for CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) permits, IRB (Institutional Review Board), BUA (Biological Use Authorization), chemical inventory, fire drills, assessments, animal protocols, radiation compliance, ethic courses, sharps containers labeled multiple ways to comply with city, county, state and federal laws, etc… The full alphabet soup that the bureaucracy throws at anyone trying to get something done. The newest joy was export/import rules, handed down by the Feds. Apparently, they were worried that the GPS in our iPhones might fall into China's hands, even though the phone was assembled in Zhengzhou.



The newly installed Export/Import Officer had taken exception to the lab's lackadaisical ways of carrying top secret technological equipment, like laptops, to the Netherlands and other suspect nations. She also wanted to update our CITES permit. The problem was that some parts of the bureaucracy did not think that the permits needed to be updated. The other problems were that I was in the middle of the Pacific on a ship, needed the permits, and was spending $250 per minute on a satellite phone call to work out the mess.

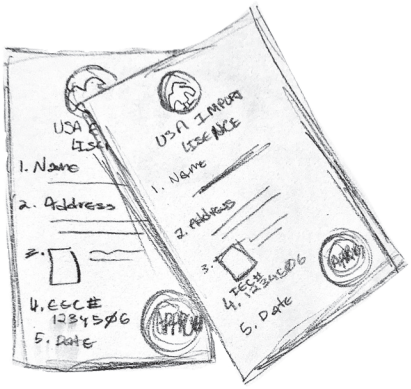
"Are you bringing back anything endangered with you?"

"No."

"It would be much easier if you were."

"So, you want me to go kill some endangered creature to make the permitting easier?", I asked.

"Yep."



My headache was getting worse and the sat phone costs skyrocketed, "Let me understand this. In the name of protecting coral reefs, we need to kill an endangered coral, to get an import permit, to get an export permit. Even though we really don't need the import permit."

"You could probably just say that you plan to kill something endangered, and then not kill it, but still get the import permit. I don't think there is a rule saying that you actually have to collect the CITES protected critter if you have the permit."

"Brilliant! Let's do that…". That's the nice thing about the bureaucracy and biology, there is always a counter rule to any rule or form.

So, an endangered coral was threatened with hammer and subsequently reprieved. This unfroze bureaucratic brakes, allowing permits to be issued, stamps to be stamped, and some very impressive looking documents FedExed to me just in time for me to stand in the "To Declare" line at LAX airport.

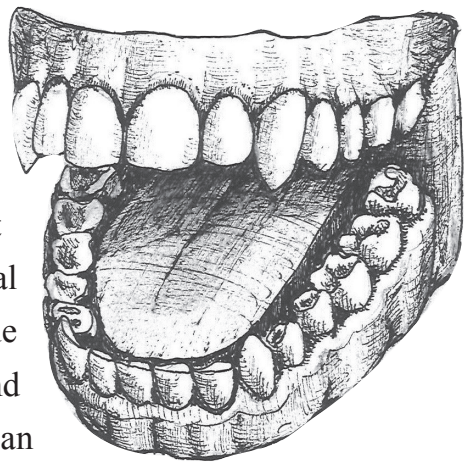
I banished my official documents to the Custom's Officer, who immediately said, "You know that you don't need an import permit for these…".

Nature has created bureaucracies as deviously complicated as anything invented by humans. And like the DMV, these rules and regulations can be navigated and understood given some time. P.H.A.G.E.S., introduced below, are the rules necessary to understand the natural world's bureaucracies.

Chapter 4.
P.H.A.G.E.S.

Ecological systems are structured by the processes of **P**redation, **H**istory, **A**ssembly, **G**overnors (energy, matter, space), **E**xpansion, and **S**election, or P.H.A.G.E.S. for short. By learning about P.H.A.G.E.S., you will be able to explain the processes driving the biosphere, including those important to your health. P.H.A.G.E.S. is a little tricky, but worth the effort.

Predation



Predation is a major process in biology.[1] **P**redation spurs the recycling of the limiting **g**overnors of energy, matter, and space. **P**redators control the **e**xpansion of populations and affect the **a**ssembly of organisms in individual wholobionts, thereby establishing unique **h**istories. **P**redators influence ecology and evolution by **s**electing[2] organisms that can evade their attacks.

1 **P**redation includes things like a wolf eating an elk, an elk eating a willow tree, and a virus eating an elk. There are specialized terms for these interactions, like carnivory, grazing, and parasitism. Ultimately, it is still one organism eating another (or at least a part of another).

2 When one of the P.H.A.G.E.S. concepts is used in the text, the first letter of the term will be bolded to attract the attention of the reader.

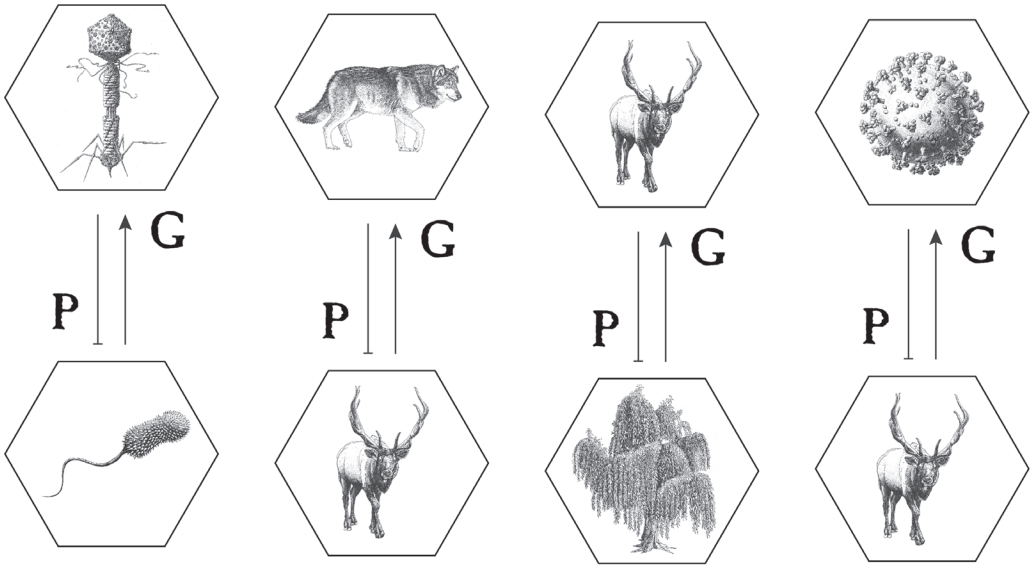


Figure 4.1. Different Types of Predation. Predators eat other living creatures, which lowers the number and/or biomass of the prey item (indicated by the terminal arrowhead). Different types of **p**redation include virulent viruses, carnivores, and grazing. The nutrients and energy are used to construct more of the **p**redators' number and/or biomass (G).

When imagining **p**redators, we think of some majestic animal with seething viciousness like a shark or a wolf. However, viruses are by far the most abundant **p**redators on the planet and they are deadly killers. Apex **p**redators like a wolf kill a couple of elk every month or so.[3] This means that the approximate 300,000 wolves on the Earth kill something like four kilograms (kg) of elk every minute and no humans at all. Viruses, on the other hand, kill over 60,000,000,000 kilograms of cells every minute.[4] And this figure doesn't include the millions of humans and other animals that die

3 Two elk per wolf per month, about 300,000 wolves globally, and each elk weights about 300 kgs.

4 It is unfair to compare all of viruses to one subset of apex **p**redators. As we will see, large **p**redators have extremely strong influences on ecosystems. The take home point is that we also need to consider the viral **p**redators, which are traditionally ignored in almost all the ecological literature.

from viral infections every year.[5] The truth is you are continuously attacked by viruses and never by a wolf or shark.

Over the last 30 years, we have learned that viruses are the world's most successful **p**redators in terms of numbers and biodiversity. Viral numbers are literally bigger than astronomical. Viruses outnumber the stars in the universe by 10 million times. Written as a traditional number this is 10,000,000,000,000,000,000,000,000,000,000 or 10^{31} in scientific notation. This means that if viruses and wolves took up the same space on Earth, wolf-sized viruses would cover the surface of 10^{17} Earth-sized planets. But we won't be knee-deep in viruses anytime soon because they are also incredibly small. Literally millions of viruses could sit on the head of the preverbal pin.

Viruses vanishingly small sizes kept them hidden from humanity until the late 1800s. Their role in ecological systems was not really considered until the 1990s when new technologies were developed to study the microbial world. This has been an incredible intellectual breakthrough. Imagine hiking through Yellowstone National Park and not being able to see the wolves, elk, or trees. How would you figure out what was going on when suddenly part of your arm got bitten off? Out of a science fiction story, yes, but figuring out an invisible **p**redator based on the visible effects on its prey is exactly what has been going for most of human history. We have prayed to gods and blamed bad spirits because no-one understood that it was viruses and their microbial minions that were maiming and killing us. The most abundant **p**redators in the world remained hidden because they are microscopic, biological entities that are not even living by some definitions.

The early virologists were mostly interested in how viruses influenced their host and we still have a host-centric view of them. Rather than viewing viruses as important life forms in their own right, their near

5 Humans are very successful **p**redators in terms of food consumed. Each of us eat about 2.5 kgs of food per day and there are almost 10^{10} of us. This means that humans are eating about 17,000,000 kg of food every minute.

invisibility still makes it hard for humans to incorporate them into our world view. This host-centric focus is reflected in classic virus names. To understand these names, you need to know that there are three main types of cells in the world: *Bacteria*, *Archaea,* and *Eukarya*.[6]

Bacteria are small, single cells found almost everywhere. Archaea are also small, single cells that are usually found in harsher environments like Yellowstone's hot springs and the deep ocean. Even though Archaea and Bacteria are very different in terms of evolutionary **h**istory and cellular biology, they are small, and we collectively refer to them as *microbes*. Eukaryotic cells can be small single cells, called *protists*, but they also make up multi-cellular organisms like animals and plants.

Page 57, Figure 4.2. The Tree of Life without most viruses and microbes. The term microbe is used to refer to anything that you need a microscope to see. This includes Bacteria, Archaea, and single-cell Eukarya. Bacteria and Archaea look similar under the microscope but are different from each other at the molecular level. Eukarya have internal membranes, including a membrane that surrounds the genomic DNA called the nucleus. Protists account for most of the eukaryotic biodiversity. This classical tree of life misses most of biodiversity, the viruses, and includes the fact that all macrobes are wholobionts.

6 I hate to do this, but there is some subtle terminology and notation that should be cleared up. When referring to the taxonomical groups of Bacteria, Archaea and Eukarya, they will be capitalized. When referring to groups of these organisms, they will not be capitalized. For example, a flask filled with bacteria. There are both historical and scientific reasons for making these distinctions...sorry:(

Millions of different viruses infect Bacteria, Archaea, and Eukarya cells. Luckily, most viruses cannot infect cells from the other cell type. So, a bacterial virus won't infect an archaeal or eukaryotic cell and *vice versa*. In fact, this relationship between viruses and their host is even more specific. A virus that infects a dog usually won't infect a human, even though both are made up of eukaryotic cells. A bacterial virus that infects an *E. coli* will not infect a very closely related *Salmonella* bacteria. This *tropism* is why there were only a few bacteriophages to attack the *A. baumannii* that were killing Tom Patterson. Tropism is also why the bacteriophages would not harm Tom's human cells; bacterial viruses only bacteria.[7]

Most viruses—like tobacco mosaic virus (TMV) or human immunodeficiency (HIV) virus—are named after the cell types they infect, the type of disease they cause, or both.[8] However, bacterial virologists have more fun, and these viruses often get imaginative names like "corndog," "whatsapiecost," "bumblebee," and "hankypanky."[9] We are going to mostly discuss the bacterial viruses and their function as **p**redators in the human wholobiont and other ecosystems. However, many of the processes are shared by all viruses. To be clear in the text, *bacterial viruses* (or bacteriophages), *archaeal viruses*, and *eukaryotic viruses* will be used to refer to viruses infecting these Domains of life.

7 There is evidence that some viruses can transfer DNA between very different organisms. Chiura, Hiroshi X. "Generalized gene transfer by virus-like particles from marine bacteria." Aquatic Microbial Ecology 13.1 (1997): 75-83.

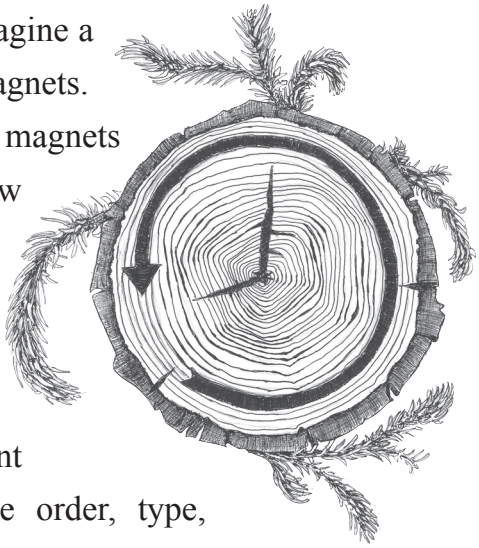
8 Tobacco mosaic = plant + description of disease; human immunodeficiency = animal + description of disease.

9 If you want to discover and name a new virus, join a SEA-PHAGES group.

Figure 4.3. Every organism on the Tree of Life is actually a wholobiont; assemblages of the viruses, other microbes, and the macrobe. Numerically, there are more viruses in each wholobiontic individual, and most of the biodiversity of a wholobiont is found in the viral fraction.

History

The second important concept in P.H.A.G.E.S. is **h**istory. Every wholobiont and ecosystem has a unique **h**istory. In the case of humans, each and every individual wholobiont has been exposed to different viruses and microbes over the entire course of their life. Everyone has eaten different things and has a unique medical **h**istory (e.g., vaccines, antibiotic treatments, eating and exercise habits, etc.). An individual's personal genetics means that each of us has a unique immune system and physiological response. All of these dimensions, as well as other known and unknown dimensions that haven't been considered, influence **h**istory.



As an illustration of **h**istory, imagine a basket filled with differently shaped magnets. Subtle differences in the shape of the magnets will determine the polarities and how the magnets will arrange themselves. The timing in which the magnets are added to the basket will ultimately determine their final arrangement. This is analogous to the development of an ecosystem or wholobiont. The order, type, environmental conditions, and millions of other dimensions all determine the final outcome. **H**istory matters.

Each person's **h**istory strongly affects their current wholobiont and often explains why advice on manipulating our health often only works on a subset of people. *Eat raw red meat like our paleo ancestors!* versus *Red meat will kill you!* may both be true depending on someone's **h**istory. If your wholobiont has **a**ssembled with a bacterial species that processes the steak into a compound called trimethylamine n-oxide, TMAO for short, then red

meat could lead to heart disease.[10] If you don't have the TMAO-generating bacteria, then red meat is one of the most nutritious things you can eat. **H**istory leads to different responses in different people.

Humans try to control the **h**istory of our viral and microbial magnets by vaccinating kids at certain ages, which protects against many pathogenic viruses and some bacteria. Currently, we don't control the other living inhabitants of our bodies; the so-called "good" viruses and microbes that make up the human *virome* and *microbiome*, respectively. For example, almost everyone has Epstein–Barr virus (EBV), and not getting EBV as a kid can lead to infectious mononucleosis later in life. Everyone also has various herpes viruses and Torque Teno Viruses (TTV) circulating in their blood.[11] Our guts are packed with *Bacteriodes* and *Firmicute* bacteria. These symbionts are acquired haphazardly from our families, friends, and strangers. In the future we will take more control of wholobiont development and **h**istory by inoculating with specific viruses and microbes. The only way to safely and productively control our **h**istory will be through personalized approaches and understanding the rest of the P.H.A.G.E.S. concepts.

Finally, when considering how life forms interact over time, it is useful to differentiate between *acute* and *chronic* associations. Acute means short. Chronic means long-lasting. Most people are chronically infected with EBV and TTV. In contrast, you are acutely infected with influenza virus when you have the flu. Your body will clear the flu viruses in 10-14 days, unless the flu kills you of course. **H**istory influences all aspects of the wholobiont including the next ecological process of P.H.A.G.E.S. - **A**ssembly.

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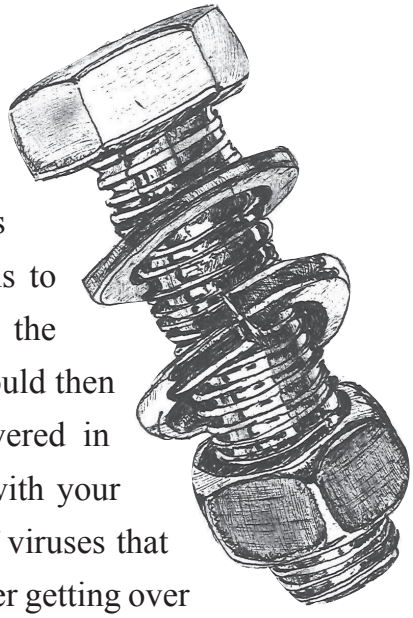
10 TMAO is actually produced by lots of different bacterial species. And these different bacteria eat lots of different foods. Damn those complications! Koeth, Robert A., et al. "Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis." Nature Medicine 19.5 (2013): 576.

11 Breitbart blood virus reference.

Assembly



If you had microscopic vision and looked at your hand, then you would see hundreds of thousands of eukaryotic cells (i.e., your human cells) arranged in patterns to create skin, bone, muscles, blood, and all the other parts of your flesh. Zoomed in, you would then notice that your human skin cells are covered in bacterial cells. And if you really squinted with your Superman eyes, there would be a myriad of viruses that infect both the human and bacterial cells. After getting over the shock, you might observe that the viruses and microbes are not passively sitting on your hand; rather they are squirming, chemically calling out to each other and the human cells, latching onto one another, feasting on secreted molecules, and multiplying. The viruses and cells of your body are an **a**ssemblage that makes up a personalized ecosystem and is as vibrant as Yellowstone or a coral reef.

There are, however, subtle differences between an environmental ecosystem and an organismal one, which is why we use the term *wholobiont* to talk about the **a**ssemblages of viruses and microbes living with individual animals and plants [12]; thousands or even tens-of-thousands of dissimilar life forms assembled together make a wholobiont.

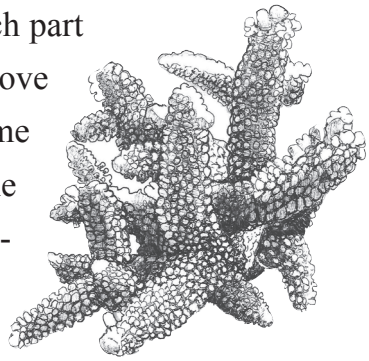
The term wholobiont does not imply whether these relationships are beneficial or harmful. At the most basic level, living things do not care about helping others, so why would it be advantageous to be a wholobiont

12 Two dissimilar organisms living in close association are engaged in a symbiosis; think sea anemone and clown fish. The problem with symbiosis as a term is that it has become loaded with a number of connotations about the strength and types of relationships between the symbionts. It is also usually limited to talking about a few symbionts. In reality, we have no idea about these implied dimensions, and it is essential to remember that wholobionts contain thousands to tens-of-thousands of viruses and cell types.

if all the residents are selfish scrabblers? The answer is that cooperation often results in all the partners doing better. And shifting symbionts in and out increases the robustness of the entire wholobiont. In a very real way, a wholobiont is much greater than its parts.

Microbes often protect macro-organisms, or *macrobes*, from other microbes, including pathogens. For example, wild mouse wholobionts thrive throughout the world. It is also common to raise microbe-free, also known as *gnotobiotic*, mice in the lab. Place any gnotobiotic mouse in the wild and it will rapidly die from infections. Wild mice are already heavily colonized by microbes and this means there are few spaces (see **g**overnors below) for pathogens to infect the animal. In fact, there are no naturally germ-free animals or plants and wholobiont **a**ssemblages of viral and microbial symbionts are essential for macrobes' health.

Different **a**ssemblies provide flexibility in function. For example, coral wholobionts that live in nitrogen-rich part of the oceans have bacteria that process and remove nitrogen-rich compounds (e.g., ammonia). The same coral animal, in nitrogen-depleted regions of the oceans, **a**ssembles with bacteria and fungi that recycle nitrogen.[13] This flexibility in the **a**ssemblage means that the same coral animal can live in very different parts of the ocean.



One of the more fascinating things about microbial ecology is that the same bacterial species may do the same job in different ecosystems. For example, one of the bacterial species that ferments cheese, called

13 Siboni, Nachshon, et al. "Geographic specific coral-associated ammonia-oxidizing archaea in the northern Gulf of Eilat (Red Sea)." Microbial Ecology 64.1 (2012): 18-24. Wegley, Linda, et al. "Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*." Environmental Microbiology 9.11 (2007): 2707-2719. Beman, J. Michael, et al. "Distribution and diversity of archaeal ammonia monooxygenase genes associated with corals." Applied Environmental Microbiology 73.17 (2007): 5642-5647.

Lactobacillus, may also ferment food in your gut. If your wholobiont lost its gut fermenters, then you could acquire the *Lactobacillus* spp. from cheese and regain the fermentation functions. This means that most of the time, the wholobiont doesn't fall apart when one viral or microbial species is lost. Rather the ecosystem adapts by **a**ssembling with another virus or microbe. This also means that much of the time there are no "magic bullet" viruses or microbes to change health status, despite the claims of probiotic companies.

This flexibility in which viruses and microbes can perform a function like fermentation or nitrogen metabolism in the wholobiont means that it is important to determine what *jobs* the viruses and microbes doing. Microbes produce as much as 10% - 30% of the energy animals like humans get from their food, as well as some of the essential vitamins.[14] The fact that different viruses and microbes can do the same job means that the main difference between you and another human isn't your DNA but the viral and microbial symbionts living with you. This unique **a**ssembly gives shape to your wholobiont's biological narrative that makes you, you.

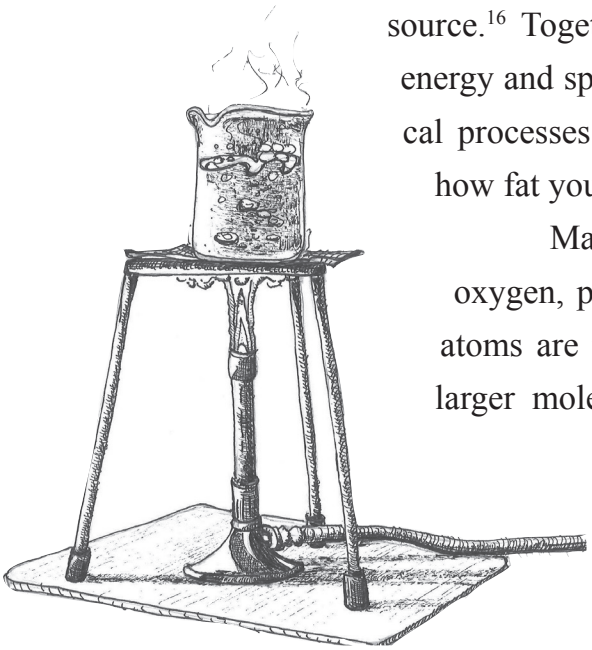
GEMS: The Governors of Energy, Matter, and Space

To survive, each symbiont in the wholobiont **a**ssemblage must get enough energy, matter, and space; these are the **G**overnors in P.H.A.G.E.S.[15] **G**overnors are physical factors that slow-down or speed-up living processes. Matter makes up the universe: elements that form more complicated molecules and mixtures. Energy is the forces that move matter around. And space is the physical area that energy moves matter around in.

Living things compete for the **g**overnors in order to live. In biology, water is the most ubiquitous form of matter and sunlight the primary energy

14 Turnbaugh, Peter J., et al. "An obesity-associated gut microbiome with increased capacity for energy harvest." Nature 444.7122 (2006): 1027.

15 Remember GEMS for the **G**overnors are Energy, Matter, and Space.



source.[16] Together, the availability of matter, energy and space **g**overn the rates of biological processes like how fast a tree grows or how fat you will get.

Matter is stuff. Carbon, nitrogen, oxygen, phosphorus, hydrogen and other atoms are the building blocks that makes larger molecules like water, lipids, DNA and proteins.[17] Some matter can be extremely limiting. Desert plant and animal wholobionts are water limited. Large terrestrial animals will search far and wide for the sodium and chloride in a salt lick. And without iodine, humans get life-threatening goiters.

Viruses and microbes are key to rapid recycling of matter and preventing an ecosystem's biological or ecological processes from stalling. Matter released in poop is quickly broken down and recycled. When an animal or plant dies, a community of viruses and microbes will chew up the corpse and release the matter. Every living organism on the planet is dependent on matter recycling by these microscopic life forms.

Energy and matter are inextricably tied to each other. Animals use

16 This might not be true. There is a reasonable possibility that ionizing radiation from the center of the Earth may actually be the largest energy source for the biosphere. We don't know for sure, because we have not sampled the *deep, hot biosphere* (coined by Thomas Gold) very extensively. Basically, this biome is everything a couple of meters below land or the sea floor to the magma. The direct counts that we do have suggests that deep, hot biosphere has thousands to millions of microbial cells per gram, which means there might be ~10^{29} cells total making this biome as big or even bigger than everything on the surface. These microbes would be getting their energy from ionizing radiation (e.g., split water) and distillation fueled by heat created by this radiation (e.g., cracked seawater, compressed carbonate, *et cetera*).

17 Much of the time, we call this stuff nutrients. This is a confusing terminology because nutritious is used to mean both the nutrients and energy. To keep it simple, we will just use matter and avoid the term "nutrients" as much as possible.

energy to move and find new sources of matter like that salt lick. Plants use energy to tear apart stones to find limiting matter like iron and phosphate. And the average human eats about one metric ton of food per year. This food contains both matter and energy. The matter in the food is used to build bones, teeth, blood, brains, and the rest of the body. Every organism uses energy to rearrange matter and run biological processes.

Manipulating energy is one of the most important jobs that microbes do in the context of wholobionts. That's because Bacteria and Archaea have evolved many more energy-manipulating metabolisms than eukaryotic cells. In a very real way, resident microbes expand the metabolic potential of wholobionts, letting humans, other animals, and plants thrive on an amazing variety of energy and matter sources.

Almost all animals ultimately get their energy from plants and other photosynthesizers that make sugar and oxygen.[18] The oxygen is released into the atmosphere or surrounding water and the plants turn many of the sugars into more complicated molecules like cellulose, which is just a bunch of sugar molecules strung together.

Cellulose is the main component of wood. Because of a biochemical trick, the sugar molecules in the cellulose are relatively resistant to degradation. This allows plants to construct forests of trees and grassy savannahs out of sugar. In turn, elk, cows, and termites can access the energy in the cellulose because they have microbes living in their guts that breakdown the cellulose and release sugar. Without the microbes, these animals would starve to death. By **a**ssembling with microbes, animals can get the energy out of plant material.

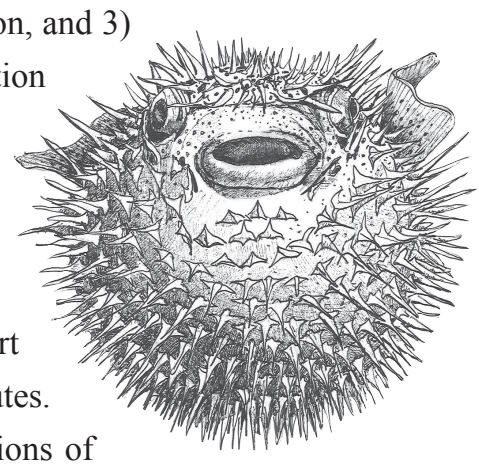
18 There are a number of wholobionts that get their energy from chemotrophic (i.e., chemical-eating) Bacteria and Archaea, rather than phototrophic (i.e., light-eating) algae and plants. The most famous chemotrophic animal wholobiont is the giant tube worms in hydrothermal vents. These wholobionts are indirectly eating energy released by nuclear decay in the deep Earth, which causes the extreme heat of the mantle. In turn, this heat cracks seawater into different chemical compounds that are ultimately used by the chemotrophic microbes to produce sugar and feed the tube worm wholobiont.

Predators like sharks and wolves would die without the grazing wholobionts and their associated viruses and microbes. It is only relatively recently that humans have figured out how to live on plants alone. Specifically, we had to develop farming and food processing (e.g., fire, grinding, and fermentation) to make the energy in plants accessible to our human cells.

In addition to energy and matter, the other **g**overnor is space. All organisms need physical space. Cells have sensors that tell them when they have reached the limits of space and they stop dividing.[19] If an ecological system runs out of space, then no new organisms can live there. Many organisms, like trees, corals, beavers, and humans, build more living spaces in a process called *niche construction*. We'll come back to this later.

Expansion

Expansion, especially exponential **e**xpansion, is another powerful process in biology. There are three important dimensions of **e**xpansion: 1) the time between generations, 2) the number of offspring (i.e., virions, kids, daughter cells) produced each generation, and 3) the variation introduced by replication (explained more in **S**election section below).



The time for each generation is a major dimension of **e**xpansion. Viruses and bacteria can have very short generations times, as short as ~10 minutes. In contrast, humans have long generations of about 20 years.

The second major dimension of **e**xpansion is the number of progeny

19 A cell that ignores the space **g**overnor to stop dividing is a cancer cell.

per generation. In terms of offspring, some viruses produce over 1,000 new virions each generation, whereas Bacteria and Archaea only divide into 2 sister cells each generation. A human female can produce over a dozen offspring over a generation; the average is about 2.4 at this moment.

As an illustration of how exponential **e**xpansion works, consider a human family that has four kids every generation. After five generations (~100 years) the family will have grown from 2 to over a thousand. Rabbits, the famously fast **e**xpanders, can have litters of 4-6 baby rabbits three times a year.[20] Give a rabbit three years and the original population of two will have become over 20,000 rabbits. Bacteria have shorter generation times (i.e., they are faster at reproducing), and some can double about every 10 minutes. That means that one bacterium could go through 144 generations in one day. This could produce an unimaginable number of 2×10^{43} daughter cells in one day.[21]

Viruses, however, are the exponential **e**xpansion winners. They can replicate as fast, or faster, than a bacterium and they produce anywhere between 25 to 10,000 new viruses per generation. That means one virus, could in theory, produce so many viruses in a day that your calculator just returns an error (e.g., 25^{144}). Worldwide there are about 2.5×10^{25} new viruses produced every second. Got that? 25,000,000,000,000,000,000,000,000 every second.

In nature, exponential **e**xpansion is limited by the rest of P.H.A.G.E.S. While those fecund rabbits could theoretically produce 20,000 rabbits in three years, this never happens in the real world. In the P.H.A.G.E.S. reality, there would still be a total of two rabbits three years later because the exponential **e**xpansion of the rabbit population is limited by the **g**overnors, as well as the **p**redatory wolves, hawks, and viruses.

20 Some rabbits can have up to 14 kits per litter and are capable of having a litter a month.

21 Youle, Merry, Matthew Haynes, and Forest Rohwer. "Scratching the surface of biology's dark matter." Viruses: Essential Agents of Life. Springer, Dordrecht, 2012. 61-81.

The third essential dimension of **e**xpansion is variation. Biology is not perfect and during each **e**xpansion event, some variations are introduced. The variations that you hear most about are changes to the DNA code and are called *mutation*s. Humans and bacteria have mutations in about 1 out of a 100 million letters of their DNA code per generation.[22] Viruses have mutations in about 1 out of 10,000 letters.[23] All of this variation is fodder for **S**election.

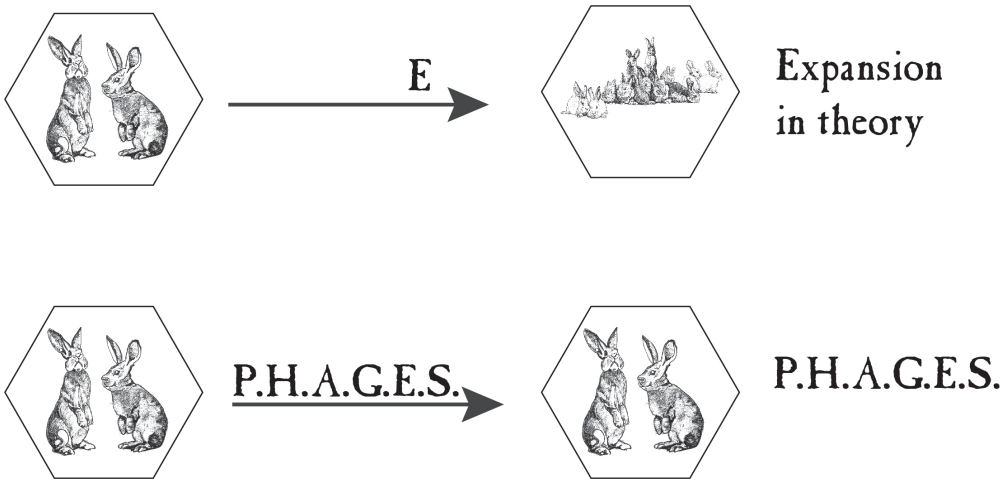


Figure 4.9. Power of Exponential Expansion versus the Power of P.H.A.G.E.S. In theory two rabbits can produce 6 babies every four months. If half of those baby rabbits become adult rabbits, then they will go on produce 6 babies of their own. After 3 years, there will have been 9 generations. And each female rabbit will have produced three more female rabbits. This is like multiplying 3 x 3 x 3 x 3 x 3 x 3 x 3 x 3 x 3, which equals 19,683 new rabbits (like my wife, we can just ignore the males for this calculation). The other way of writing this is 3^9 or 3^9 or 3 rabbits raised to the power of 9 generations. The other P.H.A.G.E.S. processes severely limit this exponential **e**xpansion such that only 2 rabbits will actually survive through year 3. These severe limitations **s**elect for the "fittest" rabbits.

22 Scally, Aylwyn, and Richard Durbin. "Revising the human mutation rate: implications for understanding human evolution." Nature Reviews Genetics 13.10 (2012): 745.

23 Viruses have a very large range of mutation rates. There is an interesting discussion of why in Duffy "Why are RNA virus mutation rates so damn high?." PLoS Biology 16.8 (2018): e3000003.

Selection

Expansion means that organisms compete for the **g**overnors, to avoid **p**redators, and to be parts of **a**ssemblies. Also, during **e**xpansion replication is never perfect and creates variants.[24] Coupling this variation with competition results in **s**election, the **S** of P.H.A.G.E.S.

Selection is the process by which one individual is picked over another. There are lots of different types of **s**elections. *Artificial* **S***election* occurs when a human breeder chooses which animals or plants will be mated to produce offspring. Let's say the breeder decided to only mate short, yellowish dogs with breathing problems to other short, yellowish dogs with breathing problems. After a while, all the puppies will be short, yellowish dogs with breathing problems, and you have Pugs. This is a very strong **s**election, because the human breeder has explicitly only chosen one type of dog for mating.

Another important type of **s**election is *Sexual* **S***election*. If the short, yellowish dogs with breathing problems were left on their own, then some of them might find very large, Great Danes sexy. Over time, the short, yellowish dogs with breathing problems would be mixing-n-matching with the large, Great Dane types. The more the Pugs prefer Great Dane types, the faster Great Pugs will appear.

The most famous **s**election is *Natural* **S***election*, introduced by Alfred Russell Wallace and Charles Darwin in 1858. If individual wolves are competing with each other for the elk, then variation, introduced during **e**xpansion, will mean that some wolves will be a little better at hunting and killing elk. These wolves will have more access to the energy and matter **g**overnors represented by the elk and will **e**xpand a little more by producing more offspring. Because of the massive potential of exponential **e**xpansion, very small variations can have very large effects on the final numbers of the successful wolves through Natural **S**election.

24 It is important to remember that the mistakes introduced during replication are random.

The other P.H.A.G.E.S. processes are the arena upon which Natural **S**election occurs. Instead of competition for **g**overnors, the wolves might experience **s**election driven by a viral disease. That is, **p**redation by viruses on the wolves. Or one wolf might, because of **h**istory, be **a**ssembled into a pack that isn't very good at hunting. The scenarios are complicated, with lots of moving dimensions. That's why evolutionary biologists often compress all of these details into a dimension called *fitness*. We have reasonably complete mathematical descriptions of the different **s**elections and related concepts like fitness. And we could spend the rest of this and 100s of other books arguing about them. Instead, let's go back and differentiate between two types of **s**election processes that are important as we try to understand wholobionts and other ecosystems: evolution and *acclimatization*.

Artificial, Sexual, and Natural **S**election are all evolutionary processes. That is, variations are differentially passed on to the next generation. There are also a large number of **s**election processes that do not involve inheritance between generation. For our purposes, we are going to group these processes under the moniker acclimatization. The difference between evolutionary **s**election and acclimatization is a little subtle, so let's revisit Tom Patterson's story. All humans have an immune system that is heritable. The immune system is under evolutionary **s**election.[25] Over the course of his life, Tom's immune system developed to kill viruses, microbes, and parasites that he encountered. This immune response was built by **e**xpansion and **s**election of immune cells. However, none of Tom's hard-earned immunity against *A. baumannii* will be transferred to his children. His specific immune responses were produced via **e**xpansion and **s**election, but they are not heritable between generations and are an example of acclimatization. The distinction between evolution and acclimatization processes will become more important to our discussion later.

25 The three pillars of evolutionary theory are variation, **s**election, and inheritance. Without inheritance, **e**xpansion and **s**election are not evolutionary.

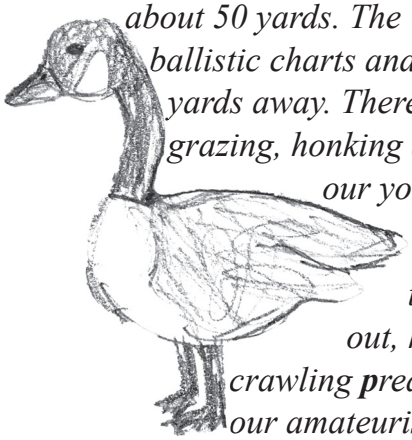
The Goldilocks Line, P.H.A.G.E.S. and Wanting to Tear Your Hair Out

Biology is complicated. Many people throw up their hands and do one of three things: 1) try to come up with simple, causal explanations, 2) dig so deeply into one subject that they become "the expert", or 3) accept defeat and decide that it is just too complicated. Don't despair just yet. And don't go the route of simple causal relationships; this is the kind of thinking that has led to tens-of-thousands of diet books and the concurrent expansion of everyone's waistlines. Likewise, an expert that only sees their very narrow view of the world is the same person writing those useless diet books. Any biological system can be understood by applying the Goldilocks Line and P.H.A.G.E.S. thinking. It just takes some patience and practice.

Acute Assemblies

Section II

The Charge of Rosebud



Distance was the problem. Effective shotgun range is only about 50 yards. The Canadian Geese had read the same ballistic charts and sat in the middle of the field, hundreds of yards away. There they frolicked in their fowl ways; grazing, honking and in general mocking us. It wore on our young, wanna-be-goose-killer minds.

*At first, we tried belly crawling through the cow pies and snow. Geese, it turns out, had been watching for foxes and other crawling **p**redators for millennia. They laughed at our amateurish attempts, flying away with dismissive honks.*

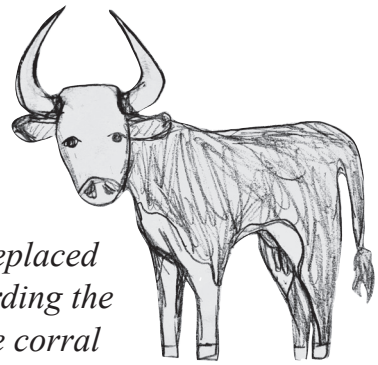
Decoys were next. Franklin and I set out plastic geese and then laid in the snow under a sheet. The thermodynamics of an Idaho winter quickly took over. As frostbite set in, we would stand up and try to restore circulation by jumping around every 30 minutes or so. Our black-and-white speckled tormentors chose these interludes to do flybys.

Even more annoying were the cows. They thought the sheets and decoys made up some sort of bovine playground. First came the sniffing and knocking over of decoys. Then they would lick and pull at the sheets. Finally, as cows are always doing, they added green poop to the pristine decoy site. There is no mercy in a cow's heart.

The herd was led by Rosebud: 2500 pounds of purebred Hereford with foot long horns and the absolute Queen of the Field. She moseyed, as only cows can mosey, through flocks of geese and the decoys with equal impudence.

"It's like geese aren't afraid of cows." chattered Franklin as he

did jumping-jacks in a futile attempt to avoid hypothermia. "Rosebud just walks amongst them like..."



Frozen neurons started to thaw. And so was born the Cow Hunters. Not to actually hunt the cows, rather we would ride them into the battle.

The frozen cow shit covered sheets were replaced with red blankets while mom was not guarding the linen closet. Rosebud was tempted into the corral with apples and soon Franklin and I were mounted, complete with red blanket camouflage, on our cow steed.

All that was left was the charge to glory. This took a lot longer than is portrayed in the Charge of the Light Brigade. *Unlike destrier charges, a cow charge proceeds at a leisurely pace, with very little direction. And whoa to anyone who tries to speed up their cow charge with a kick to flank. You could hear the geese's laughter.*

After many false cow charges, we accidently meandered in the general direction of the geese. Eventually we were in the middle of our victims, easily within the magical 50 yards. All that remained was the slaughter.

Ever try to remain on the back of a stampeding, twenty-five-hundred-pound cow who has just had some idiot shoot a shotgun while riding her?

Like all great war plans, The Charge of Rosebud *did not proceed as envisioned. Engagement with our Canadian foes ended with only one casualty, a mud-jammed gun barrel. But we innovated and adapted. Other cow charges were successfully executed, and we got our Christmas goose. And this human adaptability is a major challenge for the natural world, we are amazingly good hunters and fisherman. We have dramatically shifted whole ecosystems by killing off other big* ***p****redators. This has effectively turned much of the world into hunting grounds dominated by viruses and humans.*

Chapter 5.
Wolves and Yellowstone

Humans are the world's greatest macrobial **p**redators. Individually, we might fear other **p**redators like wolves and sharks, but they don't constitute a threat to us as a species.[1] These **p**redators do kill our livestock and compete with us for wildlife resources. Individuals' fear of **p**redators, as well as the economic considerations,[2] has led to the systematically killing of these animals throughout the world. Of particular note, was the removal of the last wolf from Yellowstone National Park in 1926. Many people celebrated this event as a new era of safety and health for the park and its guests.

In the subsequent wolf-free years, the park itself went through a series of ecological changes. The most dramatic fluctuations occurred along the rivers, called *riparian zones.* These areas are studded with various other water-loving trees and plants, whose roots are important for erosion control.[3]

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1 Howling wolves only signals an unhappy ending for elk and their like. Wolves are not a threat to humans. There have been fewer than 20 wolf attacks over the last century and only 2 were fatal.

2 Many **p**redator populations have been severely depleted for fur and food. The most egregious at this time is shark finning, which is strongly contributing to destruction of ecosystems like coral reefs. Read "Coral Reefs in the Microbial Seas" by Rohwer and Youle if you want to know more.

3 Beschta, Robert L., and W. J. Ripple. "River channel dynamics following extirpation of wolves in northwestern Yellowstone National Park, USA." Earth Surface Processes and Landforms: The Journal of the British Geomorphological Research Group 31.12 (2006): 1525-1539. Ripple, William J., and Robert L. Beschta. "Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction." Biological Conservation 145.1 (2012): 205-213.

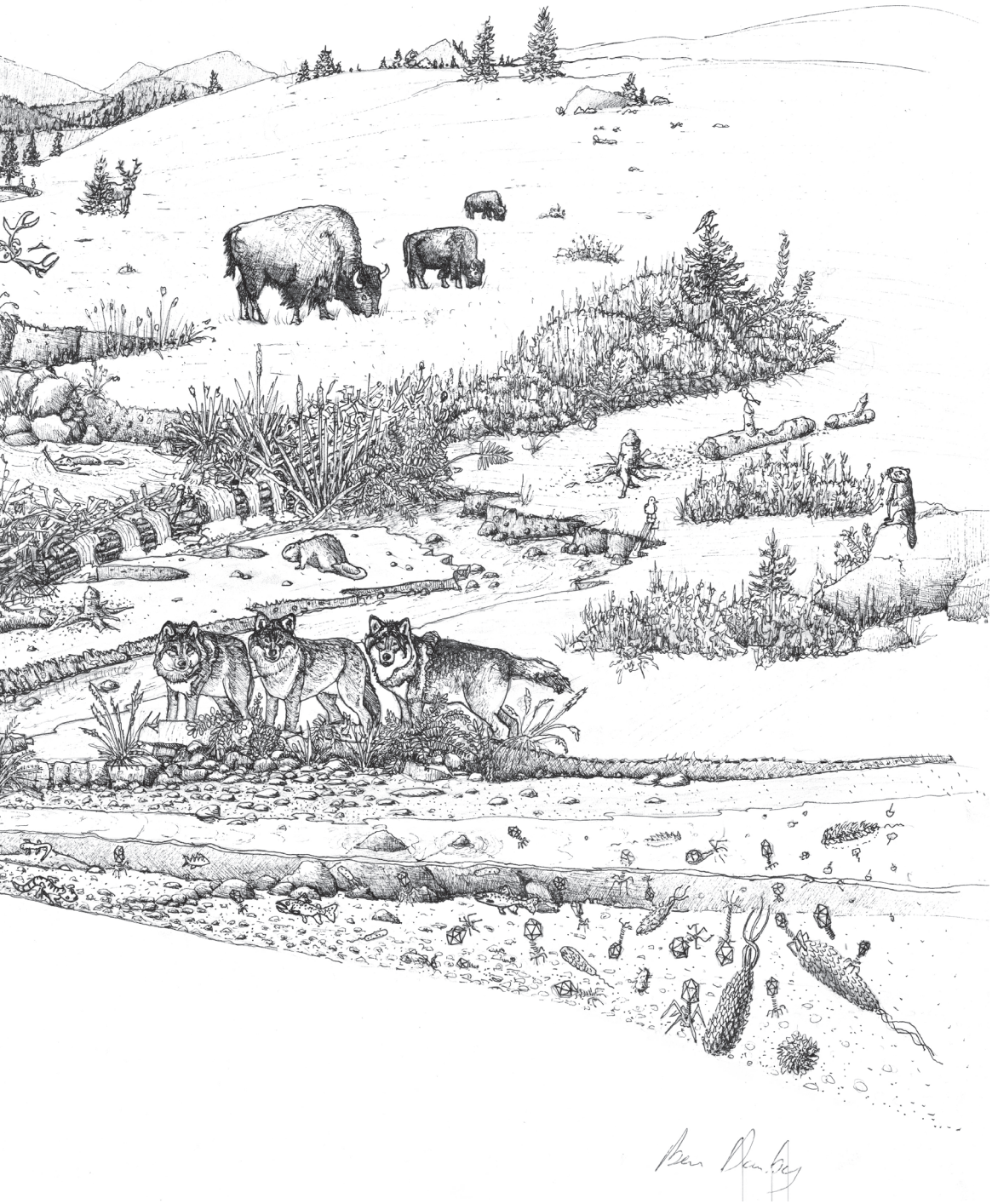
With wolves around, elk tend to avoid riparian zones because it is easier to be ambushed when surrounded by brush and small trees. However, the elk grew bolder in the wolf-free Yellowstone, and they began moving into these areas. There the elk ate tender trees like willows and aspens, eventually damaging the trees' root systems that stabilized the riverbanks. The loss of these trees also drove out the beaver that need the bark and wood for food and dam construction matter.[4]

The loss of beaver ponds and root systems, as well as the increasing number of elk hooves trampling the riverbanks, amplified erosion. Over time the crystal-clear water of Yellowstone's streams and rivers started to fill with yellow soil. The eggs of fish and other aquatic species started choking in the silt. Native fish and amphibian populations declined precipitously.

Removal of the wolves cascaded through Yellowstone in other ways. Without elk carcasses from wolf kills, the numbers of scavengers like bald eagles and bears also declined. Songbirds that depended on riparian zone willows and aspens for nesting started to disappear. Aesthetically, Yellowstone lost part of its beauty with stunted trees, water-thirsty meadow grasses, eroding soil, and fewer birdsongs. There were, however, vast herds of elk to see, and the autumn was filled with bugling bulls looking for a good fight and some lovely lady friends.

4 Persico, Lyman, and Grant Meyer. "Natural and historical variability in fluvial processes, beaver activity, and climate in the Greater Yellowstone Ecosystem." Earth Surface Processes and Landforms 38.7 (2013): 728-750.

Figure 5.1. Elk, wolves, and Yellowstone's Riparian Zones. Elk numbers **e**xpanded without wolves, and they started grazing (e.g., herbivore **p**redation) more heavily on willows that lined the streams and rivers of Yellowstone. In turn, this destabilized the banks, leading to far-reaching ecological consequences. Reintroducing the wolves (e.g., carnivore **p**redation) has restored the stream bank construction by willows (space **g**overnor).



In 1995, wolves were reintroduced into Yellowstone. The **P** in P.H.A.G.E.S. was back with a vengeance and they started killing elk, driving down their numbers. The wolf-fearing elk quickly changed their behavior and moved out of the riparian zones. Even sex changed: the boisterous, bugling bulls found out it was better to bugle less and not attract a hungry wolf pack. The declining elk numbers also meant the riparian zones started returning to pre-1920s conditions. A near-term goal of the park service is to bring back the beavers so that their ponds will raise the water table in meadows and bring back the geography of Yellowstone's "good old days".[5]

No Goldilocks in Yellowstone

Yellowstone National Park is strongly on the oxygen-rich side of the Goldilocks Line. An effective oxygen concentration of about 15% ensures that wolves, elk, beavers, and humans are all pretty happy.[6] All the extra oxygen in the atmosphere is the result of over 2.8 billion years of decoupling between sugar production and the oxygen released by plants, algae, and cyanobacteria during photosynthesis.[7] The sugar was converted to plant materials like wood and some eventually ended up as fossil fuels like oil and coal. The gaseous oxygen accumulated in the atmosphere and hydrosphere.[8]

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5 The National Park Service mission is to preserve unimpaired the natural and cultural resources and values of the National Park System for the enjoyment, education, and inspiration of this and future generations (https://www.nps.gov/aboutus/index.htm).

6 At sea level, effective oxygen concentration is ~21%. At the average elevation of Yellowstone, 8,000 feet, effective oxygen concentration is ~15%.

7 Algae is a catchall phrase for photoautotrophs that are not plants. Roughly, this means that algae do not have roots, stems, and leaves. Cyanobacteria are photosynthetic Bacteria that are sometimes called algae and sometimes not...sorry. In the **h**istory of Earth, cyanobacteria are extremely important for oxygenating the atmosphere.

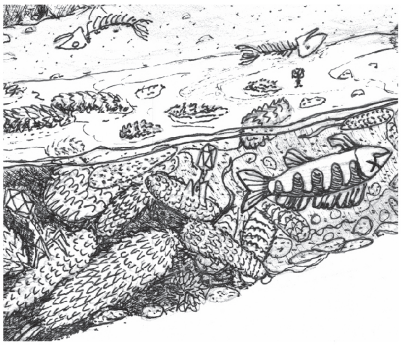
8 There was no free oxygen in the atmosphere until photosynthesis produced enough oxygen to oxidize most of the reduced compounds in the ocean and atmosphere about 2.8 billion years ago. This is creatively called the Great Oxygenation Event (GOE). Since the GOE, the atmospheric oxygen has generally increased, and carbon dioxide has gone down (from about 0.4% to 0.04%). Much of this carbon dioxide is stored in fossil fuels, which humans are now releasing back into the atmosphere by burning these old organic carbon stores…

Removal of the wolves, however, caused parts of Yellowstone to slip to the oxygen-poor side of the Goldilocks Line. The increased erosion triggered by elk moving into the riparian zones washed *organic matter* into the rivers. Organic matter is just another name for sugar and the products from sugar like wood that was stored in the yellow soil. The extra sugar in the organic matter is microbial food. Every time a bacterium eats a sugar molecule, six oxygen molecules are removed from the environment. Adding organic matter can quickly turn an oxygen-rich ecosystem into an oxygen-poor one.[9] This dynamic of increasing microbial activity by the addition of organic matter is called *microbialization*.

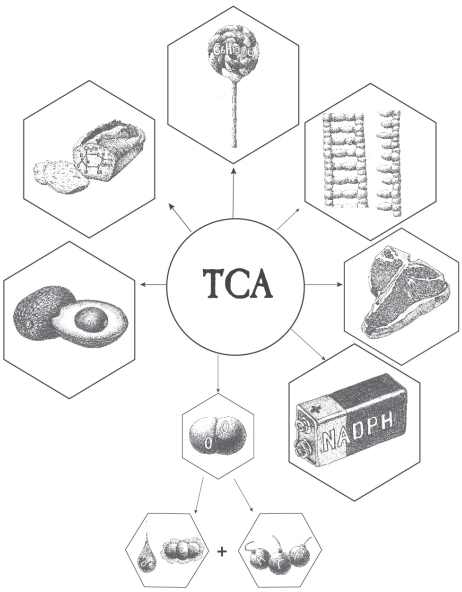
Remember the beer example? Adding lots of sugar, in the form of malt, and the yeast rapidly uses up all the oxygen. This is what happened in the Yellowstone streams and rivers; all the sugar in the organic matter led to a decrease in oxygen. This literally suffocated the fish and amphibian eggs.

9 One of the most important concepts from chemistry is Le Chatelier's Principle, which simply says that adding matter to one side of a chemical equilibrium will push the reaction the other way. Therefore, adding sugar to the respiration side will cause the system to produce more carbon dioxide and water, while using up the oxygen.

Minus wolves

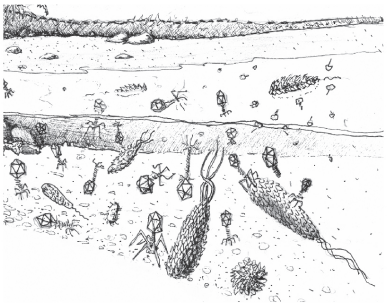


more microbes
more lsyogens
less oxygen
anabolic



Goldilocks Line

Plus wolves



fewer microbes
more viral lysis
more oxygen
catabolic

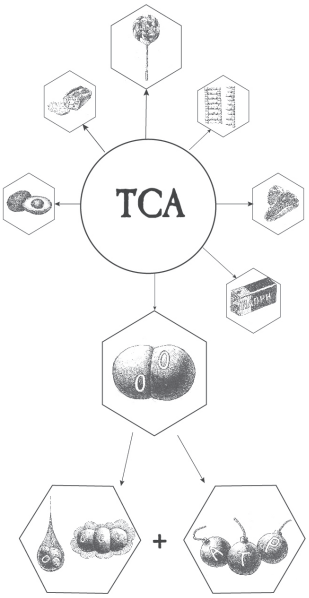


Figure 5.2. Microbialization of Yellowstone streams occurred when increased number of elk grazing led to more organic matter runoff to feed the microbes. There is so much sugar in the organic matter that microbes use up all the oxygen and become fat. This creates suboxic conditions in the streambed, which stresses the fish and amphibian eggs and larva. The high organic matter, low oxygen conditions also favor pathogenic microbes and increase incidences of disease.

P.H.A.G.E.S. in Yellowstone

Adding back the **P** in P.H.A.G.E.S., in the form of wolves, has cascaded through the Yellowstone ecosystem in a number of ways.

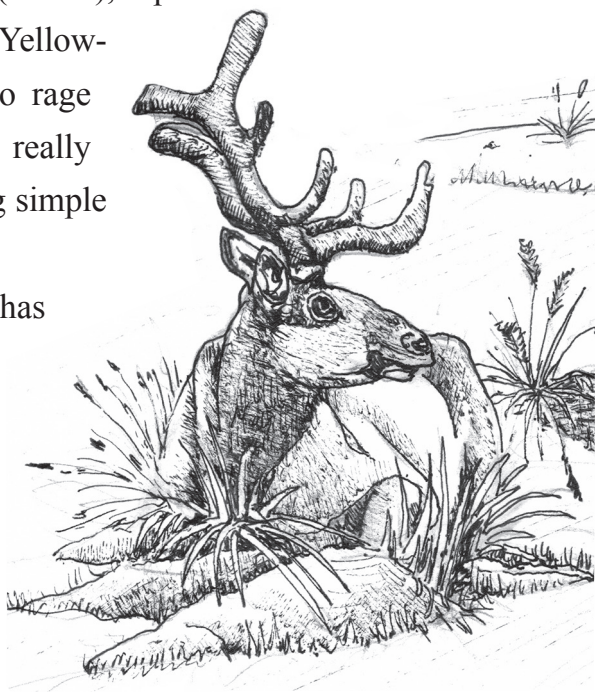
P*redators:* In addition to the wolves, humans are major **p**redators in the Yellowstone ecosystem. Controlled hunts in and out of the park regulated the elk populations for much of the 1900s. Based on other ecosystems like coral reefs, the authors also have a strong expectation that the relationship between **p**redatory bacterial viruses and their bacteria prey changed in the microbialized streams (though no-one measured this in Yellowstone). Basically, the extra organic matter encouraged the viruses to favor the temperate life cycle and hang out with their hosts as proviruses. This dynamic, called *Piggyback-the-Winner*, has important implications for diseases, and we will discuss it in more detail later.

H*istory:* Removal of wolves from Yellowstone essentially created an artificial, human-dominated ecosystem. This ecosystem was primed for dramatic changes with wolf reintroduction. This unique **h**istory also means that Yellowstone will never really return to a pre-1920's state. Instead it will continue along a unique trajectory.

A*ssemble:* The decline in aspen and willows along the riverbanks was the most obvious change in **a**ssemblage. More subtle changes occurred in the viral and microbial *biofilms* in the streams. Biofilms are assemblies build by different species of interacting microbes. You are most familiar with biofilms as the fuzz that grows on your teeth, but any rock in a clear stream is covered with a slimy layer that is also a biofilm. As the streams of Yellowstone

lost oxygen through microbialization, the biofilms on the rocks would have changed membership to use different *electron acceptors* (discussed below).

G*overnors:* As the elk numbers increased, they started to exceed Yellowstone's carrying capacity[10] in terms of energy. The energy **g**overnor was temporarily relieved by feeding the elk in winter ranges like Jackson Hole Elk Refuge (Wyoming); otherwise many of them starved.[11] The influence of the energy and matter **g**overnors in Yellowstone are even more interesting than what we have presented here. It is reasonably easy to argue that most of the changes in elk populations were caused by forest fire (energy) and changes to the water table (matter), especially in the northern range of Yellowstone. This debate continues to rage and shows how hard it is to really understand ecosystems by using simple cause and effect thinking.[12]



The space **g**overnor has also been pushed and pulled in Yellowstone; loss of the beaver dams reduced habitat for a myriad of fish, amphibians, birds, insects, mammals, as well as viruses and microbes. It has also lowered the water table, so trees dehydrate and burn easier.

10 The term *carrying capacity* refers to the population size that can be indefinitely supported within an ecosystem.

11 This is a fascinating story. Lots of people don't think the elk should be fed to re-establish the "natural order" and to ward off chronic wasting disease.

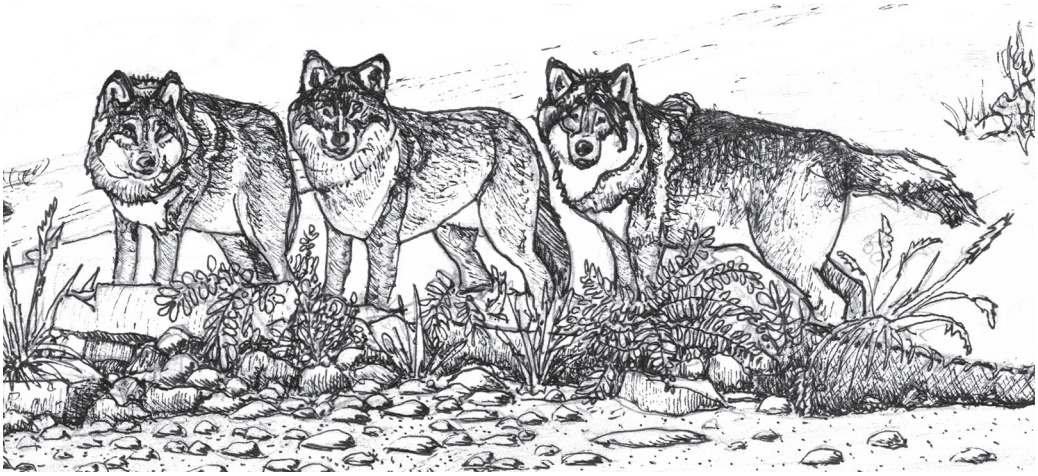
12 There is a good summary of all these moving dimensions on the National Park Service's website called "Ys 24-1 The Challenge of Understanding Northern Yellowstone Elk Dynamics after Wolf Reintroduction".

E*xpansion:* Both the elk and wolf populations showed exponential **e**xpansion in action. The number of elk in Yellowstone went from 4,000 to 18,000 in about twenty years, mostly because active management (e.g., hunting) was suspended in favor of "natural management". The wolves rapidly **e**xpanded from 20 to ~400 in the decade following reintroduction.

S*election:* Bull elk went from loudly bugling (minus wolves) to a quieter rut (plus wolves). This is an instance of acclimatization, **s**election without inheritance. The bulls learned to be quieter because of a couple of negative interactions with wolves. If you got chased by a wolf pack every time you sang loudly to a potential mate, then you would quickly give up Karaoke. Since this behavior is learned, it is not heritable. In contrast, the antlers to fight for mates is a great example of evolutionary sexual **S**election.

You as Yellowstone

By applying the Goldilocks Line and P.H.A.G.E.S. it is possible to start picking apart a complicated, multi-dimensional ecosystem like Yel-



lowstone. Note that the Goldilocks Line can be applied at any scale and can thus be applied to a stream in Yellowstone National Park to Earth to your

own body. Your tissues are relatively oxygen-rich[13], but your lower intestine is extremely oxygen-poor. The second important point is that the processes captured by the acronym P.H.A.G.E.S. are not mutually exclusive. Finally, all of the P.H.A.G.E.S. processes should be considered to make informed decisions about complicated biological systems like Yellowstone and your body. As an individual trying to make sense of life, systematically working through P.H.A.G.E.S. will help keep you from jumping to a simple cause-and-effect thinking and give you a broader, richer overview.

P.H.A.G.E.S., Goldilocks Lines, wolves, and elk may seem well and good for ecosystems like Yellowstone, but humans do not feel like they are ecosystems. It is hard to conceive of ourselves as wholobionts thronging with invisible species.[14] We can see elk and wolves, touch their fur, and smell their dusky musky scents. In contrast, there is very little connection to our internal ecosystems except by proxy. Even if your body quakes with fever chills during a viral infection, you don't actually see your foe; rather, your doctor tells you that a virus is the culprit. We still feel separate, uniquely and pristinely human. It is time to rethink human.

13 Compared to the atmosphere, the tissues of your body are oxygen low, or suboxic. This suboxic environment within the body is the result of so many cells burning oxygen and glucose. Without a constant supply of oxygen, as well as removal of carbon dioxide, your tissues would immediately go hypoxic and the pH would drop dramatically (i.e., acidosis). That's why it is so important to keep your heart and lungs working:)

14 I once told my mother about the mites the live on our hair and eyelashes. She responded, "There might be mites on your eyelashes, but not on mine!"

The Devine Decoupling

It was probably a bad idea putting a Texan in charge of wine making. Anyone from the land that produces Lone Star *as the pinnacle of the fermentative arts should immediately be suspect. But Mike assured us that he knew what he was doing and that he often made "wine". Since we didn't have any other volunteers, Mike was anointed lab vintner, and soon he was happily ordering the necessary equipment and supplies.*

The first surprise to those of us familiar with Napa, and California wineries in general, was the use of grape concentrate as the starting point.

"Don't you want to crush some grapes?", someone inquired.

"Don't worry. The concentrates work great.", was the reply from our Master Oenologist. "We can make a nice Meritage by mixing Welch's grape juice with one of the white wine varietals. My favorite is Karo syrup."

Soon the frozen grape concentrate had been mixed with water, sugar, and placed in the fermentation bottle. In went the yeast and the next morning the purple mixture was happily bubbling along as the yeast converted the sugar to carbon dioxide and ethanol.

The goal of the project was to count the viruses in the wine. We knew that viruses were essential components of all other ecosystems and assumed this would be true for wine. Samples were taken and we hit the first snag. Wine has a lot of crap in it. All the debris made it extremely hard to count the viruses under the microscope.

The wine maker went into what the labbies called "Mike's Magic" mode. Basically, he applied the scientific method to manipulate unseen forces that only he could detect. While not grounded in the physical world that the rest of us worked in, Mike was able, time and time again, to harness unseen Ley Lines to accomplish lab feats that had stymied everyone else. Many of us thought Mike's Magic arose from his frequent visits to Disneyland, but it could have just been Texan stubbornness; just keep trying stuff until something works. Basically, the same strategy that was such a success at the Alamo.

Within a couple of days Mike's Magic had paid off, and he was getting viral counts from the wine fermentation. The data were interesting. At the beginning of the fermentation, the only organisms we could see in the wine were the yeast, which rapidly multiplied as they happily chewed up the sugar and produced ethanol. At about day 10, the bubbling had completely stopped, the hydrometer showed that the wine had about 12% alcohol, and suddenly there were viruses everywhere. It looked like the yeast had finally poisoned the system enough that they were dying, and the viruses were jumping ship. We now call this dynamic Piggyback-the-Winner. For the next couple of days, the viral numbers steadied at about 10 million per milliliter of wine and the yeast continued to decline in numbers.

*Around day 14 the secondary fermentation started. Bacteria that could survive in the high alcohol mixture started to grow and along with them we saw another increase in viral numbers. Presumably these viruses were preying on the bacteria, leading to a boom bust cycle of **p**redator and prey called the Kill-the-Winner.*

Our vintner was quite happy with the results and around day 60, the bottling was initiated. As any budding wine maker knows, it's all about the labeling and

Mike's Bacteriophage Wine labels were duly produced and glued onto blue bottles. The wine was laid down to mature in the lab, and a couple of months later the first bottle was opened. And it's true, you should not put a Texan in charge of your winery…

Parts of your body, like the lower intestine, are extremely oxygen-poor. When oxygen is limiting, anabolic metabolisms, like those in wine and beer, become more important.

Chapter 6.
Your Internal River and Riparian Zones

The human body is a collection of many ecosystems defined by different geographies: your back a dry desert, your armpit a steamy jungle, your gut a dark, slow-moving river. Most of the bacterial viruses and microbes in the human wholobiont live in this river, which is formally called the *gastrointestinal (GI) tract*.

The GI river runs through a cave tunneling from mouth to anus. The walls of the cave are a barrier of *epithelial cells* that protect the inside of you from the outside world and is analogous to a riparian zone, rife with living organisms. But instead of a meadow stream with birds chirping this is a dense, crowded river. In the GI river, viruses and microbes carry out their metabolic hustles, grabbing at passing energy and matter, and fighting for space.

The mouth is the headwaters of the GI tract. The lining of the mouth is covered with viruses and microbes, but the teeth are the prime real estate. Teeth are permanent rocks in the river, and they provide important **s**pace where microbial biofilms form to scavenge the food, water, and air that is continually flowing by. How could a microbe's life get better?

Our microbial covered mouth leads to the throat cavity, also called the *oropharyngeal space*. This is a major meeting point. The viruses and microbes that are in our food join those sloughed off from our teeth and meet others trapped in mucus from the sinuses and lungs. We automatically swallow this mixture of mucosal secretions, viruses, microbes, and food,

sending them to the stomach via the esophagus. Like rafters on a river, anything that enters the path of the GI tract has one way to go: down and out the anus.[1] There are a few stops along the way.

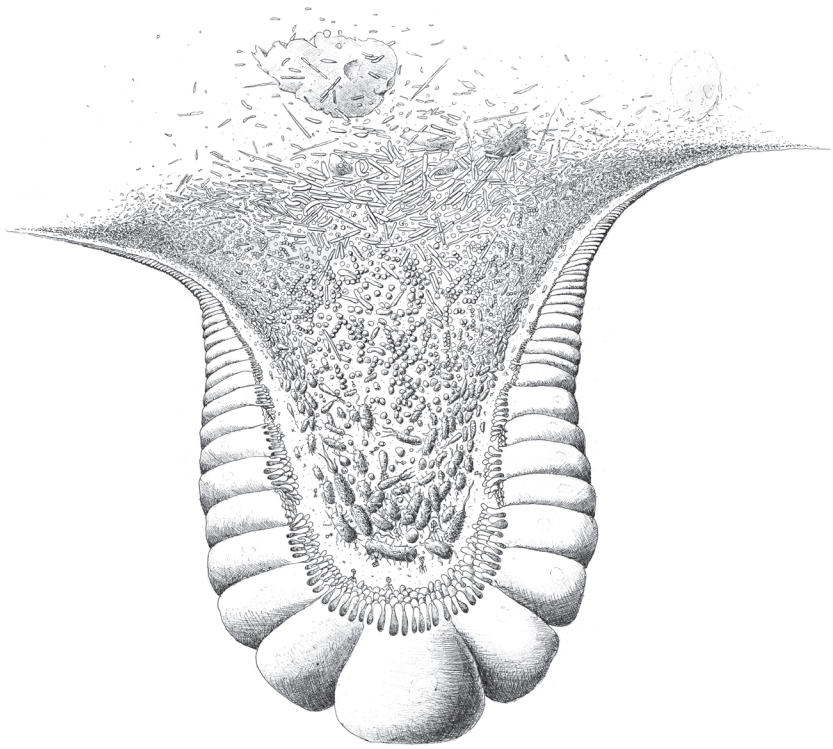
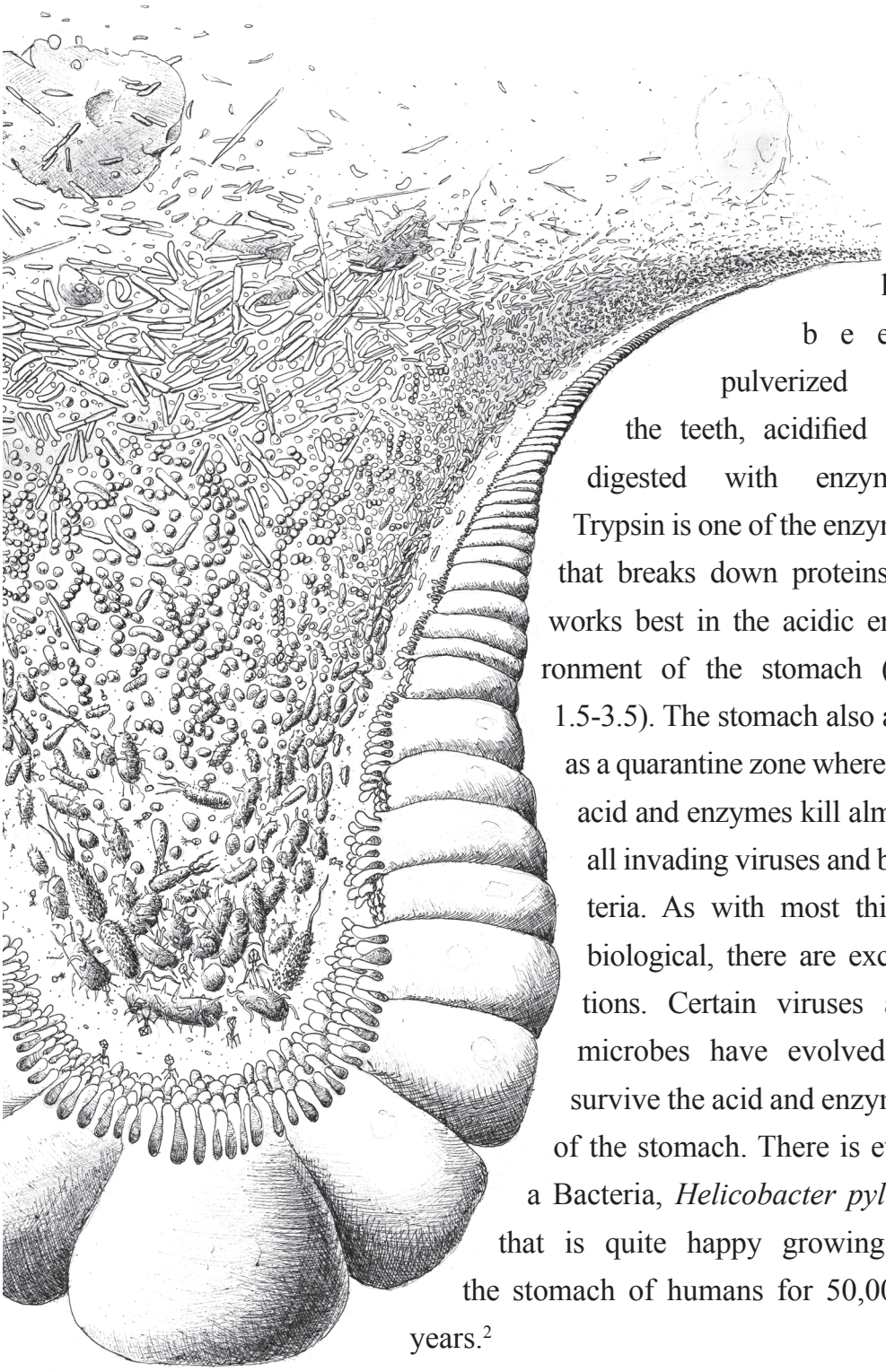


Figure 6.1. The large intestine, also known as the colon, is the major microbial ecosystem in the human wholobiont. The intestinal wall is made of human epithelial cells, which are covered with microvilli to increase the surface area. These cells are folded into bigger structures called villi, also to increase surface area. The surface of the cells is covered with mucus. This mucus protects the cells from the super-dense microbial community located in the lumen of the colon.

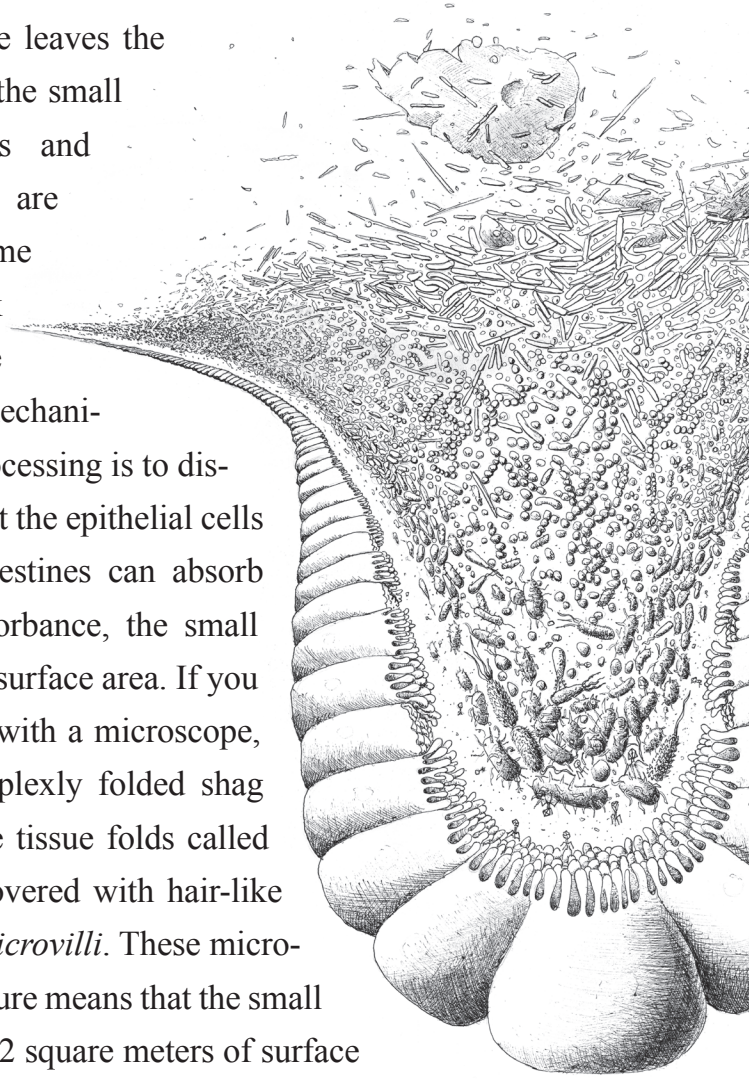
The stomach is the first large eddy in the GI river. In this acidic sluggish stretch, the gemisch from the oropharyngeal space is turned into *chyme*. Chyme is the mush you see in those rare moments when your digestive tract reverses directions and you throw up. It is the partially broken-down food that

1 With the unforgettable exceptions of vomiting and reflux.



has been pulverized by the teeth, acidified and digested with enzymes. Trypsin is one of the enzymes that breaks down proteins. It works best in the acidic environment of the stomach (pH 1.5-3.5). The stomach also acts as a quarantine zone where the acid and enzymes kill almost all invading viruses and bacteria. As with most things biological, there are exceptions. Certain viruses and microbes have evolved to survive the acid and enzymes of the stomach. There is even a Bacteria, *Helicobacter pylori*, that is quite happy growing in the stomach of humans for 50,000+ years.[2]

2 Atherton, John C., and Martin J. Blaser. "Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications." The Journal of Clinical Investigation 119.9 (2009): 2475-2487.



As the chyme leaves the stomach and enters the small intestine, bile salts and pancreatic enzymes are mixed into the chyme to further break down the food. The whole goal of this mechanical and chemical processing is to dissolve the food so that the epithelial cells lining the small intestines can absorb it. To facilitate absorbance, the small intestine has a large surface area. If you look at this surface with a microscope, it looks like a complexly folded shag carpet. The shag are tissue folds called *villi* and they are covered with hair-like protrusions called *microvilli*. These microvilli upon villi structure means that the small intestine has about 32 square meters of surface area.[3] At the base of the villi are recesses called *crypts*. Human stem cells reside inside these crypts and continually divide to make new cells. The newly made cells migrate up the sides of the villi to become epithelial cells at the surface, replacing those cells lost in the harsh conditions of the digestive tract.

3 Helander, H. F., & Fändriks, L. (2014).“Surface area of the digestive tract–revisited.” Scandinavian Journal of Gastroenterology, 49(6), 681-689. About 2 m^2 of this surface is in the colon.

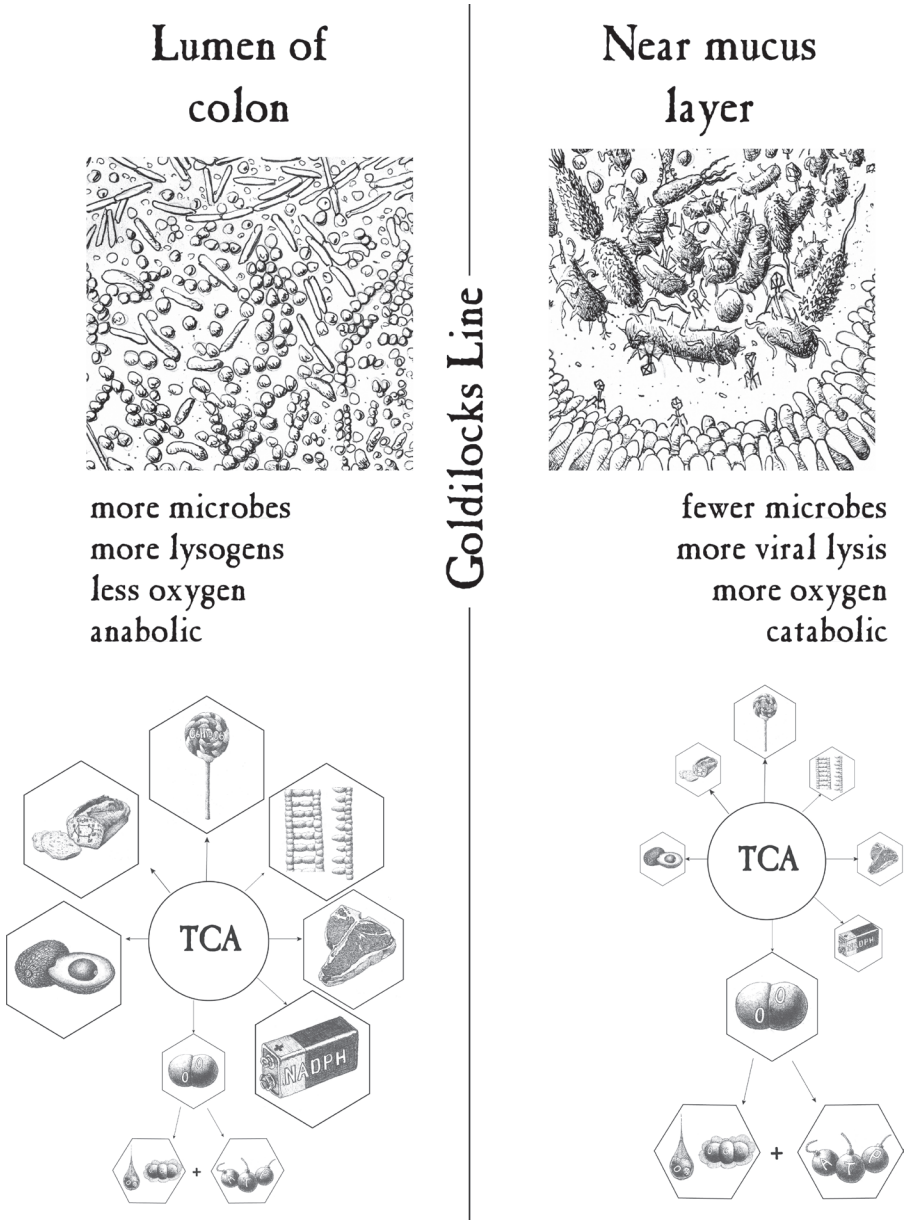


Figure 6.2. Oxygen and mucus gradients in the colon. The lumen of the colon is essentially anoxic because of all the viral and microbial activity. Oxygen increases closer to the wall of the colon where the oxygen is replenished from the blood. The Goldilocks Line occurs somewhere in this oxygen gradient. As the oxygen increases, more viruses switch to the lytic lifecycle (i.e., they are induced). Bacteriophage with mucus binding domains accumulate in the thicker mucus layers close to the human cells. BAM Immunity predicts that the bacterial viruses kill bacteria trying to predate on the epithelilium.

The small intestine also forms a protective wall against pathogens that survived the stomach, bile, and pancreatic enzymes. Some of the new cells produced in the crypts will differentiate into mucus-secreting goblet cells and Paneth cells that produce antimicrobial peptides called defensins.

The mucus is our primary protector from viruses and microbes that would like to get into our body and eat us. This slime layer coats our internal riverbanks. Mucus is divided into two layers: a dense, and imaginatively named, *adherent mucus layer* that sticks to the surface of the human epithelial cells. Over this thick mucus layer is a second, more loosely organized, *free-floating mucus layer*. The free-floating layers moves along the surface of the adherent mucus. In the small intestine, the free-floating layer is rapidly washed away. This keeps the mucus layer from clogging and maximizes matter and energy absorbance by the body.

In the lower intestine, the GI river is slower, and the mucus is thicker. It is in the lower intestine, also known as the *colon*, where most of the GI viruses and microbes are found. There are ~100,000,000,000 viruses and microbes in every gram of fecal matter in the colon.[4] The colon is a great example of the space **g**overnor. To pack so many viruses and microbes into such a tight space, GI microbes build high-rises that stretch out of the mucus into the *lumen* (i.e., the poop) where they can scavenge energy and matter.

Development of the GI Tract

The human gut structure begins forming around the second week of development. This simple tube is laid out with external epithelia that delineates what is inside and what is outside the body. Everything in the GI tract is still outside our tissues, even though it is inside our bodies.

At birth, the basic villi of the small and large intestines are in place.

4 Sender, Ron, Shai Fuchs, and Ron Milo. "Revised estimates for the number of human and bacteria cells in the body." PLoS Biology 14.8 (2016): e1002533.

But these folds are not as complexly organized in an infant as they are in an adult; infant intestinal carpets are more Berber than shag. Our human genetic stories sculpt this basic gut topography. However, it is the viruses, microbes and their byproducts that finalize the shaggy shape.[5] Remember that this is important because more folds equal more area to exchange energy and matter.

The viral and microbial colonization of the GI tract that ultimately shapes the adult gut begins possibly before[6] but definitely at birth. If the amniotic fluid is sterile, then the baby gets its first inoculation of viruses and microbes from the birth canal or the skin of their mother's breast. Babies, being babies, then chew on everything and really start the colonization process. The arrival of microbes is somewhat random and every microbial civilization in the gut shapes our personal **h**istory.[7]

Many GI microbes live close to the mucus layer, secreting anchors to hold on the ever-shifting landscape. Bacteria like *B. fragilis* homestead the mucus layer in the crypts at the base of the villi. Once ensconced there, these bacteria reseed outside colonies lost in the war zone of the upper layers. There is a specific gene responsible for *B. fragilis*' ability to colonize the crypts. *B. fragilis* protects the crypt from incoming bacteria using defensive tactics like secreting toxic molecules. *B. fragilis'* specific genetic story allows them to occupy and control a unique niche in the gut landscape and dictate who else gets to live there too. This helps shape the wholobiont's health by protecting the crypts from invading, potentially pathogenic bacteria.

5 This is a little more complicated; the microbial byproducts can also come from mom's blood across the placenta. Marceau, Geoffroy, et al. "Metabolism of retinol during mammalian placental and embryonic development." Vitamins & Hormones 75 (2007): 97-115.

6 Aagaard, Kjersti, et al. "The placenta harbors a unique microbiome." Science Translational Medicine 6.237 (2014): 237ra65-237ra65.

7 Angela Marcobal et al., "Metabolome progression during early gut microbial colonization of gnotobiotic mice," Scientific Reports 5 (June 29, 2015), https://doi.org/10.1038/srep11589.

Energy and electrons

For the human tissue part of the wholobiont, respiration is the main way to get energy: o*xygen and the sugar glucose go into our cells and carbon dioxide and water come out.* Respiration is the process of burning the glucose to release energy and is strongly analogous to a fire, where wood (basically a whole bunch of glucose molecules stuck together) is combined with oxygen to release energy. With a fire, the energy can be used to do *work* like cooking or driving an engine. Cells are doing the same thing. Burning sugar by combining it with oxygen to do work, like contracting muscles to chase down an elk. And just like a fire, cut off the oxygen or the glucose fuel and the fire dies. We call these processes suffocation and starvation, respectively.

The glucose and oxygen metabolism of human cells is the most straight-forward way of getting energy from food. It also explains why we crave sugar and are extremely fond of breathing. When working hard, our tissues run out of oxygen. When this happens, our cells continue to burn glucose, but the amount of energy released is much lower, and instead of water and carbon dioxide as end-products, lactic acid builds up in our muscles. This process is called *lactic acid fermentation*. Fermentations happen when there are not enough *electron acceptors*, like oxygen, to completely burn the glucose which is an *electron donor*. For biological fires to burn and yield energy, they must be able to give away electrons. While all this might seem a little confusing, it becomes clearer when we examine the **g**overnors in more detail.

The **g**overnors - matter, energy, and space - arise from physical laws at the sub-atomic level. Recall that atoms are the main organizing level for chemistry; putting some atoms together makes molecules and lots of molecules can make a life form. Each atom has an atomic nucleus[8] with

8 Unfortunately, there are cellular nuclei and atomic nuclei. The cellular nucleus contains the DNA of Eukaryotic cells. The atomic nucleus houses that protons and neutron in atoms.

electrons zipping around it. The atomic nucleus is made up of even smaller parts, protons and neutrons, hence the term *sub-atomic*.

Protons are positively charged.[9] This positive force means that protons really hate each other. Protons can only be crammed into the nucleus by a combination of the Strong Force (read a particle physics book if you really care) and by the shielding effects of neutrons (i.e., the neutrally charged, subatomic particles). The protons and neutrons are much, much more massive than electrons. Therefore, the mass of any atom is a function of how many protons and neutrons there are in the nucleus. The number of protons in the nucleus also determines where an element is found on the periodic table. Electrons are negatively charged, and they are attracted to the protons in the atomic nucleus. The energy for life is provided by electrons racing towards the positively charged atomic nuclei.

The Universe would be pretty boring if electrons could ever reach the protons in the nucleus. They would just stick together, and nothing would happen. Lucky for us and the Universe: 1) electrons are negatively charged and hate each other, and 2) the electrons are moving at ~1% the speed of light. Like the negative poles of two magnets, electrons are always pushing away from each other. As electrons move towards the positive charges in the atomic nucleus, they run into a space-**g**overnor problem. The volume of space around the nucleus is so small that the orbiting electrons start running into themselves because they are moving so fast; a subatomic version of a dog chasing its tail. All that negativity means that electrons really hate each other, even when it is themselves that they are running into. The combination of speed, charge, and limited space means that the electrons can only get so close to the positively charged nucleus. These regions of space in an atom where the electrons can race around the nucleus getting close to the positive charges, but staying away from themselves and other electrons, are called *orbitals*. The orbital closest

9 Just to make things a little more confusing, hydrogen ions (H^+) are also free protons.

to the nucleus can only contain two electrons. The orbital slightly further out contains 8 electrons. The bigger the atom, the more protons, the more electrons, and more complicated the orbital patterns. What is important is that the negatively charged electrons always want to get closer to the positive nucleus and "fall" into the lower orbits from higher ones.[10]

Living systems feed off energy given off by electrons moving from a higher orbital to lower ones. To make it simple, think of the electrons as little bullets that can slam into an orbital and shake things up. There is a lot of energy associated with a very small particle like an electron moving at 1% speed of light.

To visualize how this works, imagine an electron racing around an outer orbital of an atom. To get closer to the protons in the atomic nucleus, the electron needs to shed some energy. This is done by throwing off a photon (i.e., light). Therefore, every time an electron moves from a more energetic orbital to a lower energy orbital, a photon is released. The most common photons released by cells are in the infra-red (long-wave, low-energy photons). That's why night vision goggles work so well to see living things at night. The other side of the coin is that electrons can also absorb photons. When this happens, the electrons become more energized and must move to a larger orbital. This is what happens in photosynthesis; absorbance of photons from sunlight cause the electrons around water to become so energized that the water falls apart into hydrogen ions and oxygen.

Alternate electron pathways

Cells organize atomic nuclei so that electrons will move from one

10 This is a Rutherford-Bohr model of the atom (i.e., essentially Newtonian physics). The more correct version is quantum in nature. While the Rutherford-Bohr model has some problems, it is useful because it is easier to visualize and captures much of atomic theory without the high-level maths of quantum mechanics. However, there is evidence that quantum behaviors are important for biological processes, like tunneling in the photosynthetic apparatus.

orbital to another in ways that allow the energy to be harvested. And microbes are the master electron manipulators; they have hundreds of ways to steer electrons to harvest energy. Since oxygen is the world champion electron acceptor, if there is oxygen around then the microbes will use it. These are the air-loving microbes called *aerobes*.[11] If there is not any oxygen around, then the not-air-loving microbes called *anaerobes* will take over. Since there are almost no macrobial anaerobes, oxygen-poor environments are dominated by microbes (e.g., beer, sewage treatment plant, sediments) and oxygen-rich environments generally tend to be dominated by plant and animal wholobionts (e.g., pristine versions of Yellowstone and coral reefs). This is why the Goldilocks Line is so important.

As we saw in the Yellowstone example, the Goldilocks Line applies at all levels of size and organization. The wolf-free, elk-rich Yellowstone National Park was oxygen-rich, but the streams became oxygen-poor because of the erosion. Similarly, we can break a wholobiont down into oxygen-rich and oxygen-poor environments. Our teeth, for example, are covered in dense mats of microbes. The aerobic microbes live on the surface of the mats, where there is oxygen-rich air. The aerobes respire the oxygen and create oxygen-poor regions below them, and this is where anaerobes adhere to our teeth.

This **a**ssemblage of aerobes overlying anaerobes serves as a basis for how microbes colonize the rest of our body, especially the colon. The aerobes live close to human tissue because oxygen seeps out from our blood system. Anaerobes live in the virtually oxygen-less luminal void.[12]

In Yellowstone, energy moves from the sun to photosynthetic plants like willows and grass. In turn, the plants are eaten by respiring elk who are

11 Aerobe means *air loving*. These microbes can only survive and process energy in the presence of oxygen. Like our human cells, these microbes will die without oxygen.

12 Molecular oxygen (O_2) slowly diffuses into water, which is one reason why there are anoxic zones associated with standing water (e.g., a swamp).

killed by predatory wolves. At each step of this food web, waste products like shit, leaf litter, and carcasses are produced. All of these waste products represent significant sources of energy. Many animals, fungi, and microbes specialize in accessing this energy in the waste products. This is called the *detrital food web*.

Poop is one of the most important parts of the detrital food web because there is lots of it. Fecal matter is on the oxygen-poor side of the Goldilocks Line. There is so little oxygen that the fungi and microbes that are poop specialists must either colonize the surface where they can get oxygen from the air or they are anaerobes and must use alternate electron acceptors. Alternate electron acceptors can be elements like sulfur or molecules like nitrate.; they just aren't as good at accepting electrons as oxygen. Since there is a hierarchy to how well different elements and molecules accept electrons, different microbial species often use the waste products from one microbe as their electron acceptor. Eventually a chain of electron transfers is set up, going from the weakest electron acceptor to the strongest.

Even as the poop-specializing microbes munch away at the detrital leftovers, the rest of P.H.A.G.E.S. keeps churning away. Specialized **a**ssemblages of insects and microbes rapidly colonize and start harvesting the energy and matter **g**overnors in the feces. These organisms rapidly **e**xpand and over time evolutionary **s**election has made them very good at getting to and eating shit. And, of course, whenever there is some set of organisms doing well, the **p**redators will be there eating them. And the most common **p**redator will be viruses.

So exactly what are viruses and why are they so successful?

Killing THINGS

"You broke that...that...that THING!", yelled the bouncer over the music and voices. "Out!" I would have argued, but instead my long-suffering girlfriend, Anca Segall, hustled me out of the bar.

The murdered THING was a baby food jar dressed up with red felt and cotton balls to look like a Santa Claus. Originating in the basement of some demonic arts-n-crafter and migrating via an unknown route, the THING eventually ended up in an Ocean Beach thrift store, where it was bought by one Dr. Jeremy Barr. A post-doc from down under, JBarr was most happy drinking beer and annoying Americans and the THING was his latest tool for the latter. All night, as we marched barr to barr, Jeremy would take selfies with the THING and some unfortunate victim. These pictures were bound to show up somewhere on social media, never to be erased. And while your colleagues might be able to forgive a drunken debauchery involving a Santa Claus-clad Gerber's jar, they would not be so forgiving for hanging out with an Aussie; even the left-leaning academics have standards.

When we made it to the 10th bar on the crawl, it was clear that the only way to get rid of the drunken JBarr and his camera was to kill the THING. And in an unguarded moment, Jeremy abandoned his glass friend to get another beer. I picked it up, smiled serenely at the returning JBarr and smashed the THING to the ground. Rarely is a such a predation event so sweet. Little bits of the THING's body scattered across the floor, its' little Santa hat and one googly eyeball stared up before I stepped on it. JBarr wailed, "Noooooo!". My victory was short-lived, while the execution of the THING was clearly self-defense, the bouncer didn't see it that way. And California,

a somewhat backwards state compared to Idaho, Texas, and Florida, did not have appropriate stand-your-ground laws to protect me. So ended my participation in the lab's first Ugly Sweater Pub crawl. It was totally worth it.

JBarr was part of The Frat, which consisted of other luminaries like Steven Quistad, Eric Hester, and their plaid-wearing colleagues from neighboring labs. The Frat's main contributions to science were comic relief and daily taste-test reports on the newest West Coast IPAs from San Diego's many breweries. Unimpaired by any frivolous lab work, The Frat had plenty of time to come up with bad ideas, including the Christmas Ugly Sweater Pub Crawl. The goal of the crawl was to get all of the people actually working in the lab to stop working so that The Frat didn't look so terrible by comparison.

Sophie was a gifted undergraduate student who naively volunteered to do her independent research project with Jeremy. It quickly became clear that experiments went much better if Sophie was undisturbed by Jeremy's "help". This left him time to draw figures claiming credit for Sophie's findings. Somewhat surprising to The Frat and everyone else, JBarr was a secret Bronie and soon Sophie's hard work was being displayed in My Little Pony colors. Figures with yellow, orange, and sparkles were used to illustrate how bacterial viruses could protect animal intestines from invading bacteria. This novel immune system was called Bacteriophage Adherence to Mucus or BAM.

It turns out that bouncers are analogous to bacteriophage in BAM. They hang around the periphery looking for troublemakers. In the case of a drunk, it is alcohol-impaired inhibition that leads to bad behavior and broken THINGs. In the case of bacteria, it is the proviruses in the genome that leads to bad behavior like getting too close to the cells lining the GI and respiratory tracts. Once the troublemaker has been found, the bacteriophage and bouncer both move fast to remove them.

Chapter 7.
Viruses and Wolves

Nerds of all levels of expertise love to argue whether or not viruses are living. In actuality, viruses are something far more interesting than the average living organism. Cells replicate by dividing into two nearly identical halves; **e**xpansion in P.H.A.G.E.S. All of the material in the two daughter cells was in the original mother cell. In theory, a vanishing small fraction of the material in your cells is shared with the very first cell. In contrast, when a virus infects a cell and makes copies of itself, no material from the original virus is included in the new viruses. The only thing that has been transferred from one viral generation to another is the *information*.[1] Other than viruses, the only other biological entity to figure out the transfer of pure information between generations are humans. Books are a great example. Viruses just figured out pure information transfer billions of years before we did.

The term *virus* describes the most diverse collection of life forms on the planet. Physically most viruses look like lunar landers. The shell is called a *capsid* which protects the DNA text of the virus, much like the lander protects the astronauts. The analogy goes even further, because the viral lander literally lands on the surface of the cell. Capsids come in a variety of shapes ranging from spherical to bottle-shaped to spiraled rods. In the

1 The term information is very confusing. Here we mean instructions by which matter is arranged in space. In other words, the information in the parent virion is forcing matter into a specific temporal-spatial pattern, using energy, to make another virus. Information in this sense is physical and the inverse of entropy.

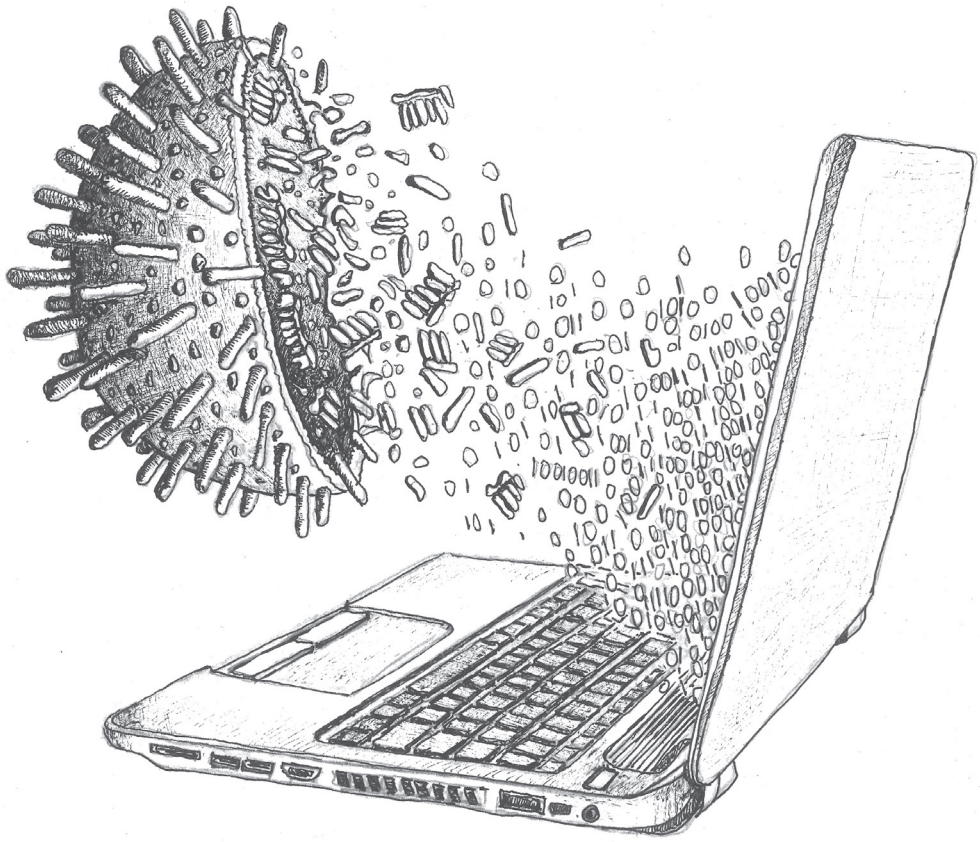
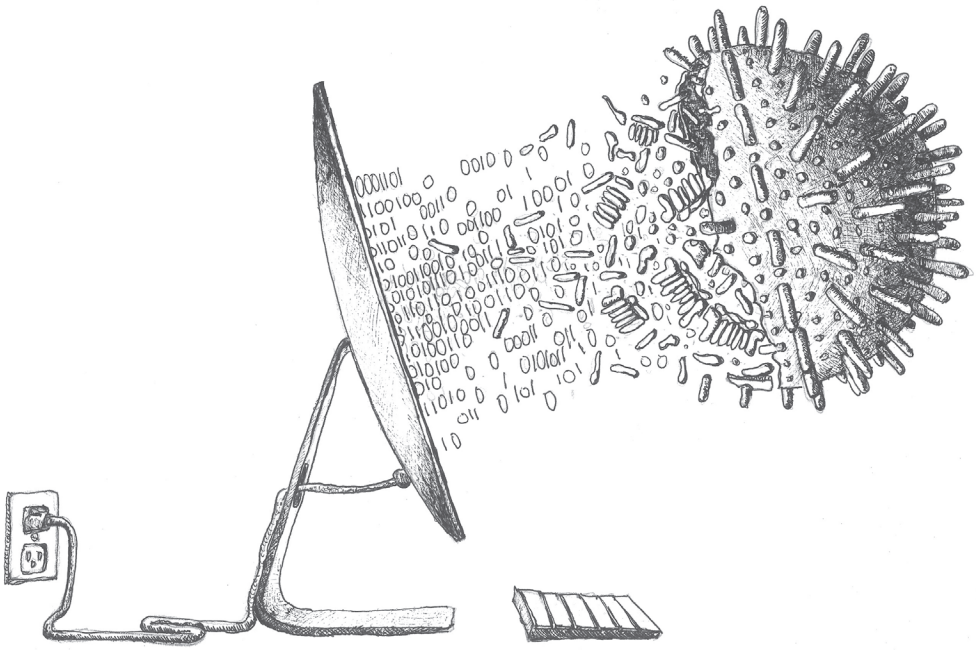


Figure 7.1 Viruses as Information: Newly minted viruses do not share matter with their mother viruses; only the information in the virus is transmitted between generations. In contrast, all cells share matter from their mother cell.

case of most bacterial viruses the virion also has a tail, which works like a syringe to deliver DNA into their cellular victim. Viruses that infect animal cells are often coated with a membrane, rather than having the syringe tail.

Every virus has a love affair with their cellular prey called *tropism*. This love affair starts with recognition; basically, the virion has proteins that recognize specific cell surface proteins and sugars on their prey. For now, just think of tropism as the interaction of the viruses with surface proteins and sugars that looks a bit like puzzle pieces fitting together.

Viruses use the unique surface proteins and sugars to find their cellular prey. To do this, many of the bacterial viruses have little legs, called



tail fibers, that "search" the surface of a cell trying to find structures that match their feet. When that match is found, the virus can lock onto the cell's surface.[2] This is how viruses hunt specific cells.

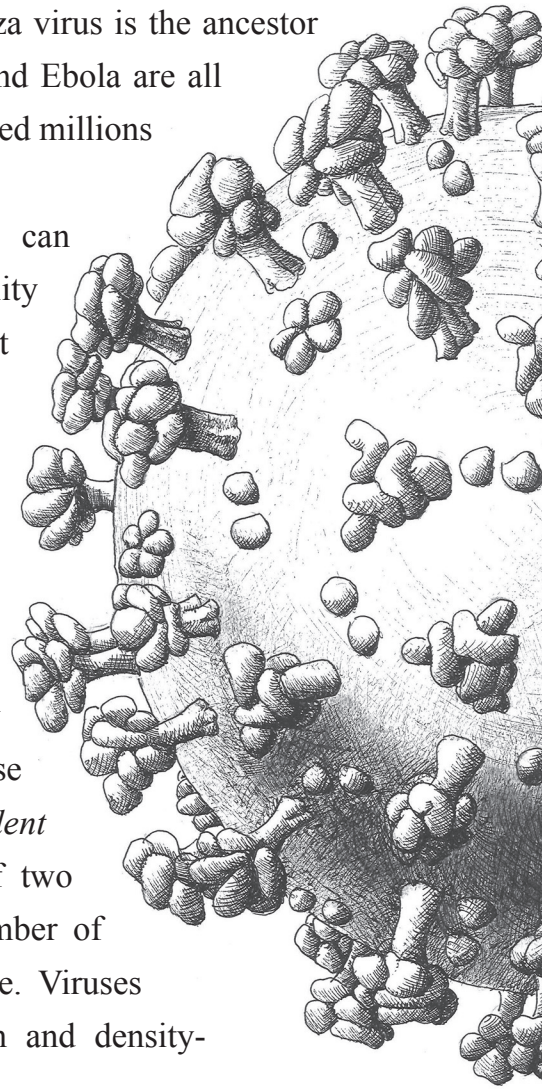
Viral tropism is often very strict. A T4 virus only infects certain strains of *E. coli*. In general, human cells can't be infected by a virus that is specific for elk and most viruses of multicellular organisms only infect certain cell types like the liver (e.g., hepatitis viruses) or immune cells (e.g., HIV).[3]

A *spillover* occurs when viruses evolve a new trophism and infect other cell types; this is a result of **e**xpansion and **s**election. Bird flu and swine flu are both influenza viruses with tropisms for bird and pig hosts.

2 Recent breakthroughs in microscopy have led to some pretty amazing photos of phage hunting. Hu, Bo, et al. "Structural remodeling of bacteriophage T4 and host membranes during infection initiation." Proceedings of the National Academy of Sciences 112.35 (2015): E4919-E4928. Wang, Chunyan, et al. "Structural dynamics of bacteriophage P22 infection initiation revealed by cryo-electron tomography." Nature Microbiology 4.6 (2019): 1049.

3 Tropism is a major way that viruses drive genetic diversity. When a virus kills a specific cell, then resistant cells are differentially **s**elected. This leads to Red Queen dynamics, which will be discussed later.

Sometime these two viruses evolved and gained the ability to infect other species including humans. When a virus jumps species, the new host's immune system usually hasn't been educated to fight the new enemy. One of the biggest spillover in human history was the 1918 Spanish Influenza Pandemic. Tens of millions of people were killed, more people than WWI and WWII combined. The Spanish Influenza virus is the ancestor of the current H1N1.[4] CoVID-19, AIDS, and Ebola are all examples of recent spillovers that have caused millions of deaths and cost trillions of dollars.



Tropism limits what cells a virus can infect. Viruses are also limited in their ability to find a host cell. This is usually dependent on relative densities. For example, if there is one virus and one cellular victim in a milliliter of water, the virus is never going to find its bacterial prey.[5] If there are a million cellular victims and 10 million viruses in that milliliter of water, then the viruses are going to kill all the cells (assuming they have the correct tropism to infect). These sorts of dynamics are called *density-dependent interactions*. Basically, if the densities of two interacting partners increase, then the number of collisions between them will also increase. Viruses have evolved to manipulate both tropism and density-dependence.

4 Memoli, Matthew J., et al. "An early 'classical' swine H1N1 influenza virus shows similar pathogenicity to the 1918 pandemic virus in ferrets and mice." Virology 393.2 (2009): 338-345.

5 This isn't strictly true. One bacteriophage and one bacterium in a milliliter of water would run into each other once in about 11,415 years according to my mathy colleague Dr. Toni Luque. Both the bacteriophage and bacterium probably won't live that long, however.

Hunting in snot

Hunting conjures up visions of a human stalking a deer or wolves orchestrating the take-down of an elk. Viruses are also hunters; they just don't use muscles and brain power to stalk and kill their prey. Rather viruses use chemical attraction, the protein puzzle pieces, and large numbers to find their prey. In the GI tract, there are vast armies of viruses hunting in the mucus, ready to attack and kill invading microbes. These bacteria-killing viruses were probably the first immune system, protecting the earliest animals more than 550 million years ago. It's like having a personalized pack of wolves hanging out in the GI tract's riparian zone, waiting for the elk to get thirsty.

When a specific bacterial species starts to expand in an environment, it becomes more common. The "winner" so to speak. As the bacteria's abundance increases, the likelihood of running into a virus that can kill it also increases (i.e., the density-dependent interactions). When the virus finally runs into one of the bacteria, all hell breaks loose because of that massive exponential **e**xpansion of the viruses. Literally, one successful viral infection can produce millions of new viruses in a matter of hours and kill most the cells in the "winning" bacterial population. This dynamic is called *Kill-the-Winner*.

Killing-the-Winner

The Yellowstone story is a great introduction to P.H.A.G.E.S. by looking at the intersection of **p**redation and **e**xpansion. **P**redators kill prey, driving the number of prey down. In turn the **p**redators start to starve, reducing their numbers. Fewer **p**redators means the preys bounce back, and now the **p**redators have more food and start to increase. This Kill-the-Winner cycling can go on *ad infinitum*.

The Kill-the-Winner cycle in Yellowstone was broken when humans removed the wolves. Without wolves the elk prospered, the trajectory of the whole ecosystem changed, and other P.H.A.G.E.S. processes became important regulators in Yellowstone. For a while human **p**redators took the

place of wolves. When this active management of the park stopped, the elk population in the wolf-free park **e**xpanded so much that they needed to be fed by humans in the winters.

Kill-the-Winner is fundamentally an ecological dynamic. In theory, this cycling can go on forever. However, getting eaten by a **p**redator kind of sucks. **P**redation also strongly **s**elects for prey variants that can escape being killed. If a microbe manages to escape its viruses by a mutation, and that change can be passed onto its baby microbes, then it is evolution and we are off to the *Red Queen's Race*.[6]

The evolutionary arms race, characterized by the Red Queen is constant. **P**redators, including parasites and pathogens, are always trying to kill their prey and each other. The very process of living leads to constant change and living things must adapt or die. All this running just to stay abreast of the constantly changing challenges is called the Red Queen's Race. Bacteriophages are great exemplars of the Red Queen's Race. Proteins on the outside of bacterial cells are used by bacteriophage to adsorb and infect their hosts. These proteins are constantly changing to evade bacteriophage attachment. Within a reasonably large population of bacteria (~10 million) there will be at least one cell with a mutated receptor that will not be recognized by the bacteriophage. The mutant cell escapes the bacteriophage attack, while all of its brethren die an explosive death. The mutant grows happily along until a mutated bacteriophage comes along and recognizes the new bacterial structure. Bacteriophage usually have large enough population sizes that at least one mutant able to "one up" its bacterial opponent is ready and waiting to infect. This cycle of winning and losing continues as bacteriophage and bacteria all run in the Red Queen's Race to simply survive attacks from each other.

6 The Red Queen refers to Lewis Carroll's line, "Now, here, you see, it takes all the running you can do, to keep in the same place." Van Valen, Leigh (1973). "A new evolutionary law". Evolutionary Theory. 1: 1–30.

It is important to differentiate between Kill-the-Winner and the Red Queen's Race. Kill-the-Winner can be sustained simply by changes in relative frequencies of **p**redator-prey densities. Just the **p**redation and **e**xpansion of P.H.A.G.E.S. The Red Queen Race is also dependent on **p**redation and **e**xpansion, but it also includes **s**election where the ability to escape the **p**redator is passed onto the next generation. It is a never-ending race because the **p**redator will be **s**elected to kill the new prey. Kill-the-Winner is an ecological dynamic (not inherited), whereas the Red Queen's Race is an evolutionary dynamic (inherited).

More BAM Immunity and Snotty Viruses

Our immune system also uses the sugars and proteins on the outside of cells to tell *self* from *non-self*. Specialized cells called macrophages[7] migrate through our body, binding to the decoration proteins and sugars on cell surfaces. If a macrophage finds a cell that does not have the right sugars and proteins to bind to, then it recruits other immune cells to help kill the invader. These other immune responders, called T- and B-cells, also look for invaders by specific binding to surface proteins and sugars. B-cells even produce little hand-grenades, called antibodies, that float through the body looking for non-self proteins and sugars. The body can make antibodies to bind and kill almost anything. One of the main reasons you get vaccines is to direct your immune system to make antibodies.

For over 200 years a concerted scientific effort has been made to understand and manipulate the human immune system. *Immunology* is one of the greatest achievements of humankind. Major decreases in human morbidity and mortality from viral and microbial infections comes direction from the study of the immune response. Hundreds of billions of dollars have been spent on understanding how macrophage, T- and B-cells, as well

7 Oh no! Not another "phage" word. In this case, these are cells the eat viruses, bacteria, dead cells, and other debris in our bodies.

as a maze of other cell types, protect us from infectious diseases. A better understanding of the immune system is one of the best hopes for treating the remaining cancers; immuno-therapy also depends on the specific binding of cell surface proteins and sugars.

With this massive research effort, it is somewhat surprising that we can find a completely novel immune system residing in humans, but that is exactly what happened in the early 2010s. This immune system is based on the bacterial viruses encoded by the microbiome of the wholobiont. It turns out that the sticky proteins of the viruses' tail fibers are also found on the capsid-head of the viruses. And sometimes, these proteins bind to the sugar groups found in mucus. This allows the bacterial viruses to hold onto, or adhere, to the mucus. For this reason, we call this type of immunity Bacteriophage Adherence to Mucus (BAM) Immunity.[8]

Mucus binding by the bacterial viruses allows them to better hunt bacteria.[9] In the lumen of the colon, there is about one virus per bacterial prey. In the mucus, however, there are about 30 viruses for every bacterium because the viruses are holding on to the mucus. Density-dependence tells us that the viruses in the mucus are much more likely to find a bacterium to kill.

BAM works because the virus' sticky capsid proteins are **s**elected to stick to the mucus. We will talk about this in more detail, but for now just remember that the mucus-sticking proteins are as diverse as our own human immune system antibodies (more than 10^{13} variants). And like our antibodies, these viruses are **s**elected to rapidly stick to the unique structures of each individual wholobiont's mucus. Over the course of your lifetime, there have been thousands of viral strains that specifically bind to your mucus through **e**xpansion and **s**election. And these viruses are busily

8 Barr, Jeremy J., et al. "Bacteriophage adhering to mucus provide a non–host-derived immunity." Proceedings of the National Academy of Sciences 110.26 (2013): 10771-10776.

9 Barr, Jeremy J., et al. "Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters." Proceedings of the National Academy of Sciences 112.44 (2015): 13675-13680.

protecting you from invading bacteria. To really understand how BAM immunity works, we need to learn about bacteriophage display and the DNA language in the next chapter.

Bacteriophages in the Blood

There are about 10^{12} bacterial viruses in the average human's colon. Many of these viruses are binding to the mucus and killing bacteria that might try to get into your body through the epithelium; that is, BAM Immunity. In 2017, we found that about thirty billion of these bacterial viruses are taken inside your body each day.[10] This is equivalent to the total number of white blood cells from your human-derived immune system!

Previous studies by our lab and others have shown that viruses, including those that infect bacteria, are relatively common in the blood of healthy, human wholobionts.[11] It was assumed that the bacterial viruses got there through holes in the epithelial barrier. For example, imagine brushing your teeth and pushing some viruses and bacteria into your blood stream.[12] When bacterial viruses were observed to bind to mucus, we decided to test the possibility that they also made it to the cell surface. Not only were the bacterial viruses getting to the epithelial cells, they were also being transported across the cells and into the body. This process is called *transcytosis.* Currently, many details about the actual cellular mechanisms are unknown. What we do know is that transcytosed bacterial viruses can kill bacteria. That means that each day 30 billion bacterial viruses are patrolling the

10 Nguyen, Sophie, et al. "Bacteriophage transcytosis provides a mechanism to cross epithelial cell layers." MBio 8.6 (2017): e01874-17.

11 Breitbart, Mya, and Forest Rohwer. "Method for discovering novel DNA viruses in blood using viral particle selection and shotgun sequencing." Biotechniques 39.5 (2005): 729-736. Gaidelytė, Aušra, Martti Vaara, and Dennis H. Bamford. "Bacteria, phages and septicemia." PLoS One 2.11 (2007): e1145. Moustafa, Ahmed, et al. "The blood DNA virome in 8,000 humans." PLoS Pathogens 13.3 (2017): e1006292.

12 BTW this does happen whenever you brush your teeth and is one reason that dentists will ask patients with heart disease to take antibiotics before extensive dental work.

blood and lymph; potentially protecting the wholobiont from pathogens.[13]

Bacterial Viruses and the Origin of the Immune System

In the last decade, we have found that bacterial viruses are patrolling and protecting the walls and insides of our bodies. In addition to the therapeutic potentials of BAM Immunity and bacteriophage transcytosis, this

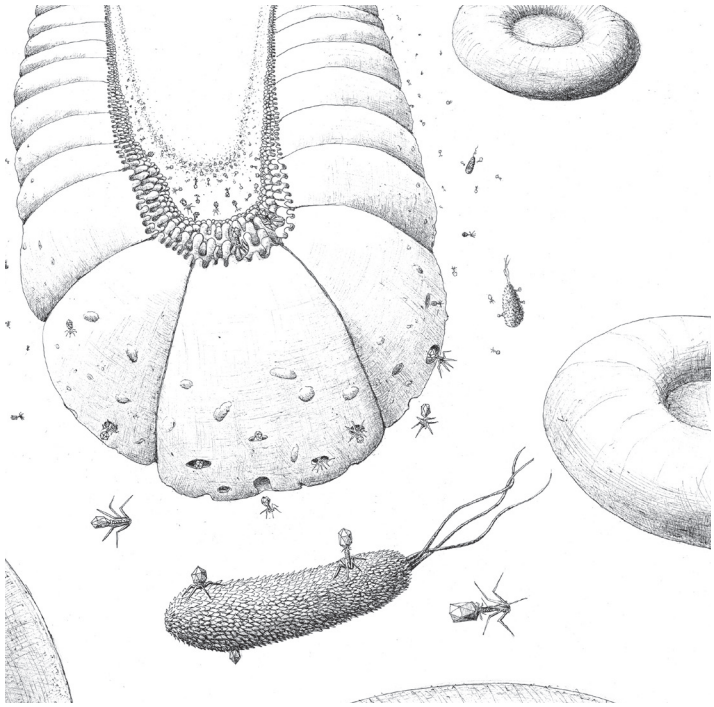


Figure 7.2. Transcytosis of phage by epithelial cells. Laboratory studies have shown that phage are transported from the apical to basal side of polarized cells. We think this means that phage are trancytosed from lumen of the gut (apical) to the lymph and blood (basal). Supporting this hypothesis is the observation the phage are extremely common in healthy human blood. Phage in the lymph and blood would be able to kill invading bacteria (i.e., defense against sepsis). Since the phage are transcytosed without any DANGER signals, the immune system would be tolerized to them (i.e., the phage would be recognized as self). This also appear to be true because no antibodies to endogenous phage are found in the blood of humans.

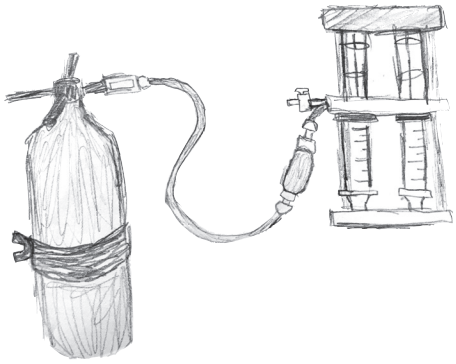
13 We actually don't know how many bacteriophage are in the body at any particular time because we don't know how fast the transcytosed virions are cleared from the body.

is just cool biology. Most animals, including the early ancestors of all of us, do not have immune systems like humans. However, all animals are surrounded by potentially pathogenic viruses and microbes. We know that the extant, living representatives of the earliest animals (i.e., corals) have BAM Immunity.[14] They probably also transcytose bacteriophage. Together, the two phenomena strongly suggest that the first immune systems actually arose from bacterial viruses.

14 Bettarel, Yvan, et al. "The versatile nature of coral-associated viruses." Environmental Microbiology 17.10 (2015): 3433-3439. Almeida, Gabriel MF, et al. "Bacteriophage adherence to mucus mediates preventive protection against pathogenic bacteria." BioRxiv (2019): 592097.

Hupsakee!

"First, hook the regulator to the tank, which is connected to the hose on the syringe. Which is connected to a filter. Oh yeah, we first need to fill the syringe with the poison to keep everything sterile. We'll let Andi do that...he's big and less likely to get a lethal dose. And I forgot the frame holding the weights to the tank. We'll just stick those together with some cable ties. Simple." My audience looked a little befuddled, almost like my instructions weren't clear. Andi and Mark kept smoking and nursing their beers; since their natural state was befuddled, I soldiered on.



"Now we need to do it right at dusk, so that the timing will be correct. Did I mention setting the timers? We'll come back to that. Since dusk is also when the sharks get most excited, we'll only put Andi and Mark in the water. Yanwei and I can supervise from the boat." This arrangement was based on sound scientific principles. Both Mark and Andi were much bigger and less likely to be eaten; this is call 'refuge based on size' by ecologists. Plus, both of them were shower-and-soap-challenged and chain smokers. Since sharks have sensitive taste buds, this combination was basically a European-based, au naturelle *shark repellent. These high offensive, protective odors are called allelochemicals by ecologists. Yanwei and I were of more of the delicate, floral ecotypes and would not be good candidates for dusk diving in shark-infested waters.*

"Now, I have designed this simple lowering system to get the sampling systems gently to the bottom. All is involved is this pulley, 16 carabiners, a few cable ties, two ropes..."

'Gooi maar them,' mumbled Mark through cigarette smoke. 'We vangen ze wel in the water.' A pause, and then the inevitable, "Hupsakee!"

Yanwei and I just stared until Andi translated the Danlish. "Just throw them and we'll catch them in the water." Helpfully, he added a, "Hupsakee!", which remains untranslatable.

I protest, "These are delicate pieces of scientific equipment, you can't just throw them overboard. Plus, they weigh about 60 pounds. What if they hit someone?"

"They had uit de weg moeten gaan." From Mark. "Hupsakee!"

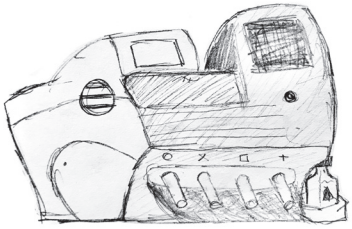
Andi translated in near-real time. "Then they should have gotten out of the way." A pause, "Hupsakee!" While not a protocol that was likely to be approved by our overly taxed dive safety officer (DSO), dropping large objects on Andi and Mark had a certain appeal. Plus the DSO had retired to bed, secure in the knowledge that no-one would be stupid enough to plan a night dive.

Having shed ourselves of needlessly bureaucratic oversight and burdensome safety equipment, Yanwei and I were soon dropping heavy equipment down to Mark and Andi. Hupsakee!

The deployments were successful at getting mid-night microbial and viral samples from the shark-infested waters and Yanwei could get to work. Up to this point, she had been sleeping between 11 pm and 5 am, but with these samples we could stop this flagrant waste of time. Yanwei was on board to run the DNA sequencer, a machine that can literally read the language of life. One goal of this cruise was to get the DNA sequence fast enough to figure out what is happening on the coral reefs while still at sea. With this data, sampling schemes could be adjusted in real time. This was pushing the technology to its limit and Yanwei had developed a number of tricks to speed things up. The conditions were less than ideal. The sequencer was latched down on the table so that it wouldn't get thrown about in the swell. Several pieces of equipment malfunctioned. And there

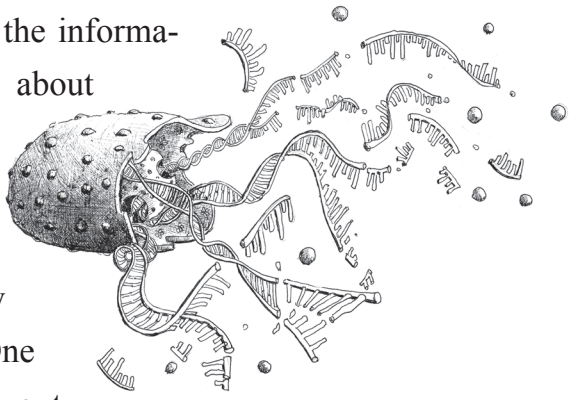
was the whole sea sick, barfing thing. However, Yanwei was able to work while lying on the floor and was soon getting the data. Hupsakee!

The challenge with DNA sequences is understanding what they are saying. That's why Rob Edwards, professor of bioinformatics, was on board with us. His job was to interpret the DNA sequences into useful terms, much like Andi translating Danlish into English. Rob designed a computer program to take the massive DNA sequence datasets generated by Yanwei's sequencer. All of this effort to get the was necessary because DNA tells the story of life if you can read it. Hupsakee!



Chapter 8.
Viruses and the Language of Life

In the early 1950's, the geneticists Martha Chase and Alfred Hershey used bacterial viruses to confirm that DNA was the informational molecule of life.[1] In their experiments, the proteins of the virion were labeled with one type of radiation and DNA inside the capsid was labeled with another type of radiation. Then Hershey and Chase let these viruses infect bacterial cells. The idea was that the differentially radiation-labeled viruses would transmit the information to make new viruses. All they had to do was determine if it was the protein or DNA that showed up in the cells. After the initial viral infection, Hershey and Chase washed away the extra viruses with a blender and found that only the DNA had been transferred into the cells. Therefore, DNA must be the information molecule, or instructions, about how to make a virus.[2]



Hershey and Chase's experiments helped initiate the scientific race to understand how DNA encodes information. One obvious thing to do was figure out the structure of DNA. The winner was Rosalind Franklin who imaged DNA

2 Hershey, Alfred D., and Martha Chase. "Independent functions of viral protein and nucleic acid in growth of bacteriophage." The Journal of General Physiology 36.1 (1952): 39-56.

using X-ray crystallography in the 1951.[3] Francis Crick, James Watson, and Maurice Wilkins then "borrowed" her results to propose the double helical DNA model. From this model, it was pretty easy to see how the molecule was copied.

DNA is a polymer, which means that it is a large molecule made of many smaller repeated subunits. This is a common theme; much of life is actually made of polymers, including DNA (made of nucleotide subunits), proteins (made of amino acids subunits), and carbohydrates (made of sugar subunits; remember the cellulose that makes up wood).

In DNA, the subunits are called *nucleotides* and represented by A, T, C, and G. The structure captured by Rosalind Franklin showed that the A, T, C, Gs form a twisted ladder, with the nucleotides pairing with each other (C-G and A-T) to form the rungs. The DNA is copied by splitting the two sides of the ladder apart and bringing in new nucleotides to form new rungs.

The process of making two copies of DNA from one is called *DNA replication* and it represents another example of exponential **e**xpansion in P.H.A.G.E.S. (i.e., two copies of DNA can rapidly become millions). Like all **e**xpansion events, DNA replication is not perfect and induces variation, which we call mutations. These mutations are one of the most important targets of **s**election.

Translating the Language

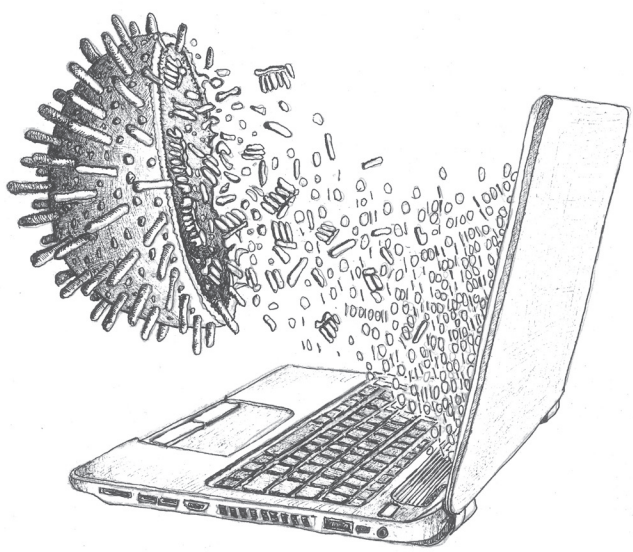
How the information in the DNA created a new virus was not so obvious. The *Phage Group* was an unofficial collection of physicists, biochemists, and biologists that set out to determine how the story in the DNA could make another bacteriophage. The goal was to use bacterial viruses to

3 Braun, Gregory, Dennis Tierney, and Heidrun Schmitzer. "How Rosalind Franklin discovered the helical structure of DNA: Experiments in diffraction." The Physics Teacher 49.3 (2011): 140-143..

figure out how life works.[4]

During the 1940s and 50s, the Phage Group and other scientists showed that the first step to build a virus or cell from DNA is to *transcribe* the information into RNA.[5] The RNA is effectively just a disposable copy of the DNA. This protects the original DNA and allows millions of copies to be made. The RNA is then *translated* into proteins. The proteins do most of the work in the cell, like turning one chemical into another. DNA-to-RNA-to-protein is the *Central Dogma of Biology*.[6]

It is probably easiest to think of the information in the DNA in terms of a language. The nucleotides are letters. And just like a language, the letters are strung together to make up words. These words are called *genes*. The magic occurs when the letters in each gene are translated into a protein. This process gives the word meaning. Some proteins convert one chemical into another. Other proteins react to photons reaching your eye



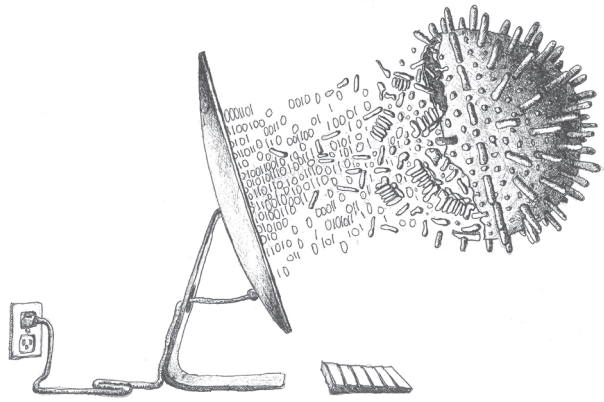
4 The Phage Group included many of the greats of molecular biology, including Seymour Benzer, Max Delbrück, Renato Dulbecco, Alfred Hershey, Salvador Luria, Matthew Meselson, Frank Stahl, Gunther Stent, James Watson, and a bunch of others. They did much of the pioneering work to determine how DNA encoded information, how DNA was manipulated by viruses and cells, and how DNA replicated. The Phage Group met in Cold Spring Harbor and trained generations of scientists in phage genetics and molecular biology. The Phage Course is still running today.

5 In RNA, the T is replaced by U.

6 Oh the joys of biology...as soon as there was a central dogma, a virus that disproves the dogma was identified. This was the Human T-Lymphotropic Virus type 1 (HTLV-I), which is a retrovirus. Retroviruses have a RNA genome, that is reverse transcribed into DNA. HTLV-I is related to the Human Immunodeficiency Virus (HIV).

and generate a signal that allows you to see. In the case of viruses, one of the genes produces a protein that makes the capsid shell [7] [8].

One of the most fascinating things about viruses, and why they were so useful to the Phage Group and other scientists, is that only the information in the DNA is transmitted between generations. Scientists regularly take the DNA chemical information in a virus and convert it into electrons on a computer (in a process called DNA sequencing). The digital information is then transformed into photons and sent through a fiber optic to a satellite station. From there, the code is sent to a satellite and beamed back down to another scientist 5,000 miles away. That scientist will then read the sequence of letters out to someone running a DNA synthesizer. Finally, the DNA synthesizer will reconstruct the chemical language in A, T, C, and Gs. This new DNA has no physical connection to the original DNA; the code has been converted into light, sound, electrons, and a chemical structure. And yet, when the newly made DNA is put into a cell, it will make an identical copy of the original virus. As mentioned above, the closest things analogous to this DNA-encoded information are human invented language and writing. In living systems, viruses are the master poets.



7 Rosalind Franklin also imaged a number of viruses using X-ray diffraction, the technique that revealed DNA's structure. Franklin, Rosalind E. "X-ray diffraction studies of cucumber virus 4 and three strains of tobacco mosaic virus." Biochimica et Biophysica Acta 19 (1956): 203-211.

8 The most important and marvelous protein in the whole world is the Major Capsid Protein (MCP). At least according to our colleague Dr. Toni Luque who studies the geometry of viruses. Twarock, Reidun, and Antoni Luque. "Structural puzzles in virology solved with an overarching icosahedral design principle." Nature Communications 10.1 (2019): 1-9.

From Language to Library

Words must be organized into sentences to convey complicated thoughts. Similarly, the protein products of genes are organized into sentences called *metabolic pathways*. We have already talked about the central metabolic pathways; these are the protein sentences that interact with the **g**overnors of energy and matter. To illustrate, let's revisit respiration where sugar is combined with oxygen to release energy, water, and carbon dioxide; the central biological fire. Like all fires, this one needs an ignition source. In living systems, this ignition is provided by the proteins in the cell. So effectively, the genes involved in central metabolism are saying, "Combust that sugar."

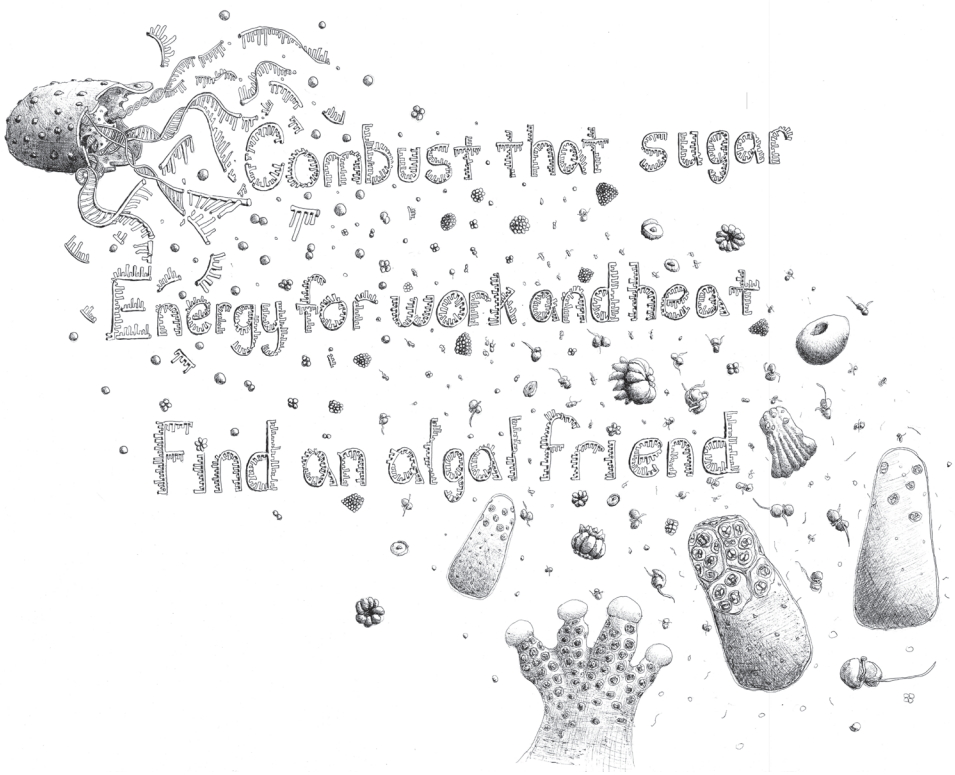


Figure 8.1. Transcribing (RNA) and translating (proteins) the DNA message builds complicated metabolic pathways that harvest energy.

Cells do not just burn the sugar and release a whole bunch of heat in a mini-explosion. Instead living systems construct complicated sentences that say, "Start burning the glucose sugar by releasing a little bit of energy that can be used to build a molecule of glucose 6-phosphate". And then another sentence is added that says, "Take the glucose 6-phosphate and turn it into molecule fructose 6-phosphate and release some energy." These sentences are added to the same paragraph until there are enough metabolic pathway sentences to build a cell. Overall the message is, "Energy for work and heat." The energy comes from photosynthesis. In a coral this means **a**ssembles with a zooxanthellae the message becomes "Find an algal friend."

The information in the DNA tells photosynthetic organisms to combine carbon dioxide, water, and sunlight into glucose and oxygen. Parts of the story tell cells to turn the glucose into carbohydrates, fats, proteins, and DNA to build more cells. Other parts of the metabolic story tell the cells to recombine the oxygen with some of the glucose and produce ATP for energy.

Not to belabor the analogy too much, but it may be helpful to think of the paragraphs as cells being organized together into chapters of tissue. And finally, the chapters into books that are the individual species. Finally, many books are found in a library, which is roughly equivalent to wholobionts and ecosystems. Together all these levels of organization contribute to the organization, and complexity, of any living story. Most people, including biologists, stop at the species/book level of organization. However, no species exists without the DNA texts of the viruses and microbes. Every organism is a library living in an even bigger biological library.

Complexity of Wholobiont

A significant scientific effort in late 20th and early 21st centuries was dedicated to determining the human DNA story encoded in the genome.

Initial estimates of the number of genes in the human genome ranged from 100,000 to a million. However, when all was said and done, humans have about 20,000-25,000 genes.[9] Approximately the same number as some of the earliest animals like corals. So where was all the complexity that gives us all the uniqueness of humans? One answer is that humans really aren't that unique.[10] A more useful answer is that complicated animals combine the same basic pieces in more complex patterns.

To give an idea of how interconnected and complex the average story-telling processes are in a wholobiont, look at the Kyoto Encyclopedia of Genes and Genomes (KEGG). There are about 22,000 gene groups (called KO; these are the words in our analogy) that belong to >1,000 main metabolic pathways (i.e., the paragraphs). This doesn't sound bad, except those genes produce over 18,000 known *metabolites* (i.e., molecules produced by metabolism).[11] And these are only the known ones. Our wholobionts also tell stories beyond the text of the human genes. When the non-human cell parts of the wholobiont, the viruses and microbes are considered, there are millions of unknown genes and metabolites.

There isn't one gene that causes you to be tall or short, introverted or extroverted, lean or curvy. Rather there are multiple genes, as well as external environmental factors that contribute to your ultimate phenotype. For example, while our DNA source text strongly influences our height, much of our actual size is due to childhood nutrition.[12] We are taller than our ancestors because we have more access to the **g**overnors of energy and

9 Willyard, Cassandra. "New human gene tally reignites debate." Nature 558.7710 (2018): 354-356.

10 It is a little bit more complicated, of course. Humans and other mammals do more with the same number of proteins than does a fruit fly. This is apparent in the interactome, which is a measure of how often proteins interact with each other.

11 Kanehisa, Minoru, et al. "New approach for understanding genome variations in KEGG." Nucleic Acids Research 47.D1 (2018): D590-D595.

12 For a fascinating and detailed read on heredity, check out Carl Zimmer's *She Has Her Mother's Laugh*.

matter and people in more affluent countries are generally taller than those in poor countries for the same reason.[13] Most of the back and forth between P.H.A.G.E.S. and the DNA code does not change the actual information. In the case of coral wholobionts, the DNA texts encode mucus-generating genes which help assemble a microbiome of Archaea and Bacteria. In turn, these microbes are essential for the wholobiont to thrive and build skeletons and ultimately reefs. The viruses, however, are happy to mess with the original information in the DNA.

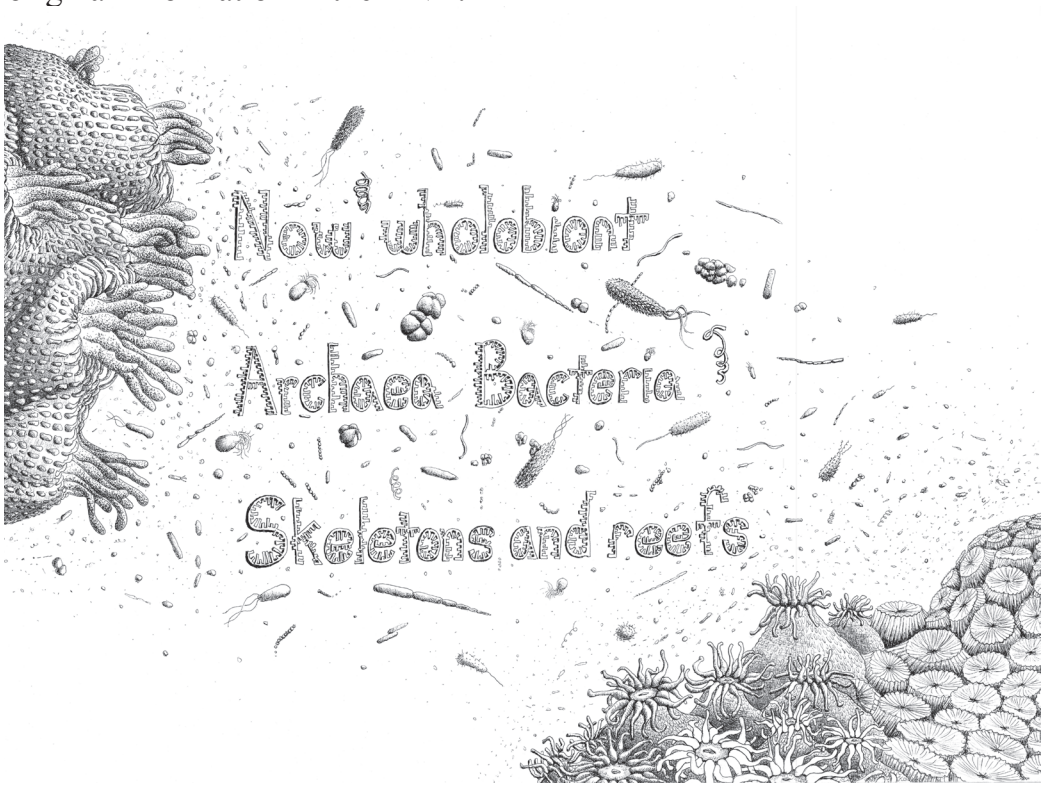
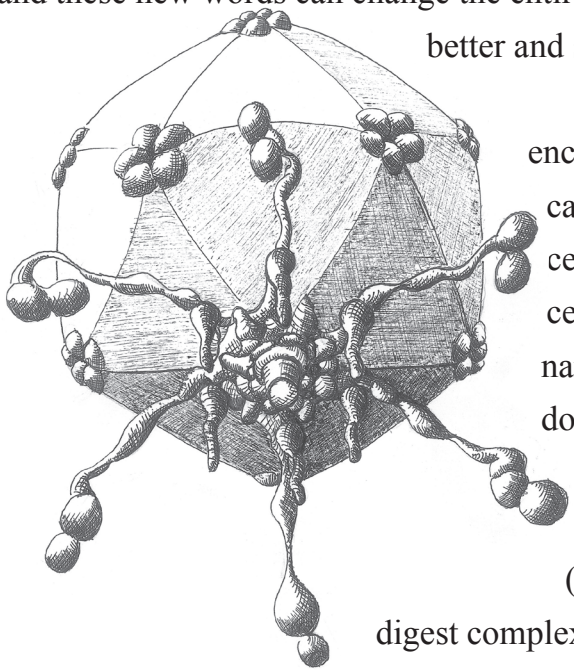


Figure 8.2. The DNA texts regulate assembly of the wholobiont. Much like a poem, however, the interpretation is situation-dependent and not strictly deterministic.

13 Peñuelas, Josep, et al. "Increasing gap in human height between rich and poor countries associated to their different intakes of N and P." Scientific Reports 7.1 (2017): 17671.

Hacking the Language of Life

Viruses alter source DNA texts with impunity. After a viral infection our cellular stories aren't just interpreted differently, they are actually transformed into new tales. It's as if the ceiling of the library opens up and thousands of pieces of paper fall in. Some have single words on them, some have strings of words. Some of the scraps fall to the floor and flutter away in a gust of wind. However, some of these papers will land on the books and these new words can change the entire story. Sometimes the story gets better and sometimes it is really bad.



Bacterial viruses strongly influence how our microbes communicate and interact with our human cells. By overlapping viral and cellular stories, there is a grander narrative for the wholobiont that doesn't rely on any singular organism's source text. Inside your colon are bacterial lysogens (provirus plus cell)[14] that help you digest complex carbohydrates.[15] Other provirus encode genes involved in pathogenesis and antibiotic resistance, and usually this isn't good for humans. Coral wholobionts stressed with too much ammonia, will assemble with viruses that detoxify the a poisonous nitrogen compound. This viral hacking is happening trillions of times every minute of every day.

14 When the viral text temporarily becomes part of the cell's genome, it is called a provirus. We'll talk about this a lot more later on.

15 Reyes, Alejandro, et al. "Viruses in the faecal microbiota of monozygotic twins and their mothers." Nature 466.7304 (2010): 334. Manrique, Pilar, Michael Dills, and Mark Young. "The human gut phage community and its implications for health and disease." Viruses 9.6 (2017): 141.

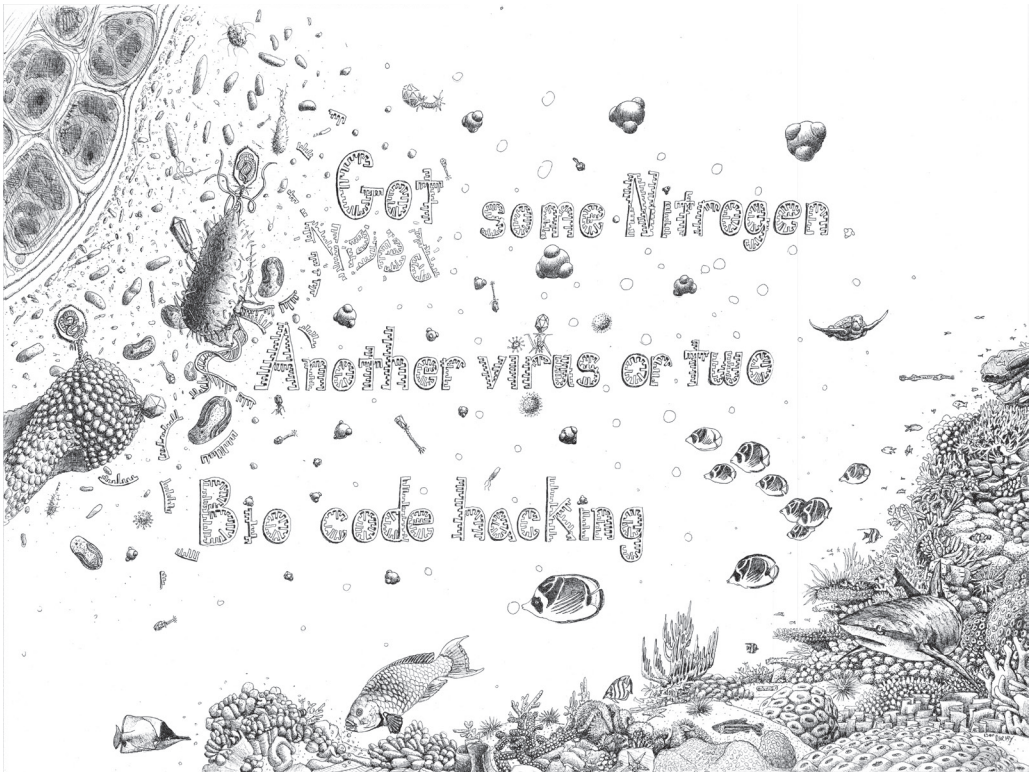


Figure 8.3. In any wholobiont, the viruses hack the DNA code. This facilitates acclimatization of the wholobiont to new conditions, but this viral hacking can also produce pathogens that harm the wholobiont.

Reading the World's Living Language

Many viral genomes are small, so the first completely known genome was bacterial virus MS2 (an RNA-based bacteriophage).[16] The first complete DNA-based genome was phiX174, a bacterial virus that infects *E. coli*.[17] And in 2002, the Rohwer lab working group introduced the idea of just sequencing all the viruses in a sample.[18] This approach eventually became known as *metagenomics* and it was important because it opened up

16 Fiers, Walter, et al. "Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene." Nature 260.5551 (1976): 500.

17 Sanger, Frederick, et al. "Nucleotide sequence of bacteriophage φX174 DNA." Nature 265.5596 (1977): 687.

18 Breitbart, Mya, et al. "Genomic analysis of uncultured marine viral communities." Proceedings of the National Academy of Sciences 99.22 (2002): 14250-14255.

the idea of reading the whole world's DNA story.

The best way to think of the metagenome is a collective of all the genomes in an ecosystem like the human wholobiont. It is equivalent to our metaphoric library of biological stories. Like an actual library, the books describing how to build the building itself is housed within.

Being able to sequence the genes of an entire ecosystem was an important breakthrough in our study of P.H.A.G.E.S. Before metagenomics, the viruses and microbes from an environment had to be laboriously grown on Petri plates in the lab. The culture-based methods are limited because many viruses and microbes are very hard to grow in the lab. These so-called *unculturable microbes* make up the majority of the microbes in the world.[19]

The culturing problem is even worse for the viruses because it is first necessary for the overworked graduate student to find laboratory conditions to raise the host cell. Then the very tired graduate student must modify these conditions so the virus can grow on the cells. This can literally take years for each virus. Not the best way to study the thousands of virus types in the average ecosystem or wholobiont.

Metagenomics avoids the challenges of culturing and allows us to directly listen to the babbling, DNA language of living systems. And what an interesting, dynamic world it has turned out to be. Early genomic and metagenomic estimates suggested that there could be 100 million viruses on the planet.[20] As we have learned more about Earth's biosphere there may be estimated 1 trillion (10^{12}) microbial species on Earth.[21] If each of these microbes is attacked and hacked by viruses, then there is an essentially infinite number of DNA texts for the rest of P.H.A.G.E.S.

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19 The unculturable microbes are not really unculturable, they are just extremely hard to culture in the lab. For example, the deepest region of our GI tracts never gets a whiff of oxygen. To culture these microbes takes special anaerobic chambers. This slows down the work and makes it much more expensive.

20 Rohwer, Forest. "Global phage diversity." Cell 113.2 (2003): 141.

21 Locey, Kenneth J., and Jay T. Lennon. "Scaling laws predict global microbial diversity." Proceedings of the National Academy of Sciences 113.21 (2016): 5970-5975.

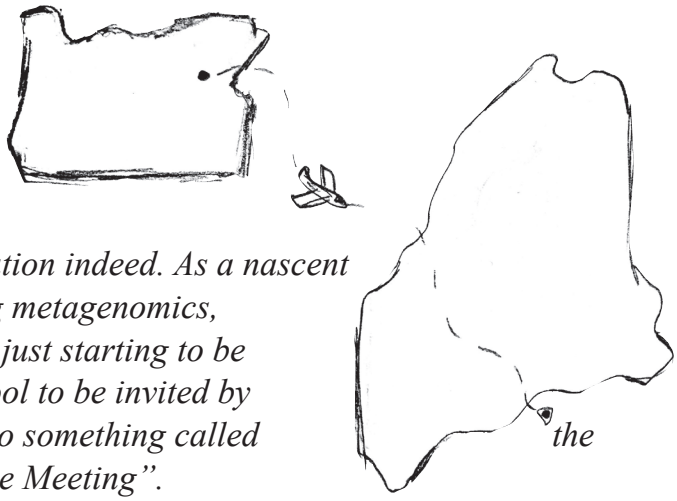
Intergalactic Phage Meeting

Dear Dr. Rohwer,

You are hereby invited to the 3rd Annual Intergalactic Phage meeting...to be held in Vinalhaven. To get there you will need to fly to Portland, take a car to Bridgeport, and then the ferry to Vinalhaven.

Sincerely,

Roger Hendrix



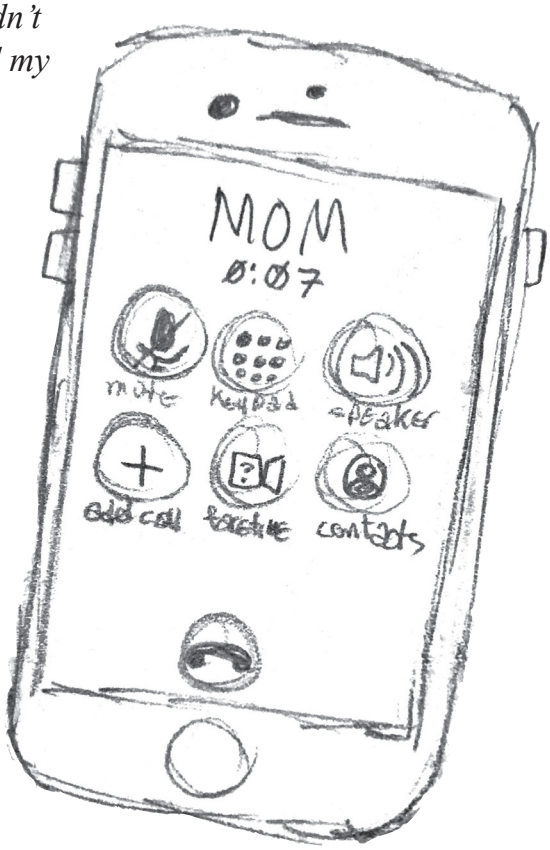
An interesting invitation indeed. As a nascent viral ecologist using metagenomics, two terms that were just starting to be used, it was quite cool to be invited by Dr. Roger Hendrix to something called the "Intergalactic Phage Meeting".

Roger was the lead author on the famous "All the world's a phage" paper, which I had read and referred to hundreds of times as I started my own journey into phage biology. A play on Shakespeare's play, this paper utilized the power of the newly emerging technology of high-throughput DNA sequencing to investigate genomic relationships between different bacterial viruses. It was really a breakthrough in our understanding of how viruses had different types of sex with each other and their microbial hosts.

To meet Roger and his colleagues at the Pittsburg Bacteriophage Institute would be great and I quickly replied "yes" without reading the email more closely. After all I knew where Bridgeport, Oregon was located; I had grown up relatively close by in Idaho. It seemed a little weird that Roger had suggested flying to Portland, Oregon, rather than the closer Boise, Idaho. Maybe there was a more direct flight from Pittsburg? I had not heard of Vinalhaven, Oregon and the fact

that Bridgeport, Oregon is basically a desert with no need of a ferry might have set off some alarm bells. However, I liked the Malheur National Forest area, so I decide to go one day early and I duly bought my plane ticket and reserved a car.

After a pleasant flight up the West Coast to Portland, Oregon and a relaxing drive through the coastal mountain range and desert, I finally got to Bridgeport, Oregon. No ferry and no Vinalhaven. I stopped at a gas station to ask directions. The clerk clearly thought I was crazy. Was the Intergalactic Phage Meeting some cruel hoax? I started to get a little worried. I was supposed to be at the meeting in less than 18 hours. So, like any mature scientist, when I couldn't figure out what to do, I called my mommy.



"No, there isn't any Vinalhaven near Bridgeport that I know of. Do you think they meant the Vinalhaven next to Bridgeport, Maine?". I was only about 3,000 miles from my destination...

With some not quite speed-limit driving, over a $1,000 of plane ticket changes for a red eye to the East Coast, and another rental car, I set the speed record for going between Bridgeport, Oregon and Bridgeport, Maine. Mainly because no-one else has bothered to go between the two places before or since.

My misguided route turned out to match well with parts of the Intergalactic Phage Meeting's talk by Roger. When bacteriophages are replicating, sequences that match each

other, like two Portlands, are recombined. This helps fix mistakes in the genetic code and is the main point of sex. However, sometimes there are two very different Portlands, one in the bacteriophage and another in the host cell. When these two strands are recombined, a mutant virus is created, much like my very circuitous route. This illegitimate recombination often adds more DNA to the viral genome. Roger and colleagues named these DNA insertions "morons" for "more on the genome". And a nice analogous to the moron that went to the wrong Bridgeport.

Chapter 9.
SEX

Vertical DNA Transfer

The **E** in P.H.A.G.E.S. is for **e**xpansion, particularly the exponential **e**xpansion of viruses, microbes, rabbits, or humans. With every generation of **e**xpansion, the DNA source text must be copied. This copying is never perfect, however. Each new source text will have some mistakes, also known as *mutations*, introduced during DNA replication. As a population grows, variations to the original text becomes more and more common. In a very few cases, the mutations are benign or even useful. However, almost all changes to the DNA text are bad.[1] That is, the stories encoded in the genomes are almost always the best for surviving in the current environment. Most organisms are the best versions of themselves and mutations introduced during **e**xpansion will lead to negative **s**election.[2]

Sex repairs the insidious creep of variation introduced by **e**xpansion.

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1 The driving forces for sex is a debate that is mostly played out with mathematical models. The best starting point for understanding sex and the necessity of recombination is Muller's Ratchet, a central axiom of evolutionary biology. Muller's Rachet basically says that deleterious mutations will irreversible accumulate in asexual populations. Recombination effectively erase the mutated DNA text and replaces it with the original story through recombinational DNA repair. Recombination can also lead to hybrid vigor via complementation. In general, recombination is the only way to escape the steady decline of a genome caused by Muller's Ratchet.

2 Roughly speaking, natural **s**election is divided into positive and negative **s**election. *Negative **s**election* refers to processes that kill off a life form. *Positive **s**election* refers to processes that increase the numbers of a life form. Negative **s**election is also called purifying **s**election, whereas positive **s**election is often called Darwinian **s**election.

The sweaty, silk sheets, and red roses kind of sex is actually about combining the genetic material from two DNA sources (i.e., the parents) to fix mistakes in the story. If one of the parent's DNA text contains a harmful mutation, then the other parent's DNA text will (hopefully) have the correct text so that the offspring survives.[3] To see how this works, we need to dive a little deeper into genomes and chromosomes.

The genome is all of the DNA text of an individual virus or cell. The genome is arranged into *chromosomes*. In our language analogy, chromosomes would be like the major subsections in a book. Some eukaryotic organisms can have hundreds of chromosomes, whereas most viruses and microbes only have one.

The chromosome-genome relationship is a little more complicated because of the *ploid number*, which is the number of copies of the genome. Most viruses and microbes are *monoploid*, which means they only have one copy of their genome, which is encoded on one chromosome. Simple. In contrast, eukaryotic cells are usually *diploid*, meaning that they have two copies of every chromosome. As an example, the human cells in your body have 46 chromosomes; 23 of these chromosomes came from your mother and 23 came from your father. This redundancy helps protects you from potentially lethal effects if one parent passes on a defective gene because there is another copy from the other parent. When a cell replicates, copies of all the chromosomes are made. Then a sophisticated cellular machine ensures that both daughter cells get the correct number of chromosomes. This process is called *mitosis*.

Sex makes everything just a little more complicated, but because of variation introduced during **e**xpansion, you can't live without it. Imagine putting two normal human cells together during sex; 46 chromosomes from

3 Inbreeding leads to accumulation of deleterious mutations. Outbreeding reduces this trend by complementing the mutated, deleterious genes with unmutated copies. The phenomena of improved fitness is called hybrid vigor or heterosis.

the mother and 46 from the father. The new cell would have 92 chromosomes, and this would be fatal to the offspring for a whole bunch of reasons. The way diploid organisms get around this problem is by replicating the chromosomes one time (92 chromosomes) and then dividing (46 chromosomes in the daughter cells). This looks like mitosis so far. However, these special cells (a.k.a., the *gametes*, sperm, eggs, sex cells) divide one more time. Now there are 4 cells with 23 chromosomes in each (i.e., they are now monoploid). Now when the parents have sex and a gamete from the mother fuses with the father, the offspring will have the correct 46 chromosomes. This unusual division is what makes the sex cells (i.e., eggs and sperms) different and is called *meiosis*.[4]

Why does sex need to be this complicated? Because during meiosis, the chromosome pairs go through recombination events, meaning that bits and pieces from one chromosome will switch places with other pieces. In some cases, deleterious mutations will be removed and replaced by a non-deleterious copy of the DNA. This is a random, but important process. Also, the recombination repair will fix many of the mutations. Together, these steps help counter the insidious increasing number of mutations into the DNA texts introduced by **e**xpansion. Finally, by having two copies of the gene in every cell (i.e., diploid), mistakes in one copy are often masked by the non-mutated copy.

Recombination fixes variations in the source text and is important for long term, faithful copying of the DNA. The DNA texts are transmitted vertically from parent to offspring and are heritable. Envision a family tree, genes from your great-grandparents percolated up through your grandparents to your mom and dad to you. Humans only transfer their genes vertically. Without a scientific break-through, it is not possible to reach across the branches of the human family tree and get genes directly from Usain

4 If you find it difficult to remember the differences between mitosis and meiosis, then remember that the "t" in mitosis stands for two cells (c.f., 4 cells in meiosis).

Bolt. Sorry to crush your Olympic dreams.[5] Also, once fertilization happens, the organism is stuck with the DNA mashup from mom and dad, the source text remains the same for life.[6]

Monoploid organisms, including most viruses and microbes, also need to engage in recombination to fix mutations introduced during **e**xpansion. There are three ways for the microbial cells to share their genes with each other. The DNA can directly pass from one cell to another through hollow, straw-like pili in a process called *conjugation*. Superficially, conjugation is most like the sex of multicellular organisms. Two cells get together and a channel is made between them. Then a copy from the donor cell is moved into the recipient cell. Recombination can occur between the two copies of DNA. When they are competent,[7] cells can pick up naked pieces of DNA from the environment in process called *transformation*. If the DNA is closely related to the cell picking it up, then there is a good probability that recombination can occur, a process called *transformation*. Finally, viruses and viral-like particles sometimes move DNA text around in ways that fix mutations in a process called *transduction*.

Horizontal DNA Transfer

Unlike the vertical transfer of DNA text within the same species (i.e., normal sex), viruses and microbes often exchange DNA with very

5 Gene therapy is starting to allow us to directly modify our human genomes. However, there is still very little chance that gene therapy will make you an Olympic quality runner because of your **h**istory. Basically, your cells grew up together and for the most part are terminally differentiated. That is, they are muscle cells, brain cells, *et cetera*. This isn't going to change by introducing a new text through gene therapy. Even introducing the text into stem cells, will not change long-lived tissues like muscles and neurons. Gene therapy is most successful with rapidly cycling tissues like blood and epithelial cells.

6 This is, of course, not completely true...oh the joys of biological complexity. The source text of some immune cells is modified. And cancers have modified source texts. And we will soon see that viruses completely mess with our source DNA text. In fact, all the cells in your body have multiple mutations. What is most important, in terms of evolution, is that the DNA text in the sex cells are as close to the original source as possible.

7 Competence is literally the ability of a cell to taking up extracellular DNA. Some cells are naturally competent. There are also tricks to make cells competent in the lab.

different species. If you imagine that family tree, then this sort of text exchange is happening between the branches (cf., vertically along the trunk and branches). Therefore, this type of sex is called Horizontal Gene Transfer (HGT). HGT allows viruses and cells to directly add DNA words, sentences, and even whole paragraphs to their DNA. This is a little bit like gaining a superpower.

A great example of HGT in the human wholobiont comes from the sushi bar. Japanese have been eating nori, the seaweed wraps in sushi, since at least 700 CE. Nori is made from an algae *Pyropia* spp. which is a wholobiont covered in viruses and microbes. Many of the nori-associated microbes are good at eating the complex carbohydrates produced by the algae. Microbes breakdown the complex carbohydrates using proteins called Carbohydrate-Active Enzymes, or CAZymes for short.[8] So, for at least 1,200 years Japanese people have been eating nori and the CAZymes genes on the associated viral and microbial communities.

The nori-eating Japanese gut is an ideal place for HGT. First, the nori has lots of complex carbohydrates that the human cells cannot access. This energy can only be released by microbes with the correct CAZymes. Second, the microbes and viruses living on the nori are adapted to seawater, not the human gut. They would never survive in the dark, dank river of the colon. However, the CAZymes genes that digest the nori-carbohydrates can survive, if they can be moved from the marine microbes to the gut microbes by HGT. This is exactly what has happened; many native Japanese have gut microbes with a nori-digesting CAZyme incorporated into their genomes. The nori-digesting CAZymes are positively **s**elected because they give the gut-residing microbes access to an additional energy **g**overnor, the nori-carbohydrates. This might even be good for the wholobiont because some of the energy from the nori will make it back to the human cells via the

8 CAZymes are also used by microbes in rumens and termite guts to digest cellulose. And not surprisingly, lots of CAZymes are encoded by proviruses.

short-chain fatty acids.[9]

The Japanese-nori-CAZyme story is a relatively clear-cut illustration of how HGT creates new stories. And there are literally trillions of these text modifiers floating around in the biosphere. Sometimes the text modifiers are simple gene words like the CAZymes, other times they are whole proviruses or other mobile elements like transposons, plasmids, and genomic islands. In all cases, they are moving good and bad superpowers around the biosphere.

MORONs and ORFans

The vertical inheritance of DNA through the Tree of Life and the HGT movement of DNA texts between different species are at odds with each other. Vertical inheritance and sex are about maintaining the *status quo* and avoiding degradation of the genomic code introduced during expansion. HGT purposefully introduces variation. How are these two diametrically opposed forces maintained? The answer lies in different types of recombination, one of the more frustrating and necessary games in biology.

Homologous recombination occurs when the DNA text of two chromosomes share lots of similarity. When 200 letters (or base pairs) are shared between two pieces of DNA, then the texts can be mix-and-matched. This is the recombination of diploid sex and conjugation between microbial cells discussed above. Homologous recombination cleans up variation and is essential for maintaining species.

Illegitimate recombination is a bit more problematic, but also essential. The CAZyme genes that moved from the nori-microbiome to the Japanese gut bacteria did so *via* illegitimate recombination. Unlike homologous recombination, the DNA texts did not need to be closely related. Illegitimate recombination only requires a very small number of

9 Hehemann, Jan-Hendrik, et al. "Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota." Nature 464.7290 (2010): 908.

letters, about 20, in the two DNA strands to be the same. In our language analogy, this means that a poem from Homer could be recombined into Catch-22. If the new text makes sense, then it may be positively selected.

The pieces of DNA text moved around by illegitimate recombination are called *morons* in viruses. The term morons come from the observation that viral genomes often have genes from other viruses and cells hacked into their genomes. Therefore, they have "more" DNA "on" them. morons were identified by comparing viral genomes. As mentioned above, whole genome sequences from the viruses were available before cellular genomes because they were smaller. Roger Hendrix, a frequent visitor to Cold Spring Harbor and the Phage Group, was one of the first to sequence a number of viral genomes. Roger and his colleagues at the Pittsburgh Bacteriophage Institute compared these viral genomic texts and identified a number of genes that appear to be hacked into an otherwise smooth storyline. It was this group that named these mobile genes morons, much to the frustration of the non-bacteriophage scientific community.

There are two reasons morons are so common in viruses. First, during the DNA replication step, most viruses express proteins that cut up the host cells genomic DNA. The purpose of this scavenging is to recycle the DNA to make more viral genomes. This means that there are lots of "free" DNA ends floating around. And if one of these ends matches the virus' DNA by 20 bp or more, then illegitimate recombination can take place. This facilitates DNA exchange between the host and their viruses.

The second reason that MORONs are so common in viruses is that virion construction requires that the right amount of DNA gets stuffed into the capsid.[10] Too little DNA and the virion is not as infective. Too much DNA and the virion will be less structurally stable. So, during the construction of the virion, the amount of DNA, not the text itself, is important. A moronic

10 Arsuaga, Javier, et al. "Investigation of viral DNA packaging using molecular mechanics models." Biophysical Chemistry 101 (2002): 475-484.

virion with host DNA can still effectively hunt and infect its next prey. It is only when the genes are transcribed that the moron is subjected to **s**election. In most cases, the morons will be bad or neutral for the virus. But in some cases, the morons will produce a viral genome more fit than the ancestor. Since this process has been happening 10^{25} times every second for several billion years, viruses have produced a biological drama surpassing anything Shakespeare wrote. In the words of Roger Hendrix, "All the World's a Phage".

MORONic Expansion

Prochlorococcus spp. are numerically the most abundant photosynthetic organisms on the planet. *Prochlorococcus* spp. dominate the open ocean and they produce about 50% of the oxygen that we breath, making them particularly interesting to microbial ecologists. One of the main questions is what is eating *Prochlorococcus* spp.? The **P** in P.H.A.G.E.S. A graduate student at MIT, named Matt Sullivan, decided to investigate this by isolating cyanophage that killed *Prochlorococcus* spp.[11] One of the coolest examples of a moron comes from these and other cyanophage.[12]

In the early 2000s we started sequencing marine bacteriophage genomes, including some that infect the host *Prochlorococcus* spp. The first set of DNA sequence data was really exciting because there was a gene central to photosynthesis, called *psbA*, in the cyanophage. While we were confirming that this was correct, Nick Mann's group reported the same gene in another type of cyanophage that infects *Synechococcus*.[13] Together

11 Just to make things a little more confusing, bacterial photosythesizers are also called cyanobacteria and the bacterial viruses that infect cyanobacteria are called cyanophages.

12 Millard, Andrew, et al. "Genetic organization of the psbAD region in phages infecting marine *Synechococcus* strains." Proceedings of the National Academy of Sciences 101.30 (2004): 11007-11012. Sullivan, Matthew B., et al. "Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations." PLoS Biology 3.5 (2005): e144.

13 Reference missing

these findings suggest that the cyanophage was making a central photosynthesis protein and subsequent studies have confirmed that this is true. So why would a bacterial virus encode photosynthesis proteins? In the upper parts of the open ocean where cyanobacteria dominate, there is plenty of oxygen. Glucose is limiting. So, to **e**xpand, bacteriophage need to keep photosynthesis going even as they kill off the host cell. Sometime in the distant past, illegitimate recombination incorporated a *psbA* gene into a cyanophage's genome. This genome was positively **s**elected. Subsequent metagenomic studies have shown that *psbA*s are one of the most common genes in oceanic viruses. Morons that help viruses and microbes acclimatize to local conditions are common.

Killing Ourselves with CoVID-19 (and other spillovers)

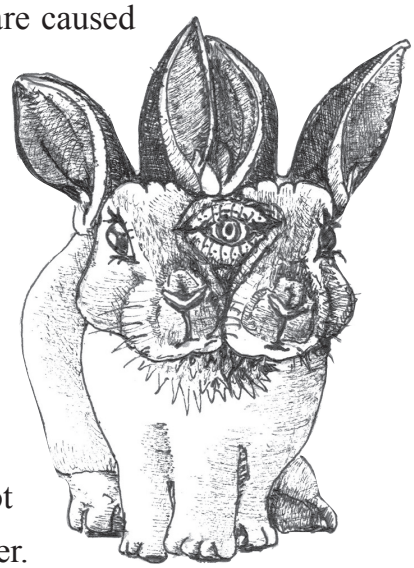
CoVID-19 and many other diseases like influenzas are human-created plagues. We do this by placing different animal species together, capturing and transporting wild animals, and butchering animals in stressful conditions (e.g., wet markets). Animals stressed by being crammed into cages, transported on noisy machines, and spending their last days in a place of death permeated with the smell of blood and feces shed viruses.

SARS-CoV-2, the etiological agent of CoVID-19, is most closely related to a bat SARS virus. We know this because the SARS-CoV-2 RNA genome is very similar to previously characterized bat SARS viruses. But SARS-CoV-2 also varies from the bat SARS at one specific part of the genome that encodes the spike (S) protein. This spike protein sticks out of the virus and allows the virus to bind and infect human cells. In SARS-CoV-2 the spike protein looks more like one from a pangolin than a bat (again this conclusion is based on genome comparisons). This chimeric

virus could have come into being via illegitimate recombination between the original bat virus and a SARS virus from either another bat or even a different species.

In the case of SARS-CoV-2, the initial analysis of the genomic sequences suggested that the bat SARS virus got together with a SARS virus from a pangolin.[14] This initial hypothesis does not appear to be true, but chimeric viral babies that form hopeful monsters are common. Any one of them can lead to a spillover.

Humans dramatically increase the possibility of spillovers like SARS-CoV-2 with poor food handling practices. Time and time again, we cram different animal species together, stress them, and pay the price with a pandemic. New influenza pandemics are caused by nearly continuous spillovers from housing birds and pigs together on small farms throughout East Asia. If we keep housing different, stressed animals together, there will be spillovers that cause deadly pandemics like CoVID-19 and the H1N1 flu.



Spillovers are entirely predictable, and we could greatly reduce their occurrences with a couple of simple rules: 1) Do not co-house different species of animals together. This applies to both domestic food animals and wild animals. And humanely treat animals by giving them space to live and not be too stressed. 2) Animals must be slaughtered in ways that reduce stress. In domestic animal production, this requires adopting humane killing protocols like those pioneered by Dr. Temple Grandin. For wildlife, this

14 K. G. Andersen, A. Rambaut, W. I. Lipkin, E. C. Holmes, R. F. Garry, "The proximal origin of SARS-CoV-2." Nat. Med. (2020) https:/doi.org/10.1038/s41591-020-0820-9. & T. Zhang, Q. Wu, Z. Zhang, Pangolin homology associated with 2019-nCoV. BioRxiv, 2020.02.19.950253 (2020).

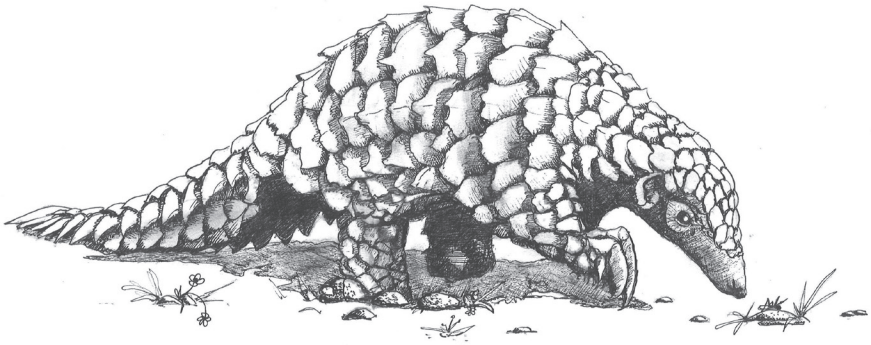
means clean kills in the field. Terrestrial wildlife should not be transported alive and slaughtered later. 3) Butchering must occur in clean facilities that reduce or eliminate cross-contamination. This is not as expensive as it sounds; many relatively poor countries have very good butchering practices. All it takes is a cinderblock building with beams from which to hang the carcasses and some drainage to wash away blood. Unused offal needs to be buried or burnt. This is important, because you don't want to feed animal products to other animals without processing that destroys viruses, prions, and other infectious agents. What is good for animals and humans is bad for spillover viruses.

In academic circles, there will be lots more debate about whether SARS-CoV-2 evolved by sex between bat and pangolin viruses, or some other viral source. This is really just an academic debate; we know that viruses evolve by recombining/reassorting their genomes. The other human CoVID-19-like diseases, SARS-CoV-1, and Middle East Respiratory Syndrome (MERS) also arose via recombination events involving civets and camels, respectively.

Bats are often the source of viruses that infect humans. There are a number of reasons why this is true, but the most important is that bats have body temperatures higher than humans. That means that bat viruses are much less susceptible to human fevers, one of the body's main virus-fighting mechanisms. Harvesting bats for meat poses a very high risk for humans.

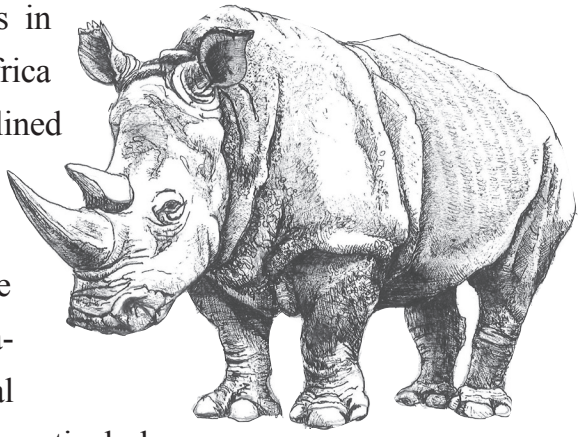
The CoVID-19 outbreak is also a reminder of the misguided killing of animals for traditional medicine. Keratin, the protein that makes up horns, hooves, and hair, does not have any medicinal properties. This has been shown

time and time again. Pangolin scales are just modified, clumped up hair, not much different than dreadlocks. The fact that the CoVID-19 pandemic could have been caused by a very small part of humanity's mistaken belief that pangolin scales will promote lactation or cure palsy is not reasonable.



Several countries have institutionalized the pangolin medical myth. Wildlife trafficking is a large, illegal business of 7-23 billion dollars per year. This relatively small business may have cost humanity trillions of dollars from CoVID-19, as well as all the suffering and death of both humans and animals.

The CoVID-19 pandemic is also negatively impacting animals in other ways. Poaching of rhinos in their last stronghold in South Africa skyrocketed as enforcement declined during the initial phases of the CoVID-19 pandemic.[15] The rhino market is also driven by the completely false belief that keratin in the rhino horn has medicinal properties. Wildlife trading, and particularly live-animal trading for falsifiable traditional medicinal claims, create condi-



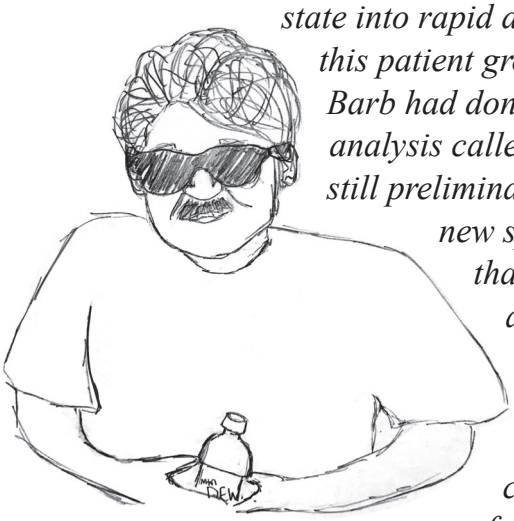
15 Overall, the CoVID-19 lockdowns restricted the movement of poachers and slowed down illegal wildlife trade.

tions that will lead to new pandemics. With some very small, economically reasonable changes, humans can dramatically reduce our risk of major pandemics and more localized epidemics. Let's quit giving the spillover viruses opportunities to have wild, cross-species sex.

The Panic

The family and I were driving into Santa Fe after the fourth Telluride Science Research Center, or TSRC, meeting on Cystic Fibrosis (CF). My phone vibrated alerting me to a text from Mike Furlan saying, "He's not slacking. It is his lungs." I knew that the message must actually be from Sharon, Mike's wife. I didn't think much about it. CFers have disease flares, called exacerbations, that last a couple of days to weeks. Maybe prophetical, but probably because I had been out of cell phone range for a while, the same message was delivered three times over the next hour.

Two days later I was back in San Diego; no Mike in the lab and I call his cell phone. Mike's on oxygen at home and was having breathing problems for over a week. He was not getting better. This was weird for him. A thought started to itch the back of my brain. At the Telluride meeting, Doug Conrad, Mike's CF doctor and Professor of Medicine at UCSD, and Barb Bailey, Professor of Statistics at SDSU, had presented data about a small set of patients "crashing". For some unknown reason, a cohort of CF patients was going from a relatively stable disease state into rapid decline. We didn't even realize this patient group existed until Doug and Barb had done some deep statistical analysis called splining. This analysis was still preliminary and when he presented the new spline, Doug had a gut feeling that there was something different about this group. He was clearly worried, and the working group had already agreed in Telluride to concentrate on this small subset of patients for intensive analysis.



I drove over to Mike's house to deliver sampling tubes, trying to be nonchalant. I even took a bottle of High West whiskey, which I had recently developed a taste for. But Sharon was onto me and wanted to know why it was so important to check Mike's samples ASAP. I waffled and told both of them not to worry, I just wanted to know what was going on.

Mike's lung function continued to decline and a day later he was sick enough to be sent to the emergency room and then to the ICU. There were two sputum samples in Mike's freezer and I drove over that night to get them. Sharon asked me straight out if the other patients with these crashing symptoms had survived. They had not.

I headed back to the lab and met up with Yanwei Lim, then one of my PhD students. Right away she started to isolate RNA and sequence the transcriptomes from Mike's samples. Transcriptomes tell us which viral, microbial and human genes are active in the sample. Samples were also sent to the Dorrestein lab at UCSD for metabolomes, which tell us what the chemistry of the sample was like. We scrambled to get supplies for the sequencing over the next couple of days. It became clear that we were unprepared to rapidly respond to this sort of emergency. Slowly the sequences and chemical data started to roll in.

In the meantime, Mike was treated with steroids, which seemed to help, and he was transferred out of the ICU.

Thanks to Rob Edwards (SDSU) and Rick Stevens (Argonne National Lab), the transcriptome sequences were put through the main bioinformatics analyses in record time. However, we were not set up to interpret the results quickly. Often it takes a year or more of intense study to really understand one of these datasets. Mike's results were initially confusing. There was evidence of both Escherichia coli *STEC F2B1 and the normal bacteria associated with Mike's CF-diseased lungs.*

The E. coli *was extremely worrying because it is an aggressive pathogen that causes dysentery through the production*

of a molecule called shiga toxin. This particular strain was closely related to the one that killed people throughout Europe in 2011 when they ate contaminated fenugreek. The problem with treating STEC is that antibiotics cause the release of shiga toxin, causing the death of human cells. This is what we think happened in Mike's case. Sometime while the CF working group was in Telluride discussing CF, Mike was exposed to E. coli *STEC F2B1. Because of the cystic fibrosis, this bacterial species colonized his lungs and caused the early exacerbation. The antibiotic treatment killed this STEC strain, but unfortunately released the shiga toxin, which continued to wreak havoc. Even as his normal microbiome re-established itself in the lung, Mike's lungs continued to atrophy.*

In the hospital, steroid treatment was able to slow down the tissue swelling for about 24 hours. Afterwards, there was too much damage and Mike started to effectively drown as blood and lymph filled his lungs. His decline was so fast that a lung transplant was ruled out. Mike was sedated, taken off life support and he died quickly afterwards.

Mike was a long-time friend and colleague. He was also a great believer that science would treat and eventually cure CF. To do this, we need to understand and apply P.H.A.G.E.S. and the Goldilocks Line in the clinic.

Chapter 10.
The CF Rapid Response

Mike Furlan's death from a horizontally acquired gene, shiga toxin, that was carried by a provirus made us realize just how little information we had about any particular human wholobiont.[1] No one had ever observed a shiga toxin-caused disease in the lungs before. There was literally no way for the doctors to know how to treat Mike. His case was entirely unique because of his **h**istory. To help address this lack of knowledge, we started building the Cystic Fibrosis Rapid Response, CFRR for short. The stated goal is to sequence the metagenomes and metatranscriptomes from patients experiencing severe exacerbations in 24 hours or less. The main challenge is how to use the DNA and RNA information. In Mike's case we would have used different antibiotics to treat the infection to keep the provirus from activating the shiga toxin. In other cases, we would need to provide new weapons to the doctors.[2]

Tom Patterson's recovery following bacteriophage therapy opened some therapeutic doors for CF patients and others. Doctors and patients heard about Tom and other successful bacteriophage therapy interventions and were less worried about treatments that involved adding a virus. Dr. Steffanie Strathdee, Tom's wife and tireless advocate for using bacteriophage therapy,

1 Güemes, Ana Georgina Cobián, et al. "Cystic fibrosis rapid response: translating multi-omics data into clinically relevant information." MBio 10.2 (2019).

2 Doctors have new drugs to fight CF. A company called Vertex set out to find modulators and correctors that would improve function of the mutant CFTR protein. The FDA recently approached a triple drug therapy for CF that partially restores CFTR function in most patients.

gave talks, wrote a book, and founded iPATH to promote bacteriophage therapy in the local hospital. Importantly, the FDA was providing guidance about acceptable clinical practices.

More successes were reported. Graham Hatful and colleagues used the SEA-PHAGES library of bacteriophage, isolated by undergraduate students all over the world, as a starting point for treating a patient with a common CF pathogen called *Mycobacterium abscesses*. Anca Segall at SDSU has led an effort to isolate bacteriophage to kill emerging CF pathogens identified in the CFRR, namely *Achromobacter*. Ampliphi, a company in San Diego, treated a number of patients with bacteriophage to kill the most common CF pathogen, *Pseudomonas aeruginosa*. And instead of just looking at the patient outcome, they asked us to apply the CFRR methods to investigate what happened before, during, and after the treatment. This work showed that bacteriophage injected into a patient's blood stream will get into the lungs and kill some bacteria.

One of the most interesting bacteriophage therapy approaches came out of Paul Turner's lab. He targeted bacteriophage to kill *Pseudomonas aeruginosa* so that they were either bacteriophage sensitive or sensitive to antibiotics. Paul is taking advantage of **s**election, specifically he has isolated bacteriophage that uses an outer membrane protein called porin M to attack *Pseudomonas aeruginosa* (i.e., that trophism thing again). Porin M is important to the bacteria because it is one of the ways that the bacteria develop antibiotic resistance. If the bacteria accumulate mutations during **e**xpansion that allows them to escape the bacteriophage, then that bacteria will be sensitive to antibiotics. Conversely, if the bacteria uses Porin M to escape the antibiotic, then the **p**redator bacteriophage can kill the bacteria. It is an evolutionary Catch-22 for the bacteria.[3]

3 Chan, Benjamin K., et al. "Phage **s**election restores antibiotic sensitivity in MDR Pseudomonas aeruginosa." Scientific Reports 6 (2016): 26717. Of course, since this is biology there are exceptions! Burmeister, Alita R., et al. "Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance." Proceedings of the National Academy of Sciences 117.21 (2020): 11207-11216.

These and other studies are providing the data to build a more scientific approach to bacteriophage therapy. One goal of the Viral Information Institute (VII; pronounce "vee") at San Diego State University is to build out the scientific platforms to better understand and inform bacteriophage therapy. This is cutting-edge personalized medicine that will take years to perfect but will provide additional treatment options for some CFers. Right now, it is possible to build a toolbox of bacteriophages and other viruses to kill or otherwise manipulate target cells. For example, in the CFRR metagenomic data there are endolysins and tailocins that could be used to kill bacteria. These living nanomachines are already doing everything envisioned by Dr. McCoy's nanobots, so we should be using them.[4] Our enemy's enemies are our friends.

Being Careful with Bacteriophage Therapy

As knowledge accumulated about CF patients through the CFRR, numerous issues with bacteriophage therapy started to pop up. The first main problem being that bacterial viruses were often the cause of severe/deadly exacerbations. The shiga toxin that killed Mike was encoded by a provirus. This was just the tip of the bacterial-viruses-aren't-always-good iceberg. Bacteriophage were moving genes into and around the CF lungs microbiome including morons that encode antibiotic resistance, virulence factors, and other genes important to promoting the disease.[5] In one CFRR, we found a virulence gene, called *zot*, that was normally associated with the deadly diarrheal disease cholera. Possibly the most concerning findings from the CFRR has been that numerous severe/deadly CF exacerbations were associated with completely unknown bacterial viruses with no known virulence genes. These unknown, viral-encoded genes were contributing to the worst-phases of CF, but we don't know how. In turn, this raises flags for

4 Schuch, Raymond, Daniel Nelson, and Vincent A. Fischetti. "A bacteriolytic agent that detects and kills *Bacillus anthracis*." Nature 418.6900 (2002): 884-889.

5 Willner, Dana, et al. "Case studies of the spatial heterogeneity of DNA viruses in the cystic fibrosis lung." American Journal of Respiratory Cell and Molecular Biology 46.2 (2012): 127-131.

Figure 10.1. Which virus is good or evil? Bacteriophage help control bacteria numbers and add essential genes to wholobionts, while others create pathogens that kill wholobionts. This Janus nature of viruses of all types makes them frustratingly interesting life forms.

bacteriophage therapy; if we want to use bacterial viruses to kill bacteria, we need to ensure that the bacteriophage will not make things worse.

Bacteriophage-encoded superpowers that may harm patients are a major concern with bacteriophage therapy. Currently, it is not possible to look at a bacterial virus in the microscope or at its genome and know if it is dangerous. Basic tests that ask simple questions like, "Does this bacteriophage kill human cells?" or "Does this bacteriophage activate proviruses that will kill the patient?" are often not being asked before trying bacteriophage therapy in the clinic. A drug would never be used in the clinic without these basic safety tests and neither should bacteriophages.[6] Another challenge is determining how many types and numbers of bacteriophages should be added to a therapeutic cocktail. Like all drugs, more is not necessarily better; bacteriophages have different effects at different concentrations. Instead of the simple, cause-and-effect thinking that bacteriophage will just kill the bacteria, we need to be scientists and figure this stuff out. Hopefully, P.H.A.G.E.S. and the Goldilocks Line will help. These concepts are essential for the more complicated chronic assemblies described below.

6 At the very least, human tissue culture cells should be treated with the final bacteriophage preparation to make sure that the cells are not harmed. It would be even better to put the raw bacteriophage lysate (bacteriophage plus the blown-up bacteria) and see if this is harmful to the tissue culture cells.

Chronic Assemblies

Section III

Get Back to Work

The Small Bar was crowded and rock-n-roll loud. My third IPA was in front of me and I was absorbing bullshit from one of the other regulars. West Coast IPAs are strong, and the BS was starting to make sense. Or it could have been that I was exhausted mentally, Mike having died a couple of hours earlier.

Even with the bustle, BS, and buzz, one question kept plaguing me, "What else could have been done?"

My long-suffering girlfriend eventually pulled me out of the bar and poured me into bed at home. Sleep was fitful and I ended up downstairs on the couch writing an obituary.

The obituary took all night and most of the morning. Hungover and drained, I sent it to my lab. Of course, they already knew that Mike was dead, but they also needed to hear it from me. The next days passed in that fuzzy way of jetlag.

There were things that we could have been done better. First, we had been unprepared to rapidly diagnose a disease using our research tools. To be useful, the doctors needed to get the data much faster. Reagents and people had to be ready to go in a couple of hours, not a week. Second, we had to quickly understand what the data were telling us and identify confirming tests; the recursive process of reassess, respond and reorder had to become fluid and fast. Third, we should have had samples banked from before the exacerbation started. The CF patients needed in-home sampling kits.

Much more could be done, but that would not help Mike.

I asked Mike's widow if she would like his obituary to be printed in the Cystic Fibrosis Research Institute (CFRI) newsletter. CFRI had sponsored a lot of the lab's work. Edits were made, and Mike's obituary was sent to Sue Landgraf, CFRI's Executive Director. Her response:

"This brings tears to my eyes. I'm so, so very saddened and shocked by Mike's passing. Oh, how I hate CF. Mike should still be with us. My daughter should not have nearly died and have had to have a double lung transplant."

She reminded me, that yes, Mike's battle was lost. But he, Sue, her daughter, and all the CFers were fighting a war that they had every intention of winning. It was time to get moving again.

To do this we need to get deeper into P.H.A.G.E.S. and understand the chronic relationships within ecosystems and wholobionts.

Chapter 11.
A More Temperate Path

Viruses are often **p**redators that blow up cells in acute relationships. However, temperate viruses that form chronic, long-term proviral **a**ssemblies with their hosts are more common. Temperate viruses are also major creators of new biological stories through Horizontal Gene Transfer (HGT); some of the biggest changes in the evolution of life are the results of virally created texts. The placenta, for example, was created when a virus moved a piece of DNA into an early egg-laying mammal similar to a platypus.[1] Temperate viruses also regularly move virulence factors and antibiotic resistance texts around, making some bacteria better pathogens. The temperate viruses are both creators and killers. Understanding this Janus nature of viruses is key to understanding how humans are harming ecosystems like coral reefs and their own wholobionts through the processes of microbialization and Piggyback-the-Winner.

After infection, some temperate viruses will form a stable DNA circle, also called a plasmid, in the host cell. Others build a small linear viral chromosome that will hang out in the host cell. Other temperate viruses will perform *recombination* to insert their viral genome directly into that of the host cell. When a temperate virus **a**ssembles with a host cell, its allegiances change. Instead of killing the host cell, the virus now must protect itself and the host against other **p**redators. The host cell temporarily allying

1 Cornelis, Guillaume, et al. "Retroviral envelope gene captures and syncytin exaptation for placentation in marsupials." Proceedings of the National Academy of Sciences 112.5 (2015): E487-E496.

with a virus is called a *lysogen*. A temperate virus inside a host cell is called a *provirus*. Viruses make a serious sacrifice when becoming a provirus, namely the number of progeny per generation goes from an average of 25 (i.e., the burst size) to 2 (i.e., regular cellular division). This is a major damper on the **e**xpansion part of P.H.A.G.E.S. for the virus. Why would a virus ever find this decline in reproductive potential advantageous? The simple answer is that viruses and hosts form **a**ssemblies when living is good for the lysogen. We will come back to this soon.

Sorry, we need a little more terminology. Proviruses get out of the lysogen by re-entering the lytic cycle. This is called *induction* and it is usually caused by intracellular changes that signal the provirus that the host cell is in trouble. Basically, the viruses are rats leaving a sinking ship. DNA damage, for example, is a great inducer of proviruses. Most everyone has experienced this type of induction event; getting too much sun at the beach activated the herpes proviruses in your lips and caused a cold sore. Or maybe the sun activated the proviruses in your skin bacteria and caused a pimple to form. Other environmental signals that trigger induction are things like starvation for energy or matter **g**overnors.

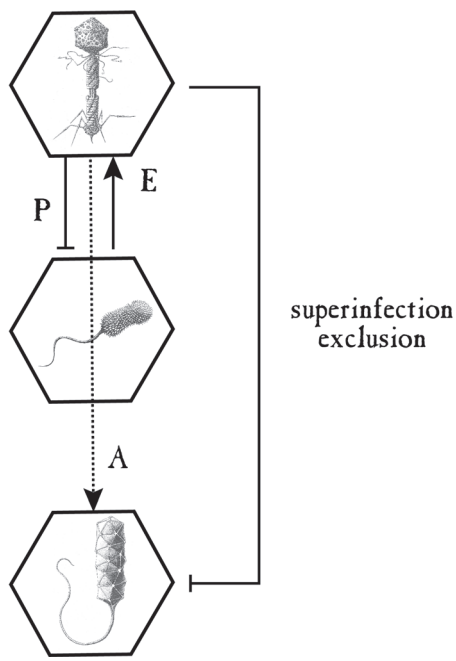


Figure 11.1. Superinfection Exclusion: Proviruses protect the lysogen from other viruses through a variety of molecular mechanisms collectively called superinfection exclusion.

In general, proviruses are not the best housemates. They tend to burn down the house and kill everyone when things get a little tough. So, it is reasonable to ask what does the host cell gain from this uneasy *detente* with temperate viruses? First, most temperate viruses make the host cell immune to other viral attacks. This is a big plus in crowded conditions where the cell is basically guaranteed to run into multiple viral **p**redators. The process of a provirus protecting its host cell from other viruses is called *superinfection exclusion*. Any crowded ecosystem, say with more than 1 million cells per gram, favors lysogens protected via superinfection exclusion.

The cells in wholobionts are so crowded together that superinfection exclusion is an essential dimension of any successful **a**ssembly. The advantage of superinfection exclusion has everything to do with the massive exponential **e**xpansion potential of viruses. If one cell in a crowded system gets infected by a purely lytic virus, then that virus is going to kill all, or most, of the related cells in a very short time.[2] This is Kill-the-Winner run amok. However, if cells are resistant to virulent viruses through superinfection exclusion, then they can fend off this attack. This is part of Piggyback-the-Winner, which we will discuss later. Superinfection exclusion is one reason why every cell in your body, whether it is human or microbial, is a lysogen carrying one or more proviruses.

The second reason lysogens are successful in crowded conditions are the protists. Protists are the other major **p**redator group of microbes. Protists are single-cell Eukaryotic cells like amoeba and paramecium. In the ocean, protists kill as many microbes as the viruses.[3] Our immune system

2 This is called a viral or bacteriophage lysate and it is a common laboratory protocol. Some of the scarier viruses like Smallpox, Ebola, and SARS also behave as purely lytic viruses, killing their human hosts and causing pandemics.

3 Fuhrman, Jed A., and Rachel T. Noble. "Viruses and protists cause similar bacterial mortality in coastal seawater." Limnology and Oceanography 40.7 (1995): 1236-1242. Alonso, M. C., et al. "Role of ciliates, flagellates and bacteriophages on the mortality of marine bacteria and on dissolved-DNA concentration in laboratory experimental systems." Journal of Experimental Marine Biology and Ecology 244.2 (2000): 239-252. Johnke, Julia, et al. "Multiple micro-predators controlling bacterial communities in the environment." Current Opinion in Biotechnology 27 (2014): 185-190.

has cells, called macrophages, that engulf microbes just like protistan **p**redators. Obviously, a provirus hanging out in a lysogen does not want to get eaten by an amoeba-like creature. So, proviruses carry weapons to kill the protists and macrophages.[4] These weapons are basically superpowers to kill eukaryotic cells, which we call *virulence factors*. The shiga toxin that killed Mike Furlan was a provirus-encoded virulence factor.

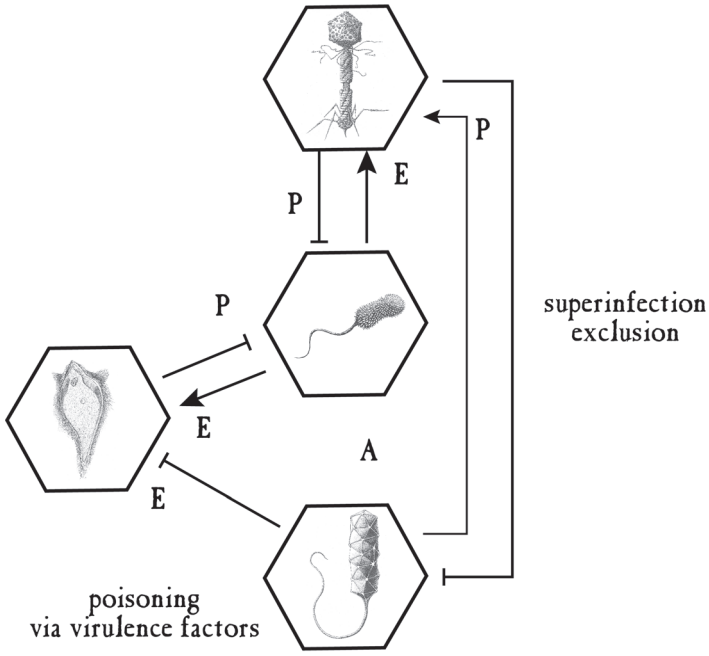


Figure 11.2. Virulence Factors Kill Protists: Proviruses need to protect the lysogen from protists. One way they do this is by the production of virulence factors that kill protists. Since protists are eukaryotic cells, many of these protist killing proteins are also virulence factors that can kill animals, including humans.

In a lysogen, the proviruses' interest are aligned with their host cells. Therefore, they also often carry additional genes, called MORONs, that will give the lysogen an advantage over its neighbors. Many of these virally encoded biological stories have to do with how the **g**overnors are

4 Brüssow, Harald. "Bacteria between protists and phages: from antipredation strategies to the evolution of pathogenicity." Molecular Microbiology 65.3 (2007): 583-589. Erken, Martina, Carla Lutz, and Diane McDougald. "The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment." Microbial Ecology 65.4 (2013): 860-868.

processed. For example, many CAZymes are encoded by proviruses. Other proviruses encode morons that protect the host cells against stressors like antibiotics in diseased systems like the CF lung.[5]

My long-time colleague Dr. Linda Wegley-Kelly showed that the microbes associated with coral reefs carry biological stories that explained how they adapted to varying local conditions (i.e., the function diversity), even though the types of bacteria remained the same (i.e., the taxa).[6] The local adaption genes are moving between different microbes on proviruses and the other agents of HGT. In this way, the same coral wholobionts can live in extremely different water conditions. Similarly, every human wholobiont has its own, unique virome. This allows us to adapt to varying conditions via **a**ssembly of viruses, microbes, and human cells. The adaption of organisms by horizontally acquiring functions, or by physiological changes, is called *acclimatization*. The uneasy alliance between chronically infected lysogens and proviruses[7] is one of the major causes of unhealthy coral reefs and humans. To understand how this links together, let's go SCUBA diving.

5 Willner, Dana, and Mike Furlan. "Deciphering the role of phage in the cystic fibrosis airway." Virulence 1.4 (2010): 309-313.

6 Kelly, Linda W., et al. "Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors." Proceedings of the National Academy of Sciences 111.28 (2014): 10227-10232.

7 I have been avoiding the chronic life cycle of viruses for the sake of simplicity. Strict chronic viruses do not lyse the host cell when they reproduce. Instead they "bud" out of the cell, leaving it intact. This form of **p**redation is usually called parasitism because the chronic viruses don't kill, they just steal energy and matter from the host cell. These types of viral infections are really important, but we are going to ignore them for this book. Sorry chronic viruses...

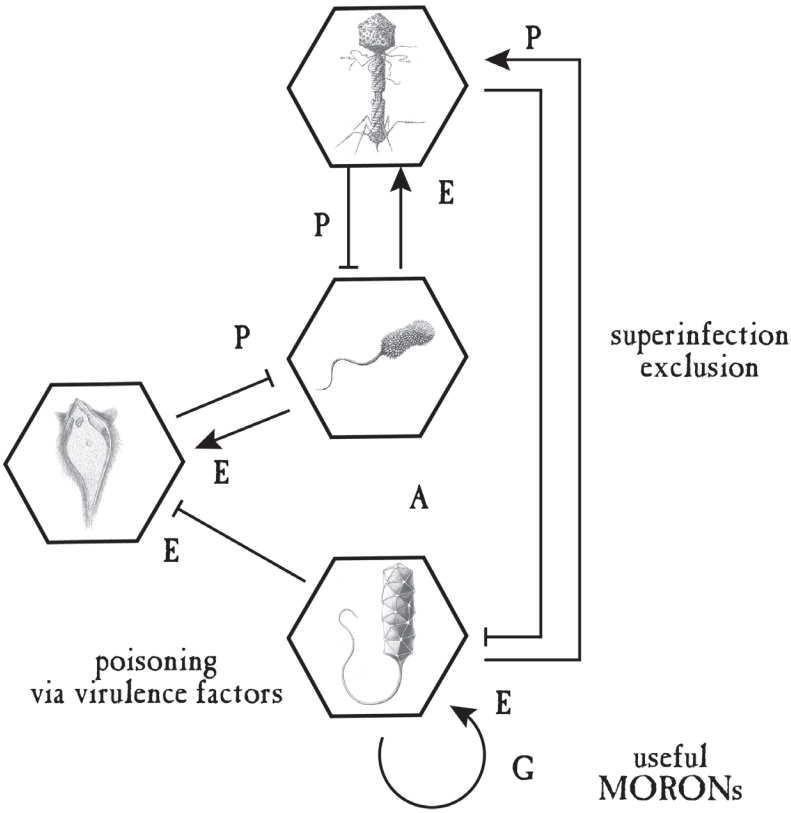
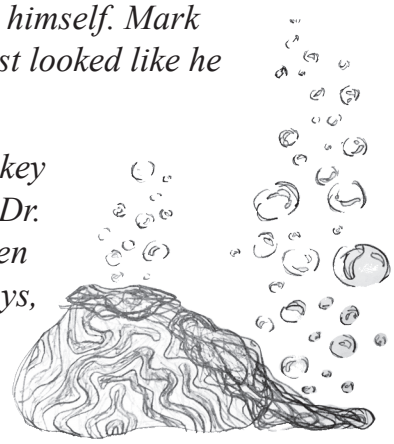


Figure 11.3. MORON-Mediated Acclimatization to Local Environments: Many proviruses also encode genes that help the lysogen acclimatize to local environmental conditions.

Bubble Hunting

"Bubbles? You want me to hunt bubbles?" Mark Vermeij looked at me askew. "Why?"

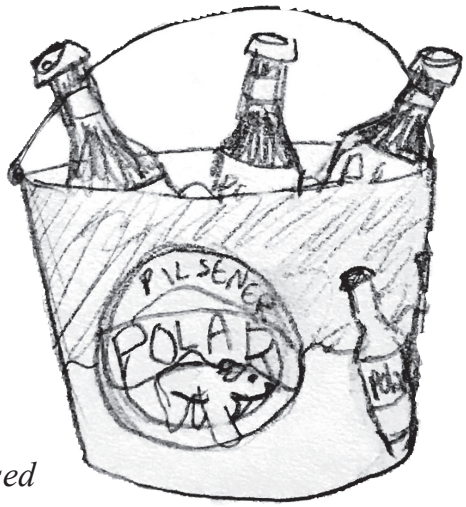
A bruisingly big Dutchman, with a wicked scar running down from his scalp, though one eyebrow, and down one cheekbone, Mark looks a lot like Jason Momoa's Aquaman. In actuality, he's much better underwater then Aquaman. Besides being amazing at underwater jobs and one of the world's best naturalists, Mark has the unofficial job of keeping the microbiologists from killing themselves while in the field. Since this is effectively like keeping toddlers playing with chainsaws alive, he is understandably nervous about new ideas. It didn't help that earlier on the trip, one of our team had decided to eat a poison beach apple and nearly killed himself. Mark quickly downed another Polar; it almost looked like he needed the alcohol.



"Well the bubbles on the algae are the key to coral reef decline." A grimace from Dr. Aquaman, so I continued quickly. "When the oxygen bubbles away, the sugar stays, and feeds the bacteria. They quickly use up the oxygen, which is why we are seeing hypoxic conditions on parts of the reefs."

I was telling Mark things he already knew. He had helped collect samples and set up experiments showing that sugars from algae could kill corals. And we had hunted hypoxic zones on the reefs on reefs in two oceans. Now was the time for the hard sell. "And these hypoxic zones encourage the bacteriophage to create opportunistic pathogens."

"Uggh...not the stupid viruses again. I need another bucket of beer." Mark may have heard about viruses one time too many since viruses are the main topic of discussion on long sea voyages.



As we walked along the beach to buy another round of Polars, I continued. "The low oxygen conditions switch the microbes to anabolic metabolisms. This tells the viruses that the cell is doing well, so they form lysogens. And these lysogens produce virulence factors to protect against protists, but can also kill corals. Basically, coral reef decline is caused by the viruses turning the bacteria into pathogens. It's analogous to brewing beer."

Mark finished another Polar. "So, do we have bubble hunting tools?"

"I'm glad you asked! All we need is 50 feet of PVC tubing, some cable ties, a few minor explosives to shake the bubble loose, dive gear...". His scarred eyebrow started to twitch. It was time for another beer.

Chapter 12.
Coral, DIVAs, and Piggies

Aesthetically, coral reefs are some of the most beautiful places in the world.[1] They are also important sources of fish protein for humans. And herein lies the problem. Coral reefs around the world have been fished extensively.[2] In turn, the fishing has dramatically changed the normal **p**redator-prey dynamics at both the macrobial and microbial scales. Most simply, removal of sharks and other big **p**redators has switched the energy **g**overnor. Sugars from photosynthesis that previously sharks are now feeding microbes. There are more microbes and these microbes are lysogens carrying proviruses with virulence factors. This phenomenon of moving an ecosystem from macro-organism dominance to microbes is called *microbialization.*

Microbialization of coral reefs

Sunlight is in abundance in the shallow waters where corals thrive. The corals are animals and cannot capture the energy in the photons. Instead corals **a**ssemble with single-cell algae, called zooxanthellae, that convert the sun's energy to sugar and oxygen. In turn, the coral animal uses the sugar and oxygen to build the skeletons that make the reef. Over millions of years,

1 Haas, Andreas F., et al. "Can we measure beauty? Computational evaluation of coral reef aesthetics." PeerJ 3 (2015): e1390.

2 Williams, Ivor D., et al. "Differences in reef fish assemblages between populated and remote reefs spanning multiple archipelagos across the central and western Pacific." Journal of Marine Biology 2011 (2011).

coral-zooxanthellae wholobionts have built the largest biological structures in the known Universe, coral reefs.

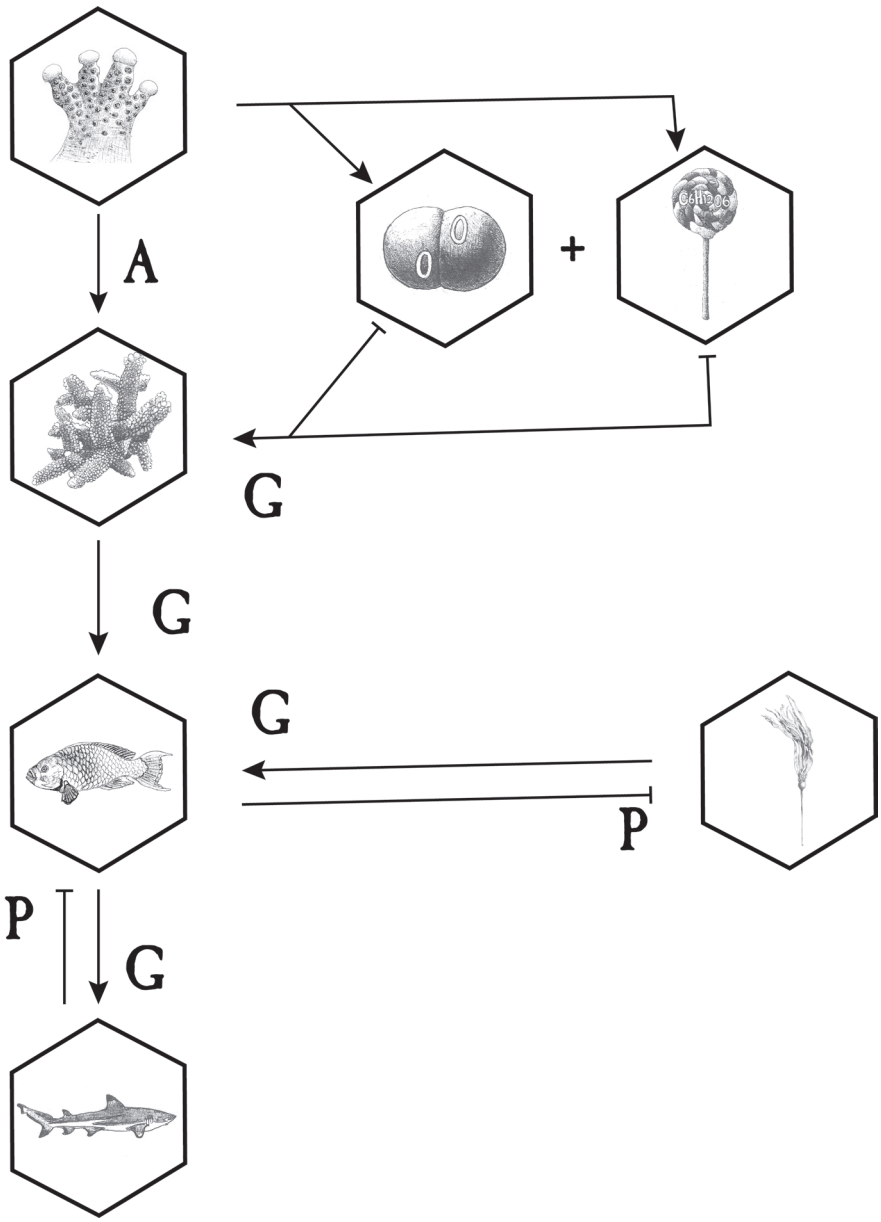


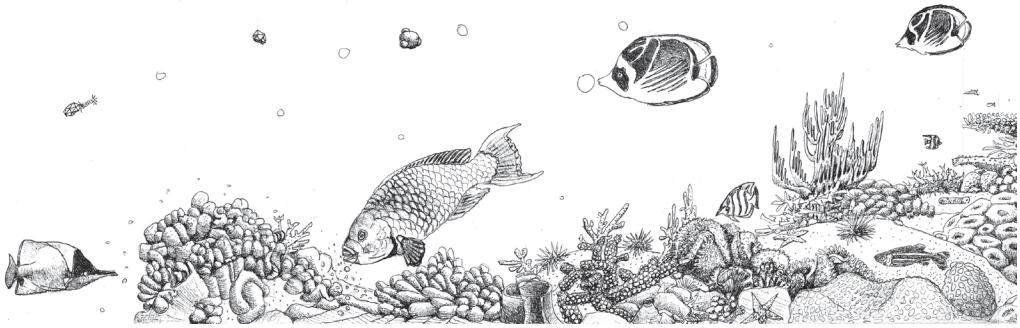
Figure 12.1. Healthy coral reef P.H.A.G.E.S. flowchart. Coral wholobionts build reefs which creates holes for fish (space**-G**overnor). These fish graze on seaweeds (Predation via grazing) and are eaten by sharks and other carnivores (carnivore Predation). The coral animals obtain reef building energy via photosynthesis (energy**-G**overnor from photosynthesis) carried about by single-cell algae called zooxanthellae (zoox; **A**ssembly). Since the zooxanthellae are inside the animal, the oxygen and sugar are coupled which facilitates catabolic **G**overnors.

The coral-zooxanthellae wholobionts are covered in mucus, just like our GI tract. And just like our internal rivers, the mucus is home to a multitude of viruses and microbes. There are over 100 million microbes per square centimeter of surface on healthy corals.[3] And like our GI microbes, the coral's microbiome produces bacteriophage for BAM Immunity and occupy space to keep invading microbes out. Other resident coral bacteria convert the inorganic nitrogen in the surrounding sea water into organic nitrogen, effectively creating a vital material out of air. Other microbes recycle phosphate, iron and other limiting materials. By **a**ssembling with different microbes, many of them carrying provirus-encoded superpowers, corals build reefs all over the tropics.

Coral versus seaweed wholobionts

The coral-zooxanthellae wholobionts are not the only organisms that extract energy from sunlight on coral reefs. There are literally thousands of other types of algae trying to find their place in the sun. For our purposes, we will just lump all of this amazing algal diversity together under the moniker "seaweed".

Seaweeds compete with the corals for space on the reef and are normally kept in check by grazing fish and invertebrates like sea urchins.[4] The



4 The **p**redators in P.H.A.G.E.S. includes the grazing herbivores. Sometimes it is useful to differentiate between meat-eating and plant-eating predation. Most plant-eating predation doesn't kill the victim, whereas almost all meat-eating predation kills prey. That being said, grazing, browsing, nipping are all types of **p**redation, or parasitism, on plants.

grazers preferentially eat the seaweed because it isn't housed in a rock skeleton like the zooxanthellae living with corals. All of the grazing keeps the seaweed well-groomed and small. And lots of tasty, grazing fish and invertebrates attract the **p**redators. On a healthy coral reef, the grazing invertebrates and fish must be careful not to stray too far from protection of the coral because a hungry shark or grouper is always lurking a fin flick away. This means that a healthy, pristine coral reef has a lot of apex **p**redators like sharks and groupers.[5] As a SCUBA diver on a pristine reef, the main thing you see are the corals and big **p**redators. Everything else is hiding in the reef spaces.

The **h**istory of most coral reefs has been so dramatically changed by fishing that almost no-one knows what a pristine reef looks like, including most coral reef scientists. This is called *shifting baselines*. Yellowstone also suffers from shifting baselines; no one living remembers what the park looked like before the wolves were removed. But we have better written records about Yellowstone; almost nothing was recorded from the world's coral reefs before industrial fishing did its damage.[6] Not knowing what a pristine ecosystem looks likes has been a major problem for both coral reef science and human health because in both cases we don't even know what an undisturbed ecosystem is. This is a major challenge for conservation and restoration goals because just like the discussion of "Health" in Chapter 2 we don't know what our target might be.[7]

Big **p**redators are important to coral reefs because they require a lot of the energy. A rule of thumb is that about 90% of the energy is lost as waste heat every time it is transferred from one organism to another.

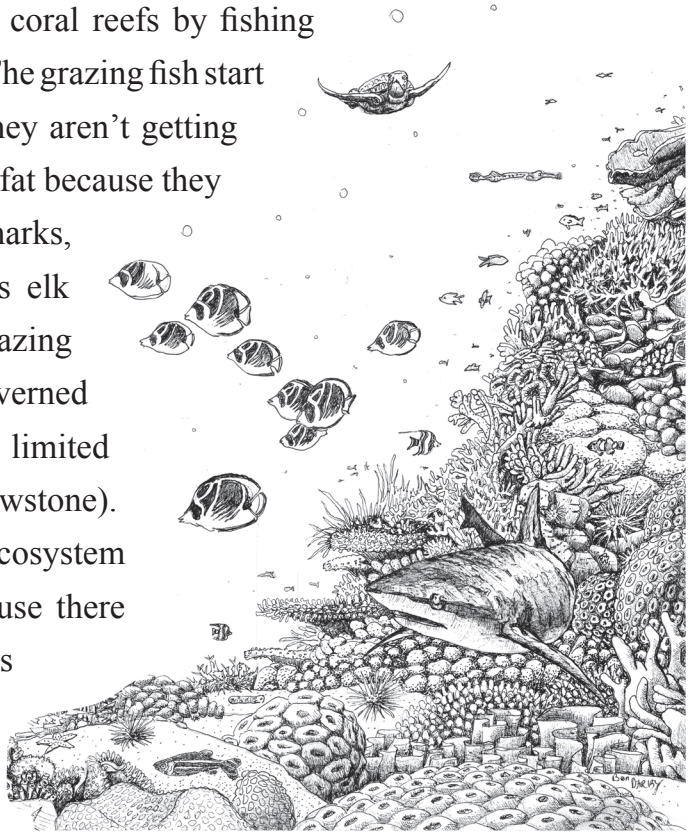
5 Traditionally the large **p**redators are referred to as apex **p**redators; the idea being that they don't have any **p**redators that eat them. This is a little outdated, since we are aware that the very small **p**redators, like viruses, are constantly killing and parasitizing the supposable apex **p**redators.

6 SCUBA gear and our exploration of the underwater world occurred at the same time as industrialized fishing, mostly because of technologies developed during the world wars.

7 Jackson, Jeremy BC, et al. "Historical overfishing and the recent collapse of coastal ecosystems." Science 293.5530 (2001): 629-637. Tito, Raul Y., et al. "Insights from characterizing extinct human gut microbiomes." PloS One 7.12 (2012): e51146.

That means that a 100-pound shark represents 1,000 pounds of grazing fish which represents 10,000 pounds of seaweed.[8] Ultimately, it takes a lot of sunlight to fuel a shark.

Humans mess up coral reefs by fishing out the apex **p**redators.[9] The grazing fish start to live longer because they aren't getting eaten. They get lazy and fat because they aren't worried about sharks, much like Yellowstone's elk without wolves.[10] The grazing fish become space-**g**overned (cf., elk became energy limited in the wolf-free Yellowstone). The fished coral reef ecosystem has become stuck, because there are no 100-pound sharks killing the grazing fish and groupers eating the invertebrates. Because these grazers are not being eaten, grazing **p**redation goes down and the ungrazed seaweed expands. Worse, big seaweeds are unpalatable to the



8 This isn't very accurate and is only used for illustrative purposes. In actuality, energy consumption follows scaling laws summarized in the Metabolic Theory of Ecology (MTE). Using MTE, we calculated that a pristine coral reef fish community need ~2.5 milliWatt of Energy per cubic meter versus ~0.3 on a degraded reef. This is a 10X decrease in energy required by the fish, which means that there is lots of potential energy leftover for feeding the microbes. McDole, Tracey, et al. "Assessing coral reefs on a Pacific-wide scale using the microbialization score." PLoS One 7.9 (2012): e43233.

9 This most insidious version of this overfishing is shark finning.

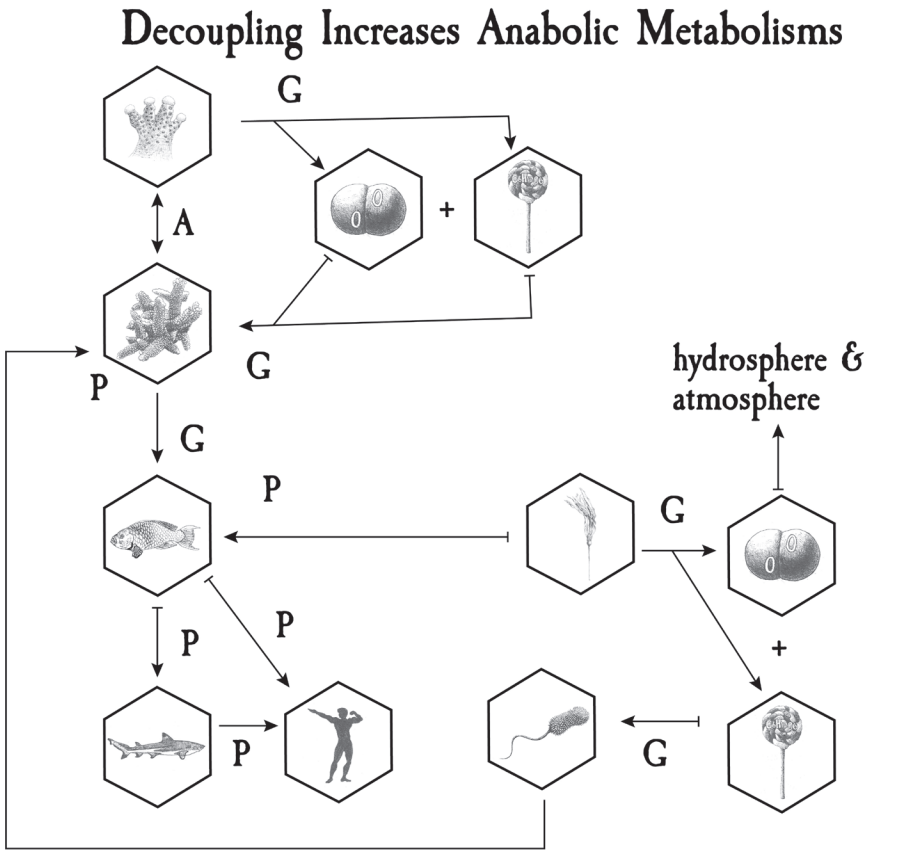
10 Another major killer of corals is the beautiful crown-of-thorns starfish (CoT). During a CoT outbreak, waves of these echinoderms will eat their way across a coral reef. CoT appear to be increasing and the cause is probably **p**redator release. Specifically, overfishing of the **p**redatory triton snails has probably contributed to these outbreaks. Cowan, Zara-Louise, et al. "Known predators of crown-of-thorns starfish (*Acanthaster* spp.) and their role in mitigating, if not preventing, population outbreaks." Diversity 9.1 (2017): 7.

remaining grazing fish and invertebrates.[11]

As grazing **p**redation decreases, the amount of incoming sunlight remains the same. This means that photosynthesis happily continues, churning out sugar and oxygen. And this is where the wholobiont trickery coupled with the Goldilocks Line comes into play. When photosynthesis is carried out by coral-zooxanthellae wholobionts, the oxygen and sugar are immediately captured by the surrounding animal tissue. The animal then uses this energy to build coral reefs. However, the seaweed has no surrounding animal tissue. As oxygen is produced it forms bubbles which are eventually swept away by the current, leaving behind a lot of sugar.[12] And only the microbes, with their anaerobic metabolisms like fermentation, can eat this extra sugar. The decoupling between oxygen and sugar is the ultimate cause of coral reef decline. Another example of how crossing the Goldilocks Line dramatically changes ecosystems.

11 It is common for prey species like plants and algae to produce chemical defenses to discourage **p**redatory grazers (e.g., make them taste bad, poisons, *et cetera*). The most famous are the tannins, which protect against ruminants. Heady, Harold F. "Palatability of herbage and animal preference." Rangeland Ecology & Management/Journal of Range Management Archives 17.2 (1964): 76-82.

12 Silveira, Cynthia B., et al. "Biophysical and physiological processes causing oxygen loss from coral reefs." Elife 8 (2019): e49114.



1) Fishing by humans reduces grazing pressure on algae.
2) Algal photosynthesis leads to decoupling between oxygen & sugar.
3) The excess sugar increases bacteria abundances, which leads to increased incidences of coral diseases.

Figure 12.3. Microbialization of coral reefs and P.H.A.G.E.S. Predations by humans reduces grazing by fish. In turn this increases photosynthesis by seaweed. The oxygen bubbles away and sugar increases. This encourages anabolic metabolism and increased bacterial numbers. More bacteria means more coral disease.

DIVAs and Beer

Once seaweeds get to a certain size, they stop growing. The seaweed continues to produce sugar and oxygen, and the gaseous oxygen bubbles away. This leaves a lot of energy, in the form of sugars and more complex

carbohydrates, that feeds the *Dinner Is Very Available,* or *DIVA, microbes*. DIVAs are the speed eaters of the microbial world. Their exponential expansion quickly uses up much of the remaining oxygen and starts to suffocate the coral reef.

The oxygen bubbling away, plus the rapidly growing DIVAs who are metabolizing oxygen, means that degraded reefs don't have enough oxygen.[13] In other words, there are lots of electron donors (e.g., sugar) and not enough electron acceptors (e.g., oxygen). Too little oxygen means that sugar taken into the cell can only be partially broken down, leaving a number of intermediates of metabolism called primary metabolites. And when there are lots of primary metabolites, cells start to build things in a process called *anabolic metabolism*.

You are intimately familiar with anabolic metabolism, even if you don't realize it. Humans least favorite anabolic metabolism is strenuous exercise. The reason you go to the gym and lift weights is to cause your muscles to become anaerobic (i.e., oxygen-poor). Without the oxygen as a final electron acceptor, your muscle cells start to ferment, and lactic acid accumulates in the tissue. The anaerobic exercise also causes a bunch of primary metabolites to build up. In turn, the cells use these primary metabolites to build more muscle fibers and you become stronger.[14] This is anabolic metabolism at its most basic. And yes, unfortunately anabolic and anaerobic looks very similar and are kind of hard to keep apart.[15] Luckily, they are related, if there isn't enough oxygen (i.e., the oxygen-poor side of the Goldilocks Line) then the ecosystem is anaerobic and anabolic metabolisms (i.e., building with primary metabolites) are more common.

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13 We are already seeing massive suffocation events caused by microbialization on coral reef. Altieri, Andrew H., et al. "Tropical dead zones and mass mortalities on coral reefs." Proceedings of the National Academy of Sciences 114.14 (2017): 3660-3665.

14 The cheat is to use anabolic steroids to cause this change.

15 Anabolic is derived from "ana" in Greek, which means to ascent or build up. The "an" in anaerobic means "without" and the "aerobic" refers to air and life. So, anaerobic means "life without air". The beginning of both words with "ana" is therefore just an unfortunate coincidence that has been confusing students for over a century.

The second example of anabolic metabolism that you are intimately familiar with, and the reason you need to go to gym in the first place, is beer. Recall that the energy in beer is just sugar made from captured sunlight in the barley seed. A brewer puts that sugar in some water with yeast and seals off the system so that no oxygen can get into the vat. In this vat, the yeast has lots of electron donors and not enough electron acceptors, so it starts fermenting. The ecosystem in the vat is strongly on the oxygen-poor side of the Goldilocks Line. Luckily for all of us, the yeast produce alcohol when they run out of oxygen (cf., lactic acid in human tissues). Unlucky for us modern humans, but not our starving ancestors, the yeast also produces a bunch of primary metabolites that our bodies love to use for building beer-bellies. Using P.H.A.G.E.S., we would say that excess energy (stored in the ethanol) and matter (stored in the primary metabolites), means that space becomes the limiting **g**overnor. Our bodies respond by building more space in the form of belly fat.

The degraded coral reefs are just like that beer. The extra sugar and not enough oxygen mean that the microbes start to grow and they both get fatter and more numerous. The apparent quandary is that we know that more abundant microbes should be more susceptible to their viral and protist **p**redators. The **P** in P.H.A.G.E.S. should be killing the DIVA winners. But this is not what we observe on degraded coral reefs. Instead as microbes get fatter and more abundant the relative number of viruses decreases. Why?

Piggybacking-the-Winner

Piggyback-the-Winner occurs when cell numbers get high enough that a runaway viral infection would kill all of the hosts, leaving the free viruses without a new cellular home. Under these conditions, the temperate viruses are more successful than purely lytic ones because they are hiding out and guarding their cellular selves, the lysogens.[16] To protect themselves

16 There is another relationship, called Piggyback-the-Loser, which occurs when the number of hosts are very rare. We are not going to talk about Piggyback-the-Loser because we don't understand many of the details.

the proviruses make different proteins to stop other viruses and protists,[17] superinfection exclusion and virulence factors, respectively.

To be an effective life cycle, the viruses need to know that it is a good time to start behaving in a temperate fashion. This is when the host cell is growing well and there are lots of primary metabolites around. That is, the anabolic growth associated with a degraded reef.

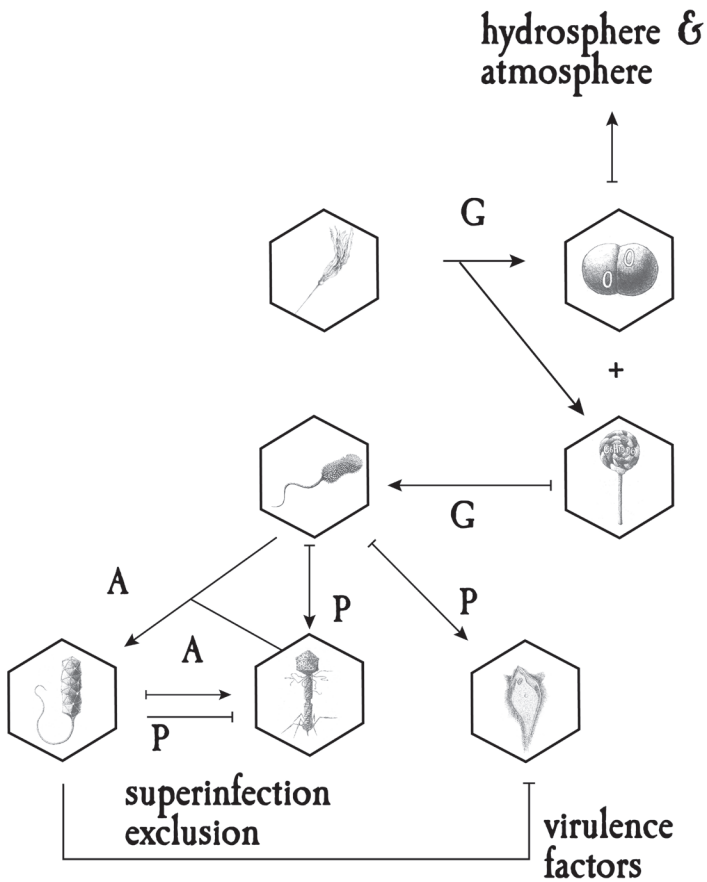


Figure 12.4 Piggyback-the-Winner flowchart. Flesh seaweeds and turf algae produce sugar and oxygen. The oxygen bubbles away thereby creating a sugar-rich, anabolic environment. In turn, this favors temperate behavior by the viruses. These resulting assemblages of piggybacking proviruses and cells both protect the lysogen from other viruses though superinfection exclusion and protists via expression of virulence factors.

17 Remember that protists are single-celled, eukaryotic microbes. Many protists are **p**redators that eat bacteria. These include the nano-flagellates, amoeba, ciliates, paramecia, and a whole bunch of others.

Figure 12.5. Predation of sharks and groupers by humans from coral-dominated reefs leads to unstable, transitional reef ecosystems. Transitional reefs are characterized by soft corals, which do not build reefs, and other filter-feeders like sponges and ascidians. Most of the reefs of the world are in these transitional states and almost any perturbation, like a strong hurricane, disease outbreak, water temperature event, will shift the ecosystem to the seaweed-dominated reef state. The loss of oxygen through ebullition and the subsequent rise of piggybacking viruses and microbial hosts helps stabilize this undesirable reef state (i.e., the Goldilocks Line). Notice how **S**pace for grazing fish and invertebrates decrease as the reef flattens.

At first Piggyback-the-Winner doesn't seem so bad: the viruses **e**xpand more slowly (i.e., less progeny per generation) and fewer host cells die in the process. However, there is always another dimension to consider in biology. In this case, it is the protists. The proviruses also needs to protect the lysogen against the protistan **p**redators, so it makes weapons that kill or incapacitate them. These weapons are virulence factors like the shiga toxin that killed Mike Furlan. The provirally encoded virulence factors are usually proteins that disrupt essential functions like RNA and protein synthesis. Because of the differences between eukaryotic and bacterial cells, these virulence factors only kill the eukaryotic cells. On a microbialized reef the anti-protist virulence factors encoded by bacterial proviruses also kill the other eukaryotic cells like those of the coral animals. So lysogens protecting themselves against protists are also pathogens that kill animals. As the DIVA with their piggybacking proviruses kill coral, they increase open space on the reef and more seaweed grows.[18]

Yes, it is complicated. The Goldilocks Line and P.H.A.G.E.S. will help you work through this complexity. All the moving dimensions means that strong mathematical and statistical models are also needed to describe these changing ecosystems. Recognizing this biological complexity is important because there are no simple solutions to manipulating wholobionts and ecosystems. For example, once there is lots of seaweed on a coral reef, it isn't enough to add back sharks. Going from a degraded reef back to a healthy one is something we haven't figured out yet. Similarly, Yellowstone will not return to pre-human conditions just by adding back wolves. There is a big

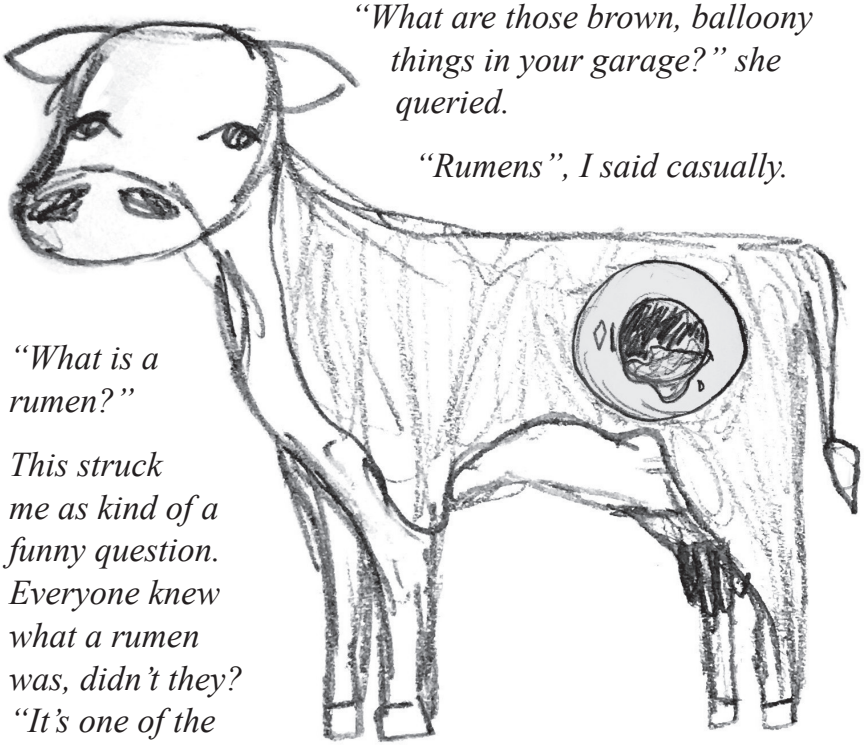
18 This is a positive feedback system we call DDAM for Disease, DOC, Algae, and Microbes (DOC stands for Dissolved Organic Carbon...don't you love acronyms within in acronyms?). Ungrazed macro- and turf algae (caused by overfishing) feed DOC to microbes, which increase in numbers. The microbes become more pathogenic because of piggybacking viruses and kill corals. This creates more space for more algae. This is a positive feedback loop that replaces coral reefs with algal reefs. Since it gets confusing referring to different types of algae, it is more convenient to use seaweeds when referring to the fleshy macroalgae and turf algae. By DDSM doesn't sound as cool:)

difference between understanding why an ecosystem has changed versus knowing how to manipulate the ecosystem to a desired state.

Finally, this biological complexity is relevant to you and your health decisions. Just like different states of Yellowstone and a coral reef, there are many different forms of the human wholobiont. Our challenge right now is that the modern lifestyle is encouraging DIVAs and piggybacking viruses. Humans are currently microbializing coral reefs, as well as themselves. The end result is we are getting fatter and less healthy.

Love Ruminations

I thought that a quick trip from college to the family ranch would be a fun outing for my newest soon-to-be-girlfriend. We parked the car and headed towards the house.



"What are those brown, balloony things in your garage?" she queried.

"Rumens", I said casually.

"What is a rumen?"

This struck me as kind of a funny question. Everyone knew what a rumen was, didn't they? "It's one of the stomachs from a cow."

"That is so gross! Why are there cow stomachs in your garage?" Her voice might have gone up a few notes.

"My dad is doing experiments on them." I thought, "Why else would you have rumens in the garage?", but it seemed a bad idea to voice this out loud.

"Where do you get the cow stomachs?!", her voice was definitely getting shriller.

"The slaughterhouse. You see when they gut the cow, the intestines and stomach fall out, and you can just walk up to the pile and pick out the rumen. We tie off the esophagial groove, and then load the whole bloody mess onto a truck, and bring it back here to the garage." She seemed slightly confuddled, so I helpfully continued, "Then you put a one-way value into them so that the gasses, most methane, can escape. As long as you keep feeding them, rumens can last a month or more without the cow."

Slightly green, she queried in a voice that had now dropped a full octave, "Whhhy?" Maybe she had a speech impediment. Best not mention it...

"Currently we are testing how bicarbonate changes the digestion of high moisture corn. It's cheaper to start the experiments in isolated rumens and then move into cattle for the final trials. In fact, the cows should be the next stop on the Bar Diamond tour."

My parents had started their combination animal nutrition lab and research feedlot in rural Idaho after meeting at the University of Nevada, Reno. This was the Golden Age of animal nutrition, with the goal of moving cattle off the rangelands and into feedlots in the grain belt, so there was lots of work. The research feedlot was a marvel of 1980's technology, with separate feeders for each animal that was operated through electronic tags merely 10 pounds in weight. Through these futuristic devices, the food each cow ate was closely monitored. But the real selling point to feed companies looking to test their products were the rumen fistules. If these didn't woe my female companion, then I must have no insight into true romance.

"Just step over that stream of manure, we'll get that cleaned up later. Maybe you should have worn boots. No worries, we can always rinse your high-heels with the hose after the tour. Okay, let's get this little guy into the squeeze-chute." The 800-pound steer, tagged #83, was one of the orneriest animals in the facility and he wasn't cooperating. It was almost like he didn't appreciate being stuffed in a metal box so we could probe

and prod his insides. Go figure. Since his recalcine nature was making me look bad in front of my little love bird, I gave #83 a little encouragement with the electrical Hot Shot. He jumped forward, where his head was caught with the chute's hydraulic clamp. His struggling to free himself was quickly suppressed by using the squeeze function of the chute. Now that my bovine "volunteer" was behaving, I could show off the jewel of this manure-coated, romantic tour.

"To monitor how different feed stocks are digested in the rumen, we just surgically cut a hole through the cow's body wall and into the rumen. These are stitched together and they soon heal to create a fistula, or window, into the rumen. Pretty cool huh?"

Eighty-three didn't think this was "pretty cool" and blew a big wad of snot onto the ground in front of her manure covered toes. She quickly moved to the side of the chute.

"We put this rubber cannula into the fistula. This gives access into the rumen. Here let me show you." I pried the cannula open so this lucky lass could get a good look into the rumen. "Look here!", I encouraged her. "We put pH probes and oxygen meters in here and take samples of the rumen fluid."

Even I found rumen fluid a little hard to deal with. Basically, rumen fluid is a soup of viruses and microbes happily digesting whatever the cow ate. The high microbial activity means that the rumen is an anaerobic rotter that produces nutritious short chain fatty acids, as well as odiferous volatiles like methane. By comparison to rumen

fluid, manure smells like a bouquet of flowers.

As my own delicate little flower reluctantly leaned forward to get a better view of this seething vat of grossness, #83 decided to perform a "rumen burp". Since the rumen has evolved to move around 50 plus pounds of rumen fluid, it has a lot of muscle. Mr. Bovine-Not-So-Pleased-to-be-Hot-Shotted, used all of that considerable rumen muscular force to push about 20 gallons of rumen fluid out the fistula and drench my rapidly dwindling love hope in persona.

As she took her third shower, it is surprisingly hard to get rumen fluid smell off your skin, I bagged her clothes and shoes. Though naked, I didn't think this would be good time to mention any amorous sweetnothings to her. Instead I left her some of my mother's clothes to wear.

As I drove my newly ex-girlfriend back to college, the conversation was reduced to, "Would you mind rolling down the window, something really smells..."

Free-standing rumens and fistulated animals were just part of the scientific effort to feed a rapidly expanding human population. This effort was known as the Green Revolution and it massively increased the quality of life for humans. However, like all most too-good-to-be true changes there were also many undesirable consequences that we are now dealing with. On of the most insidious is overnutrition; basically, instead of starving we are getting too much food and paying the cost in obesity and the associated chronic diseases.

Chapter 13.
Constructing the Fat American

We've discussed the colon as a congested, slow-moving river filled with viruses and microbes. Some of your internal inhabitants are loosely attached to the mucus layer to resist being pushed out during defecation. They form wobbly skyscrapers extending from the oxygen-rich, mucus basements near the human epithelia into the strongly anaerobic regions of the lumen. The viruses and microbes in these filaments, as well as those in the appendix, reinoculated the incoming chyme from the small intestine. This turns the mostly sterile, water-laden chyme into a dense microbial, water-parched landscape in about 24 hours. During this time, much of the water is reabsorbed by the body and the microbes process the left-over food into primary and secondary metabolites. Many of these metabolites, like *short chain fatty acids* (SCFA), are absorbed by the human cells, others are released as farts and feces. These processes influence your health in a variety of ways, including how much of the energy is stored by the human wholobiont.

Creating Space

The colon's microbiome consists of billions of individual virions and microbial cells crammed in each cubic centimeter of space. This high density helps protect the human tissue, because the limiting space-**g**overnor means that any invading microbe will have a hard time finding a place to make a living. Potential pathogens will simply float down the intestinal

river and out with the feces. The space-**g**overnor is one of the reasons that food poisoning is not as common as one might expect given that food is often covered in viruses and microbes. The space-**g**overnor is also why the probiotics have very little real health effect for most people;[1] the transient probiotic microbes can't find a place to homestead.[2] Resident gut microbes, like *B. fragilis*, regulate microbial composition in our intestinal crypts and prevent newcomers from exerting an influence in the wholobiont. One downside of taking antibiotics to ward off pathogens is that many of our symbionts are collateral damage. This leaves space in our guts for other viruses and microbes to move in. These open spaces are why probiotics might help during a course of antibiotics; the hope is that the "friendly" microbes in yogurt will exclude any "bad" guys.[3]

The first considerations for microbes homesteading the human GI tract is determined by the Goldilocks Line, does that microbe need oxygen? Our mouths, particularly our teeth, are covered in abundant mats of the air-loving, aerobic microbes on the outside and the air-hating, anaerobes next to our teeth. The aerobic microbes eat oxygen, which creates an oxygen-poor layer. This oxygen-poor layer serves as habitat for anaerobic microbes, which use other metabolic pathways to break down food. This is an example of *niche construction*; by using up all of the oxygen, the aerobic microbes are creating an environment, or niche, that didn't exist before. The aerobic microbes are making more space. The creation of an oxygen-poor environment is the equivalent to how the trees of Yellowstone

1 Suez, Jotham, et al. "The pros, cons, and many unknowns of probiotics." Nature Medicine 25.5 (2019): 716-729.

2 Probiotics that are optimized to live in yogurt or ferment in a factory will never be able to successfully **a**ssemble into a wild-type wholobiont. These organisms have been **s**elected for different environments.

3 This is also why people with *C. difficile* infections who can be cured by a fecal microbiota transplant will often have a recurrence of the infection when they take antibiotics because their new symbionts that took up the space after the transplant so that the *C. difficile* couldn't expand, get knocked out, and the few remaining *C. difficile* in the system quickly take advantage of the gap.

create space for songbirds or the coral reef creates space for fish.

In our colon, aerobic microbes are as close to our mucus-covered epithelia as possible. They need the oxygen from our blood to survive. Moving away from the colon's walls into the lumen, oxygen becomes exceedingly limited, and chains of alternate electron acceptors gets setup. This is niche construction on the millimeter level.

Microbial niche construction, based on a series of electron transfers to harvest energy, was discovered by Sergey Winogradsky. He was interested in the bands of purple, red, green and black that one sees when digging into a rich sediment. Anyone can visualize this microbial niche construction in the comfort of their own home by building a *Winogradsky Column*. Basically, grab some river mud, mix it with a little bit of egg yolk, and put it in a clear tube or bottle with shredded paper.[4] After a couple of weeks, there will be the different colored bands of microbes. Each band is a rung in the electron donor-acceptor ladder where the electrons are heading towards the oxygen at the surface.

Your colon is a type of Winogradsky Column. Electron donors in the chyme are physically removed from the oxygen circulating in your blood. In the low oxygen environment, anaerobic microbes use alternate electron acceptors like sulfur. Too much of the sulfur activity and you will fart very smelly sulfur compounds. Other alternative electron acceptor metabolisms use nitrogen and iron compounds. The use of alternate electron acceptors is kind of the same as oxygen-based metabolism and primarily builds ATP. However, less ATP is made per incoming glucose molecule because the final electron acceptors are not as good as oxygen. As the alternative electron acceptor pools are used up, fermentation becomes more important.

Unlike alternate electron acceptor and oxygen-based metabolisms, fermentations are more like stuck metabolisms. The energy for the most

4 The egg yolk (sulfur) and shredded paper (carbon) ensure that nutrient **g**overnors do not become problematic in the enclosed ecosystem.

"excited" electrons is released when the electron moves to lower energy orbitals within organic molecules. When this happens, some energy is released to do work. The end products of fermentation still have a lot of energy left in them. Think about the most famous fermentation product, ethanol. Drinking too much beer will help you get fat because there are so many high energy electrons left in the alcohol and the other fermentation end products. Your body uses the energy from these excited electrons to build fat.

In the human colon, oxygen depletion causes the microbes to produce a number of fermentation products. One of the most interesting classes of these metabolites is the short chain fatty acids (SCFA). Human cells love SCFA like acetate, propionate, and butyrate. Since SCFA are metabolites they can just be pulled into the human cells' metabolic pathways to build things or to generate energy. Most epithelial cells in the colon use SCFA as their main source of material for building and up to 90% of their energy. In P.H.A.G.E.S.'s speak, the SCFA provide two of the **g**overnors, energy and matter, to the human cells.

Each human wholobiont has a mixture of aerobic and anaerobic microbes living in their gut. Too many of the alternative electron acceptor bacteria that use sulfur and you won't be popular. Feed the fermenters too much food, like sugary drinks, and you will get lots of SCFA, and gain weight. Feed the system pre-fermented food like beer filled with the same types of metabolites and you will gain weight faster.[5]

Haber-Bosch Space

From the days of hunting-and-gathering to monocultures of genetically modified foods, humans have drastically increased access to energy

5 One popular diet is to only eat fermented foods. Fermented foods will have some potentially useful micronutrients like B12 and probiotic bacteria. However, calorie for calorie we would expect fermented foods to be more easily converted to fat or muscles (depending on your exercise regime) than unfermented foods.

and matter **g**overnors in food. We have supplemented this surplus with the necessary micro-nutrients like vitamins and trace minerals. Those three dimensions of nutrition, Calories, macro-, and micro-nutrients discussed in Chapter 2, are more than adequately filled. So much so, that the average American is getting too much nutrition.

How did we get here? The first big step was forming groups to hunt down big animals for large quantities of protein and fat. Then humans learned to use fire for cooking the meat, which releases more of the energy and matter **g**overnors. Cooking also released more energy and matter from grain seeds. Other food-processing methods were developed to get sugars directly from plants, including grinding and fermenting. The earliest archeological evidence of fermentation comes from Israel; probably just a tasty accident of trying to store grains that got a little wet.[6] These process released a lot more energy from grains and humans were on their way to getting lots more energy, matter, and micro-nutrients from their food.

Farming was the next big step forward and allowed humanity to build large cities and civilizations. However, farming left the human population limited by the matter **g**overnor. We simply couldn't get enough organic nitrogen to feed our crops from natural sources. Lots of tricks were used like moving to new land, crop rotations, and adding guano to farmland. But these were only temporary fixes, we were still limited by organic nitrogen. All this changed in 1910 with the advent of the *Haber-Bosch process* of converting atmospheric nitrogen into organic nitrogen for fertilizer. All this organic nitrogen allowed domesticated plants, and therefore humans, to escape a major matter **g**overnor. Since Haber-Bosch was invented, human nutrition has effectively escaped both the matter and energy **g**overnors[7]

6 Liu, Li, et al. "Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: Investigating Natufian ritual feasting." Journal of Archaeological Science: Reports 21 (2018): 783-793.

7 The energy-**g**overnor was circumvented by burning of fossil fuels.

The Haber-Bosch process led directly to the *Western, Educated, Industrialized, Rich, and Democratic,* or *WEIRD*, diet.[8] In the early 1900s, the average American had access to about 3,000 Calories per day. By 2000, that average American had access to 3,800 Calories each day.[9] Further, the types of food that we eat regularly have shifted from less-digestible "whole" foods to highly processed items that contain more easily accessed energy (e.g., high fructose corn syrup, ethanol) and matter (e.g., primary metabolites in beer).[10]

All of the extra energy and matter have dramatically changed our gut viruses and microbes. We know about these changes in the gut microbiome by comparing the metagenomes from different groups of people. European children, for example, have gut microbes most associated with high fat and animal proteins. Children from West Africa have microbiomes associated with high carbohydrate, plant-based diets that contain less animal protein and dairy. The microbiomes of people living in the industrialized societies have lower biodiversity when compared to more rural and hunter-gatherer peoples. This is also exactly what we observe on the microbialized coral reefs and severe cystic fibrosis exacerbations. As the energy-**g**overnor is released, microbial biodiversity goes down and the fat DIVAs (i.e., Dinner Is Very Available microbes) with piggybacking viruses become more common.

The WEIRD gut microbiome is dependent on excess energy in the

8 WEIRD was first introduced as a psychology term. Henrich, Joseph, Steven J. Heine, and Ara Norenzayan. "The weirdest people in the world?." Behavioral and Brain Sciences 33.2-3 (2010): 61-83.

9 Calories, a measure of energy, does not directly measure the matter-**g**overnor. Calories do capture the fact that energy from plants has dramatically increased. This increase is because the matter-**g**overnor has been released by the Haber-Bosch process.

10 United States Dept of Agriculture Office of Communications, Agriculture Factbook (Office of Communications, U.S. Dept. of Agriculture, 2001).

diet. More energy leads to the microbes that produce more SCFAs.[11] This creates a positive feedback loop of energy extraction means that a person with a WEIRD adapted microbiome will get more and more energy out of that burger and beer then someone with a hunter-gatherer microbiome. This makes losing weight even harder.

In WEIRD-fed humans, the DIVAs have access to so much energy that they start to attack our mucus layer; literally eating their host from the inside out.[12] In turn, this triggers immune responses and a state of near-chronic inflammation. Most of the non-communicable diseases (NCDs) like metabolic syndrome, type 2 diabetes, obesity, and cardiovascular disease can be mechanistically linked to excess energy consumption.[13]

Metabolic Syndrome

Many of the early studies on the human microbiome focused on the differences between lean and obese identical twins.[14] Because they have identical human genomes, studying identical twins means that differences in the human DNA text can be ignored. These studies were looking for outside influences on obesity. More specifically, on what jobs the microbes

11 Microbes that have better food also have better tools for getting more energy out of the food. This is called *co-metabolism* or *priming* in microbial ecology. The tools include more CAZymes, often encoded by proviruses. Turnbaugh, Peter J., et al. "An obesity-associated gut microbiome with increased capacity for energy harvest." Nature 444.7122 (2006): 1027. Haas, Andreas F., et al. "Global microbialization of coral reefs." Nature Microbiology 1.6 (2016): 16042.

12 Fiber really seems to be the most important thing to cultivating a microbiome that concentrates on degrading the leftover food in our colon, rather than the mucus lining. See the beautiful images in Tropini, Carolina, et al. "The gut microbiome: connecting spatial organization to function." Cell Host & Microbe 21.4 (2017): 433-442.

13 There are other medical reasons for some of these diseases, but we are specifically addressing those that are brought about through excessive Calorie consumption.

14 Twin studies are very important in scientific research. In examining identical twins (who share 100% genetic similarity) and fraternal twins (who share 50% genetic identity like any other brother or sister) the difference is that they are exposed to factors at the same point in time (unlike conventionally spaced siblings), we can answer very important questions about environmental versus genetic effects on humans.

were doing, including processing leftover food in the chyme, communicating with human cells to store energy as fat, and interacting with the human immune system. The early results were striking, almost 400 genes involved in energy metabolism were different between obese and lean twins.[15] Further, obese people's colonic ecosystems looked more like other obese people than their lean twin. Finally, mice could be made obese by transferring the microbiome from an obese human.[16] These were very exciting findings, opening up the possibility of preventing obesity through microbiology. This initial hope has been tempered by the reality of P.H.A.G.E.S. and the complication of the human wholobiont. There is no easy microbial magic bullet for curing obesity and other NCDs (Non-Communicable Diseases).[17]

The twin studies spurred a new round of studies into the human wholobiont with a dramatic increase in our knowledge in the complicated dimensionality of our health. Putting all of this work together and squinting a little, we now know: 1) WEIRD microbiomes spur positive feedback loops that increase the amount of energy extracted from food. WEIRD viruses and microbes are extracting more calories out of our food, which is ending up as fat in the human wholobiont. 2) WEIRD microbiomes produce signals that promote fat cells to get fatter. They also suppress the human-tissue derived signaling molecule that regulates how easily muscle and adipose tissues grab energy. So, in an obese wholobiont, microbes actually change how our human cells are storing fat. And, 3) WEIRD microbiomes trigger inflammation. Our human immune cells interact with the microbial cells through surface puzzle-piece proteins and other molecules to determine if a microbe

15 Turnbaugh, Peter J., et al. "A core gut microbiome in obese and lean twins." Nature 457.7228 (2009): 480.

16 Turnbaugh, Peter J., et al. "The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice." Science Translational Medicine 1.6 (2009): 6ra14-6ra14.

17 This brings us back to the points in chapter 2, simple cause-and-effect thinking will not fix complicated problems. If we can cause obesity by transferring the microbiome, then obesity is, in part, a communicable disease.

is a friend or a foe. One of the major puzzle-pieces is LPS,[18] that signal to our immune cells that bacteria have crossed the mucus barrier and are staging an attack. When LPS from the WEIRD microbiome leaks into the blood from our overfilled colons, the immune cells sound the defense alarm and rally the troops. These immune troops then attack the gut microbiome, causing chronic inflammation. One of the side effects of this war is damage to the epithelial cells. A break in the barrier allows oxygen to leak out and feed the DIVAs and allows more LPS to leak in, thereby ensuring another attack. This is a positive feedback loop to make us fatter and less healthy.

The consequences of WEIRD microbiomes are enormous. When dieting to lose weight the viruses and microbes in your guts are fighting you. They need the excess energy. In ecology, we call this resistance, which is an apt term. The WEIRD microbiome is resisting change and wants to keep the fat. All of this kind of sucks. We are left with the question: how might we deconstruct the fat American?

18 Lipopolysaccharides (LPS) is a macromolecule made of a lipid and a polysaccharide found in bacteria. Our immune system is very reactive to LPS, so much so that one of the major concerns of bacteriophage therapy is that the LPS in the lysates will trigger a deadly immune response. Removing LPS is why we got involved in the Tom Patterson case described in Chapter 1.

Useful Futility

"Please, please, pleeeeassssse...it's the first day of elk season. We can't miss it. Please, please, pleeeeassssse!"

"It is also your dad and I's anniversary." Replied my mother, who was not biting on our plan to be driven to the elk killing fields.

Rather than spend time on menial tasks like homework or chores, Franklin and I had spent the last six months shooting our bows in preparation for slaughtering, or at least, annoying elk. All that stood between us and elk jerky was my parent's mountain taxi service.

"Your anniversary is every year, not like elk season..." My mother stared at me a little incredulously as I reconsidered my rhetorical parry. "Oh yeah, elk season is also every year..."

Like any good future scientist, I quickly changed tactics. "What if we pay for a hotel room in Lowman for you and Dad?" I had her interest. Not only would she be releasing Franklin and I into the wild where we belong, but she could also get away from my little brother. The icing on the cake would be if my plan also included leaving my dad at home.

"And dinner. You will pay for dinner?"

"Of course, we'll be happy to pick up the dinner bill." At this point negotiating was easy, both she and I understood that neither I nor Franklin had any money. We belonged to the work-challenged, bow-shooting, leisure class.

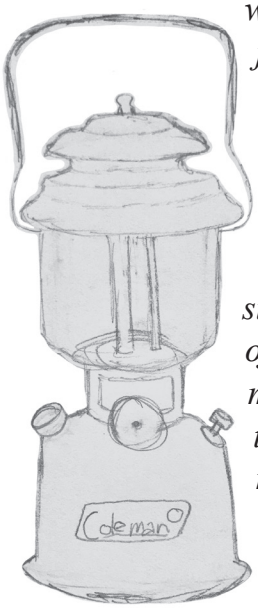
"Okay, you've got yourself a deal. Dinner and room for a ride into the mountains."

"Great! We'll get our stuff. Oh yeah, plus a little shopping.

It shouldn't take long. You and Dad will still have a great anniversary..." Our well-planned plan was coming together. Now all we needed was food, camping equipment, warm clothing, et cetera. Also, a map and some idea where we were going would be useful.

"No, if you want a ride we are leaving now. It will be close to midnight by the time we get there as is." Okay, we could do this without the food, warm clothing, camping stuff, or a map. We were budding woodsman after all. A quick survey showed that we had bows, arrows, an old canvas tent, some moldy sleeping bags, a Coleman lantern with white gas, and a case of Coca-Cola. What else could we possibly need?

Everything was crammed into the parents' mountain Cadillac, though they didn't realize it was a 4-wheel drive vehicle just yet. That bit of knowledge was to be an anniversary surprise from Franklin and I. Hours later, we were nearing the general, but unspecified, wilderness around Lowman, Idaho. Ominously, rain was coming down in sheets. Maybe a jacket would have been a good idea?



"Do you two have any idea where you are going?" queried Mom.

"Yeaaah...why don't we turn onto this logging road to the left here?" The Caddie's wheels started to spin in the mud. Luckily, the torrent of water cascading down the road washed away much of the mud slide, and my mother was able to keep the car moving with only occasional metallic bumps as the car bottomed out on middling-sized boulders. Finally, we ran into a largish river flowing through a gorge that had formally been the road.

"This looks like a good place for you to camp." Said my mother.

Franklin and I eyed the dark woods, pouring rain that was starting to look more like sleet, and considered our meager supplies. "Yes, this looks great! Thanks!" We jumped out and were immediately soaked to the bone. My mother, showing surprisingly good driving skills, though somewhat questionable parenting instincts, quickly backed down the mountain.

We set up our tent, shelter being the most important item for survival. The water immediately started to pool on the roof, which then dripped down through the WWI-era canvas onto the Civil War-era sleeping bags. Luckily, we did have a marvel of 1980's camping gear technology...inflatable mattresses! This way, as our tent rapidly became an enclosed pool, the sleeping bags would remain afloat.

"A fire" suggested Franklin through blue lips. Soaking wet wood was gathered and using our young woodsmen skills we proved soaking wet wood would not burn. "Let's pour some of the lantern's white gas on it..." This produced a barely observable, blue flame that produced no warmth and did not ignite the wood.

We needed substance to get the think cells fired up. Sugar and caffeine being the most important nutrients in survival circumstances, both of us grabbed a Coke. Luckily, this seemed to work, "Maybe we could fill the empty Coke cans with lantern fuel and then burn them for heat." This also produced almost no heat. Nonetheless, it was the only fire and we spent the whole night filling empty Coke cans with lighter fluid and burning them. Sometime even a futile fire is useful.

Chapter 14.
Useful futility

Most biological molecules are temporally unstable. To keep these delicate structures from falling apart, energy is used to keep the atoms in the right place. The more matter in your body, the more energy that your body will require to keep that matter in the correct place. This process of keeping the different pieces of matter in place, using energy, is the *resting metabolism* (a.k.a., basal metabolism). Of course, if you do anything other than just sit, even more energy is required. This is your *active metabolism* and one way that exercise is good for someone fighting overnutrition; movement uses up some of the extra energy in your diet.

Resting metabolism accounts for most of the energy used by wholobionts. Your brain is a major energy suck, as are your GI tract and muscles. Fat, or adipose tissue, is the notable exception. Many fats are incredibly stable biological molecules. Some are so stable that geomicrobiologists can identify fat molecules in rocks from bacteria that lived billions of years ago.[1] This chemical stability means that the amount of energy to maintain fat is very minimum. So even if you are carrying around 40 pounds of fat, it does not represent a major energy cost other than to move it. And, of course, that is the whole point. Evolution has **s**elected fats as the energy storing molecules precisely because they are so stable.

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1 Technically, much of this work has been done on lipids. Fats are a subgroup of lipids. Other lipids include cholesterol. Summons, RE and MR Walter. "Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments." American Journal of Science 290.A (1990): 212-244.

The body mass index (BMI) is a useful health metric because it is measuring how much energy is being stored as fat versus how much is being burned for resting metabolism.[2] And the BMI can be modified by building muscle or fat. Lots of anaerobic work at the gym will initiate anabolic metabolisms and more muscle. Lots of sitting around and drinking beer in the bar will lead to storage of energy in fat. Eating less will slow down both processes.

Some studies of humans have found correlations between body mass index (BMI) and microbial symbionts.[3] And wouldn't it be nice if there was a simple connection? If we could just change the viruses and microbes in our gut, then we could avoid the piggybacking viruses and DIVAs from the WEIRD diet. But, alas, P.H.A.G.E.S. reminds us the fix won't be so simple.

First, **h**istory and **a**ssembly mean that human wholobionts process food in both individual and culturally distinct ways. As discussed earlier, the gut microbes of Japanese people got a new DNA story from a marine microbe that helped them process seaweed energy more efficiently. Another major culture and genetic difference involves milk. Most mammals cannot process lactose after weaning. However, two separate groups of humans have acquired mutations that allow them to get energy from lactose sugar from milk as adults. One of these lactose-eating mutations occurred in northern Europe about 10,000 years ago and another in a part of Africa about 7,000 years ago. If you have ancestry from these regions, then your adult cells still make the enzyme, lactase, that will let your body access the energy in lactose without getting sick after weaning. This means if you are from these genetic groups, you most likely drink milk as a cultural habit.

Individual human wholobionts also acclimatize on ecological time scales. As our food changes, some viruses and microbes **e**xpand and undergo

2 The BMI is actually an extrapolation about the amount of fat versus muscle of the body. The relationships between the fat and muscle to energy is another extrapolation. This means that BMIs explain only about 75% of the energy usage by a particular individual. To really measure energy usage, it is necessary to put people into calorimeters.

3 There is a connection to BMI and viral symbionts too, but the research is still very nascent.

selection. If we start eating a kale-only diet, then a subset of our viruses and microbes rapidly **e**xpand and the previously happy microbiome are killed off by proviruses being induced because the bacteria would rather die than live on kale. There are so many different GI species that our gut functions despite drastic changes in the **g**overnors of energy and matter. How can we use the P.H.A.G.E.S. and the Goldilocks Line to help control our wholobionts?

Using the Goldilocks Line and P.H.A.G.E.S.

To use what we have learned so far, first identify where the Goldilocks Line is important for the system in question. It is helpful to build a table that lists the P.H.A.G.E.S. processes most important to the system in question. Roughly identify whether the processes are acute (short-term) or chronic (long-term).

P Brainstorm on a sheet of paper or chalkboard. Identify important P.H.A.G.E.S. processes for your problem. Get as many relationships down as possible and do not be too worried whether they are redundant; remember the P.H.A.G.E.S. processes are not mutually exclusive.

H Group the brainstorming scribbles into the appropriate P.H.A.G.E.S. processes in a table like this one.

A Order the processes based on relative importance within each P.H.A.G.E.S. category (i.e., the rows). At this point, start grouping and simplifying redundant processes where possible. The goal is to narrow your list to the most fundamental processes.

G Label the ranked processes within each P.H.A.G.E.S. category. For example, G1, G2, etc...

E In the third column of the table, label each process with an "A" for acute/short-term or "C" for chronic/long-term.

Now it is time to draw your P.H.A.G.E.S. figure. In this figure, things like viruses, cells, molecules, et cetera are connected to each other with the P.H.A.G.E.S. processes.

Once you have worked through this series of steps, stop and sleep on the problem. With a clear mind, start reordering and redrawing to reduce the problem to the most fundamental and concise statements possible. Make the figure as simple as possible. This is an iterative process and will take many tries for any complex problem.

Using the Goldilocks Line and P.H.A.G.E.S. to Understand and Optimize Dieting

Let's try using this approach to understand overnutrition and improve dieting. First, identify the Goldilocks Line.

Goldilocks Line in Relation to Dieting: : The WEIRD diet, as well as the less physical lifestyle of humans in the Western world, has pushed our bodies further into the anabolic region (i.e., lots of food and not enough oxygen). Anabolism is a biomass building process; without exercise the building will be fat, as well as microbialization of our guts. Build a P.H.A.G.E.S. table that captures these points.

Table 14.2. P.H.A.G.E.S. and Dieting

P	P1: WEIRD Humans eat lots more food, which means more sugar/electron donors. P2: More sugar with less oxygen favors abundant, fat microbes (i.e,, microbialization) and temperate viruses (e.g., Piggyback-the-Winner). P3: Lysogens attacking mucus under high sugar:low oxygen conditions.
H	H1: Height determined by genetics. Weight determined by eating and exercise habits. This yields the Body Mass Index (BMI). H2: Underlying conditions like metabolic syndrome.
A	A1: Some types of bacterial viruses and microbes in the gut release more energy from food (e.g., when there are not enough electron acceptors). A2: Acquisition of bacterial viruses and microbe during development.
G	G1: Increased sugar:oxygen ratio leads to anabolic metabolisms. G2: Low physical activity means anabolic products stored as fat. High physical activity (i.e., exercise) facilitates storage of anabolic products in muscle. G3: How energy is divided up in & stored in the food. G4: Increased space for food storage.
E	E1: **E**xpansion of microbial cell types. E2: For the most part, the main human cell types we are concerned with aren't replicating (i.e., they are terminally differentiated).
S	S1: Prophage bring in genses that get more energy from food.

In this case of dieting, the main consideration is the high sugar:ox-

ygen ratio driving anabolic metabolisms. Eating a WEIRD diet shifts the human wholobiont strongly into the anabolic region. Where the anabolic products are stored depends on the amount of physical activity. This is one reason why exercise is important. However, no amount of exercise will make up for too much sugar entering the human wholobiont. To lose weight, the sugar:oxygen ratio needs to be shifted to the catabolic side of the Goldilocks Line. This will release stored anabolic products as carbon dioxide and water. To get to this endpoint, the amount of oxygen must exceed that amount of sugar taken in. Adding fiber to the person's diet encourages *Prevotella* spp. at the expense of *Bacteroides* spp.

The P.H.A.G.E.S. for dieting shows something that we all know. Eat too much food and the sugar to oxygen ratios are going to favor the production of anabolic products. In turn, these produces will be stored in the body (i.e., building biomass). Reduce the amount of food, and the sugar:oxygen ratio will get smaller, fewer anabolic metabolites will be produced, and there will be less biomass building. Shift the sugar:oxygen ratio enough and the body will turn biomass into carbon dioxide and water and you will start to lose weight. This is just moving the body from the anabolic side of the Goldilocks Line the catabolic side.

The body primarily stores anabolic products as fat and muscle. Exercise increases anaerobic conditions (i.e., low oxygen) preferentially in the muscles. That is why exercise is necessary to build muscle. If you do not exercise, but take in lots of sugar, then the anabolic products are stored in fat. This is also a reason why the term anabolic often confuses people since anabolism builds both fat and muscle. Someone sitting on a bar stool and drinking a beer is taking in plenty of anabolic products and building fat. Someone exercising and drinking beer is building muscle. It is difficult to exercise enough to move all of the anabolic products from the beer into

your muscles.[4]

The Goldilocks Line is essential for controlling how the energy-**g**overnor is manipulated by the wholobiont. In humans, our brain is 2% of our weight and accounts for ~20% of basal metabolism. The brain only uses glucose and oxygen to generate the ATP necessary to run neurons for thinking. This means brain tissue is running the full TCA cycle to generate as much ATP as possible. All this metabolic work is damaging because it produces reactive oxygen species. This is why sleep is absolutely necessary to survive. The Western world is so active mentally that we are always feeling starved for sugar. This is one of the reasons that we munch so much on junk food. In a very real sense, sugar is brain food. If you spend all day staring at a computer screen and then go home and watch TV, then your brain is slurping up glucose. Since we only sleep about 8 hours anymore, this sugar demand has increased. Unfortunately, thinking hard does demand glucose but you can't think hard enough to make up for all those gummy bears.[5]

Creating Futile Cycles with Fiber

Similar to the brain, the microbiome only makes up ~2% of the body mass and accounts for 10-25% of the basal metabolism of the human wholobiont. This means that somewhere between 50-450 Calories are processed through this external organ every day. Hence the desire to manipulate the microbiome to lose weight. Too much of the energy and matter **g**overnors leads to anabolic metabolism, microbialization, piggybacking viruses, and skyrocketing obesity. Our simple response has been to decrease the energy- and matter-**g**overnors through diets. This has failed miserably by

4 Since the relationships between fat, muscle, and sugar are so central to metabolism, the body has a number of physiological controls like insulin and anabolic steroids.

5 Like wood, fat is essentially just a bunch of sugars strung together and rearranged with the molecular formula of fat is $C_{55}H_{104}O_6$. Each pound of human fat represents about 3,500 calories. So, to burn one pound per week, you need to reach a total calorie deficit of about 3500 calories per week.

any waist-line measure.

If humans are going to eat cake instead of kale, then we need to do it in way that avoids the downsides of microbialization. A straight-forward way may be to create futile cycles. These are energy-utilizing metabolisms that don't do any useful work. The easiest futile cycle is to add fiber to the diet. This favors bacteria like *Prevotella* spp., which grow well in the strict anaerobic conditions of the colon, but provide fewer anabolic metabolites to the human host. The best way to provide this fiber, unfortunately, is to eat kale and other vegetables. If you are morally opposed to vegetables, then add Acacia fiber to your diet.[6]

One future possibility for increasing futile cycles in the microbiome might be adding alternate electron acceptors. If there was enough oxygen, then the microbes would not ferment or use alternate electron acceptors. In theory, they would burn the excess energy with a lot less anabolic building, and the bacteriophage would behave more virulently and kill the bacteria, speeding up the production of waste heat. Maybe these extra electron acceptors could be added to food to ensure that catabolic metabolisms are favored. This would need to be controlled, to make sure that the extra electron acceptors were released in the colon. While the extra electron acceptors would decrease much of the piggybacking and other problems with microbialization, it might encourage some bad DIVAs who love lots of electron donors and acceptors. This and other problems that might be caused by increasing the number of electron acceptors in the gut means it isn't quite time to quit your day job and start a gut oxygenation company. However, **a**ssembling the human wholobiont does have some potential, near-term possibilities.

6 As with everything diet related, the noise around supplementary fiber is almost impenetrable. Unlike most diet recommendations, however, we have a pretty good idea about how fiber will increase weight-loss associated bacterial genera like *Prevotella* and *Bifidobacterium*. Hosobuchi, Cindy, et al. "Efficacy of acacia, pectin, and guar gum-based fiber supplementation in the control of hypercholesterolemia." Nutrition Research 19.5 (1999): 643-649.

Some Assembly Required

Humans are mammals. The very name sums up how important mammary glands and milk are to our existence. Human milk **s**elects for a specific set of viruses and microbes on ecological time scales and the composition of milk has been **s**elected on evolutionary time scales.

Most of us get our initial dose of microbes from our mother's reproductive and GI tracts during birth. This is a fleeting moment and may or may not be that important in the long term for **a**ssembly. Some studies have shown that children born vaginally have different microbial communities than those born via Caesarian surgery. The former infants were colonized with more vaginal microbes such as *Lactobacillus* and *Bifidobacterium*; while in the case of the latter, more skin associated microbes predominate such as *Staphylococcus* and *Clostridium*.[7] As is typical, the more we look, the more confusing it becomes. Now some data suggest that the inoculation starts even earlier from placental-derived microbes (this is probably not true in most cases).[8] And, of course, the longer-term inoculation comes from milk and skin viruses and microbes found on the teat, as well as GI microbes from mom. It is impossible to get all of the fecal microbes off mom's fingers and when she touches her nipples they get transferred to the skin and eventually into the milk. Biology is messy.

Breast milk is not a sterile liquid. Actually it appears that milk, in addition to being an amazing nutrition source, is also a vector for transferring viruses and microbes to the infant. There are about 15 genera of bacteria present in breast milk that are also common symbionts in the adult human GI tract. Milk is a transmitter of microbes many of which are poten-

7 Maria G. Dominguez-Bello et al., "Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns," Proceedings of the National Academy of Sciences, June 21, 2010, https://doi.org/10.1073/pnas.1002601107

8 Perez-Muñoz, Maria Elisa, et al. "A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome." Microbiome 5.1 (2017): 48.

tially beneficial, doing jobs like educating the immature immune system[9] and maturing the structure of the gut itself through the microbial version of terraforming. Micrographs of human immune cells and milk cells show bacterial structures associated with them, linking these cells as possible elements of microbial transfer from mother to infant. The mother's mammary glands are great culturing systems for microbes that feed off milk at body temperature and facilitate viral and microbial transfer to her offspring via breast milk.[10]

Human infants do not have a fully mature GI system when they are born. Maternally derived growth factors in breast milk, like *epidermal growth factor (EGF)*, facilitate gut maturation by stimulating our human cells to increase mucus production while decreasing the permeability of intestinal epithelial cells by signaling to them to crowd together. These changes in the tight junctions and mucus hinder invading viruses and microbes.[11]

EGF also helps set up niches for our symbionts, delineating space and boundaries that will be further modified by the symbionts through their fermentative abilities. The levels of EGF and other components[12] in breast milk respond to the changing needs of the infant's digestive tract first by being present in very high levels just post birth—at even higher levels when the birth is premature—when it is vital that the infant establish a healthy gut topography and strong mucus flow, then to lower levels as the infant ages and his gut's structure becomes more stable, conferring protective and metabolic powers to young human wholobionts by directly affecting the

9 Verhasselt, Valérie. "Neonatal tolerance under breastfeeding influence." Current Opinion in Immunology 22.5 (2010): 623-630.

10 Donnet-Hughes, Anne, et al. "Potential role of the intestinal microbiota of the mother in neonatal immune education." Proceedings of the Nutrition Society 69.3 (2010): 407-415.

11 Dvorak, Bohuslav. "Milk epidermal growth factor and gut protection." The Journal of Pediatrics 156.2 (2010): S31-S35.

12 For example, cytokines, secretory immunoglobulin A, and lactoferrin offer protective influence from pathogenic microbes and possible allergens. Further, amylase, casein, and folate binding protein work to make nutrients in breast milk more accessible to both human and microbial cells.

colonizing microbiota.

One of the strongest lines of evidence that Mother Nature intents us to be wholobionts is that some components of breast milk aren't directly useable by the human cells. These components must first be processed by microbes. The best characterized are *human milk oligosaccharides* (HMO), the third most prevalent component of breast milk after lactose and lipids, have the important role in selecting and nurturing the types of microbes that will colonize the gut. That is, HMOs are setting a baseline for microbial and intestinal health that will affect the infant throughout the rest of their life. EGF and HMO work together to encourage microbial colonization and growth on the lumen side of the mucus.

When the data are combined, it looks like the HMOs and lactose in human milk are primarily selecting the Bacteria species *Lactobacillus* and *Bifidobacterium*.[13] When those microbial species are not around, other related ones take their place. Formula, as well as cow's milk, select for DIVA-like microbes because they have higher sugar and protein content than human milk. Further, both these replacements are sterile. This means that formula-fed children tend to pick up DIVA microbes willy-nilly from the environment, instead of mother's mammary gland.

The evidence is compelling that we should make sure that initial **a**ssembly of the human wholobiont be encouraged towards *Lactobacillus* and *Bifidobacterium* microbes using human milk. Mother's milk is best. Banked breast milk is good. Prolacta has made a set of breast milk products that are very successful in the neonatal intensive care unit (NICU). Finally, progress is being made in this direction by massively improving formula to include less sugar and at least some HMOs.

13 This is an example of shifting baselines in the human wholobiont; we don't really know what the original human viromes and microbiomes looked like. Unlike a coral reefs, it is not clear that we even want to get to the pristine, ancestral state. Human life used to be short and brutal with lots of deadly diarrhea and intestinal parasites. Unfortunately, this is still true in much of the developing world.

Rejecting Mom

At about one year of age, many kids start to spit up mom's breast milk. Babies start to expand their diet to more solid food and shoes. This is weaning, one of the most important development steps and in the developing world weaning is where kids die.[14] Even though we know that weaning is super important, it is much less well understood in terms of microbiology.

Weaning is concurrent with important developments in the structure of the GI tract. Sphincters form between the stomach and small intestine. The stomach then acidifies, and this is one reason that many kids start to spit up their mother's milk as curdled chunks. All this acid means that stomach is essentially sterile, creating a barrier to incoming viruses and microbes.[15]

Another sphincter forms between the small intestine and the colon. Most of the viruses and microbes get pushed out of the small intestine. The mucus layer thins so that energy and matter from chyme can be absorbed easier.

In the colon, obligate anaerobes slide into the functional niches made as oxygen levels decrease to near zero.[16] These are the microbes in your colon that do all that fermentation of impossible-for-humans-to-digest food. Some of the fermentation products, like the SCFA (short chain fatty acids), are passed onto the rest of the human wholobiont. These products also influence the living wall of epithelial cells, triggering even more tight junction formations.[17]

By age two, a healthy individual will have an established "adult" gut

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14 Gordon, John E., John B. Wyon, and Werner Ascoli. "The second year death rate in less developed countries." American Journal of Medical Sciences 254.3 (1967): 357-80.

15 Many viruses, bacteria, and protists have endospores and other acid-resistant states that can resist the GI tracts' defenses; these are the pathogens that cause so much trouble.

16 Adlerberth, I., and A. E. Wold. "Establishment of the gut microbiota in Western infants." Acta Paediatrica 98.2 (2009): 229-238.

17 Sharma, Renu, Christopher Young, and Josef Neu. "Molecular modulation of intestinal epithelial barrier: contribution of microbiota." BioMed Research International 2010 (2010).

with a viral and microbial community that is nicely settled into its niches. This community remains remarkably constant over time despite transient changes that occur when taking antibiotics or dieting.[18]

Because early homesteaders and subsequent immune education dynamically arises from whatever microbes and viruses happen to be around when we are born, there isn't a magical combination of microbes and breast milk that confers health. The **h**istory of each individual wholobiont ensures that his or her specific combination of symbionts uniquely responds to changes in the environment and energy flow. The flexibility and flux of the infant gut community means that as the wholobiont responds to the environment, microbes who can do certain jobs are **s**elected.

Bacteriophage Attachment to Mucus (BAM) Immunity and Milk

One fraction we have not talked about in mother's milk is the fat globules, or cream. Since fats are stable biological molecules used to store energy, it was assumed that the fat in milk is just about getting enough energy to the baby. In part, this is true; marine mammals put large amounts of fat into their milk to rapidly bulk up their soon-to-be-blubbery infants. However, the story might be more complicated and the fat globules in mother's milk may have dual functions. Surrounding the fat globules is a thin layer of mucin proteins. Remember that some phage like to bind to mucus, specifically those that form the microbiome-derived BAM Immunity. So, the simple hypothesis is the bacteriophage are being transported by milk to initiate BAM Immunity to protect the infant. There are some data supporting this hypothesis, but lots of work remains.

This brings us to an important point. Almost all the data on human

18 Most current research focuses on microbes in the gut because their metabolic contributions are the most evident. This focus is shifting as the sheer volume of genetic influence of the bacterial viruses become known..

microbiome development is based on statistical analyses of fecal matter. The larger the datasets, the better our understanding of how different things like viruses, milk, microbes and body fat are related. These types of statistical analyses are only the first step in the scientific process. They help define hypotheses, which must be tested with experiments. The goal is to come up with the mechanisms by which things are happening. In general, understanding the mechanisms is far more powerful than a statistical description alone for predicting the future and/or manipulating a complicated system. However, we don't directly experiment on humans for ethical reasons. This means that most of our mechanistic understanding of the human wholobiont actually comes via proxies like pigs, mice, and tissue culture. P.H.A.G.E.S. is an attempt to lay out the mechanisms that will be important in any ecosystem and hopefully allow us to safely manipulate these our wholobionts and even Earth itself.

Lionfish: Friend or Foe?

Willow, age 9 and about 45 pounds, was literally shaking in her little wetsuit from excitement. As the boat stopped, she was overboard and snorkeling away from the boat. We could see 3 or 4 sharks just below her. She stopped took her head out of the water and yelled through her snorkel, "Dad! Hurry up, there are sharks!" Her excitement was contagious, and the more nervous adults started to get ready to enter the shark-filled waters around Bora Bora. Everyone who ever snorkels or SCUBA dives knows the thrill of seeing sharks and big fish.

On the same trip, Willow and I saw the magnificent lionfish hunting on the reef crest. Usually these colorful groupers move as a pair, trapping smaller fish in coral crevices by spreading out their venomous pectoral fins. Just when the small fish thinks it is safe, the lionfish gulp several liters of water into their mouth and out their gills. The luckless victim is pulled out of their coral retreat, filtered out by the gill rakers, and swallowed whole by the lionfish.

Though Bora Bora is still an underwater wonderland, the signs of reef decline were everywhere if you knew what to look for. There were sharks, but only a few of them. They congregated at the tourist boats to get a fish snack, but on the reef proper there were 1 or 2 sharks where there should have been 10-100s. Similarly, the big groupers were mostly absent. Many of the corals were looking pretty rough with dead zones caused by bleaching and diseases. The Tahitians were aware of this decline as evidenced by the coral gardens advertised as reef restoration.

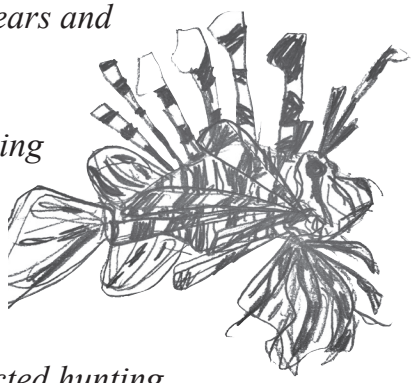
Several months after the Bora Bora trip, I once again broke Willow out of kid prison (a.k.a., school) and escaped the email-filled halls of academia to go work in Curacao. This time we

took a trip around to the East Point, where some of the highest coral cover in the Caribbean can be found. One reason is that getting to East Point is fairly rough, with largish swell and lots of wind chop. Willow was gamely dealing with the up-and-down and side-to-side tumbling, but starting to look a little green.

I gave her some quality dad advice, "I always find it better to eat something dry with a little salt. Here, try these Cheez-Its. They should be great."

In her pre-teen naivety, she still listened to Dad and ate a couple of handfuls. About then, the boat turned around a small point and the sea got really rough. Everyone was hanging onto anything they could as waves broke over the stern and soaked us. After a nice pool of seawater was sloshing around our dive gear and feet, Willow lost control of the her Cheez-It filled stomach. The orange-colored vomit was quickly mixed with the seawater to cover dive gear and feet. Several adults turned green, creating a positive feedback loop of vomit water leading to vomit to more chunky water. All we could do was hold on as this experiment in emulsification played itself out around our feet and dive gear. Finally, we reached calmer water, and with the resilience of a kid, Willow was first into the water. The rest of us were trying to deal with Cheez-It encrusted dive gear. She stuck her head out of the water and yelled, "Lionfish, Dad! Hurry up."

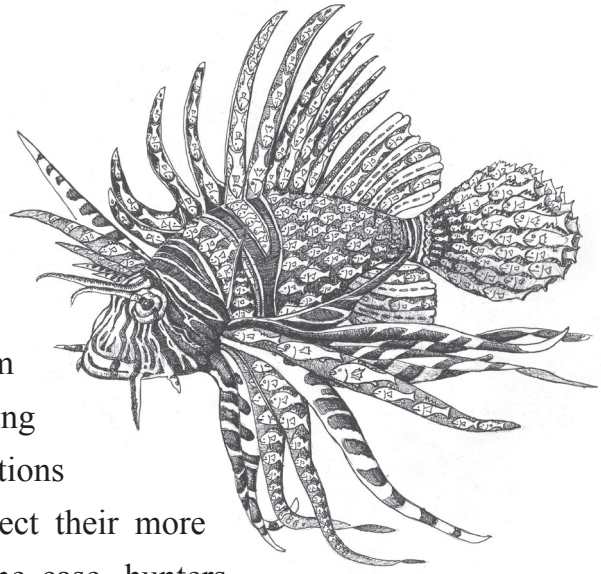
Several others got out their hand spears and jumped in the water. They beelined towards the lionfish and killed them. Willow asked me, "Why are they killing the lionfish? I thought they were good for the reef."



Willow was correct, lionfish are probably good for the degraded reefs of the Caribbean. The unrestricted hunting

of lionfish in the Carribean is justified because they are invasive species that eat native fish. But this simple, cause-and-effect misses the point that the shifted reefs are missing apex predators. As we move into the future, we will need to harness P.H.A.G.E.S. and consider alternatives like letting invasive species do some ecosystem engineering for us.

Chapter 15. The Phuture



P.H.A.G.E.S. and Lionfish

Hunting by humans has dramatically changed our nutrition and food webs ranging from Yellowstone to coral reefs. Shifting baselines means that our perceptions of these ecosystems do not reflect their more pristine states. In the Yellowstone case, hunters, ranchers, and biologists alike did not anticipate many of the consequences of re-introducing wolves. These top **p**redators terrified and killed so many elk that the whole ecosystem responded, and many people want to kill off all the wolves again. Similarly, in the early 2000s a top **p**redator entered the mostly **p**redator-free Caribbean coral reefs and started killing large numbers of little fish. The response was similar, people started a campaign to kill off these predatory lionfish. However, as we saw in Chapter 11, overfishing has led to coral reef decline through microbialization and piggybacking viruses. Might the **e**xpansion of **p**redatory lionfish in the Caribbean be a good thing for these coral reefs? Let's use the Goldilocks Line and P.H.A.G.E.S. to build our arguments.

Goldilocks and Coral Reefs: Removal of fish has reduced grazing pressure on turf and fleshy macroalgae. These seaweeds use photosynthesis to make sugar, which stays on the reef, and release oxygen, which bubbles off the reef. This creates an oxygen-depleted ecosystem, with happy microbes and sick corals.

Table 15.1 P.H.A.G.E.S. for Lionfish on Caribbean Coral Reefs

P	P1: Fish and invertebrate grazers eat algae. P2: Higher trophic layers, like sharks and groupers, eat the grazers.
H	H1: Most coral reefs are severely overfished, even though most people don't recognize this fact due to shifting baselines.
A	A1: In oxygen-poor conditions, bacteriophage form lysogens (rather than lyse host cells). The proviruses encode virulence factors to protect against protists, thereby creating opportunistic coral pathogens.
G	G1: Space for fish is provided by corals. More space/holes, more fish. G2: 90% of the energy is lost at each trophic level.
E	E1: Invasive species expand rapidly because their **p**redators and parasites are absent.
S	S1: Lionfish in the Caribbean have escaped **p**redators

There is plenty of data and stories about how many other fish the lionfish are eating, and there is a fear that they will eat all the fish in the

already overfished Caribbean.[1] There is another problem: lionfish are also invasive meaning that they came from the Pacific and are not native to the Caribbean. There are often lots of problems with invasive species. Often an invasive species has escaped its normal **p**redators, so the populations can rapidly **e**xpand. And many times, the prey items don't have defenses against the invader. While many invasive species are bad, there are some invasive species that are really useful, like honeybees.

From the P.H.A.G.E.S. point-of-view, letting the invasive lionfish **e**xpand in the Caribbean is probably a good idea. First, lionfish could be that trophic layer of top **p**redators that is needed on Caribbean coral reefs. Second, lionfish are invasive species so no-one feels inclined to protect them. They are also tasty, so lionfish can be exploited as a fishery. Third, lionfish are beautiful underwater. If you are going to be invaded, then be invaded by something that is pretty.

A P.H.A.G.E.S.-based response to the lionfish invasion of the Caribbean would be something like this. Since **p**redation on seaweeds and grazers is essential for coral reefs, put a size limit on lionfish catches. For example, eat any lionfish smaller than 12 inches. This will lead to more fish tacos and leave a pool of large, mama lionfish to replenish the population.[2] At the same time, enforce a strict moratorium on fishing any native Caribbean groupers or sharks. These populations will need decades to re-establish themselves. This plan has the advantage of providing humans with a valu-

1 In the words of the NOAA webpage (http://www.sailorsforthesea.org/programs/ocean-watch/lionfish-invasion): "Lionfish are voracious predators and are taking the already threatened Caribbean reefs by storm. Lionfish are non-selective feeders, and with virtually no natural enemies in the tropical western Atlantic they've invited themselves to an all you can eat seafood buffet. Lionfish have been observed consuming 20 small fish in a 30-minute period and prey up to 2/3rd of their own length. Impressively, their stomachs can expand up to 30 times their normal size after a meal. Mark Hixon et al (2009) determined that a single lionfish can reduce juvenile fish populations by 79% in just 5 weeks."

2 P.H.A.G.E.S. is already kicking in and controlling lionfish. Their rapid **e**xpansion is slowing down because of the energy- and space-**g**overnors (i.e., running out of food) and **p**redation by a parasitic skin disease.

able protein source while re-establishing native **p**redator populations and avoiding DIVAs and piggybacking viruses of a microbialized coral reef. In the far future, when the Caribbean coral reefs stabilize, aggressive lionfish eradication programs can be used to reestablish the "natural" ecosystems. As **h**istory reminds us, these recovered reefs will not be the same as those before humans showed up, but they will be beautiful for diving and fulfill vital reef functions like fisheries, natural products, and coastal protection.

What does P.H.A.G.E.S. tell us about managing the lionfish population? Recall that **e**xpansion has three main dimensions: 1) the time between generations, 2) the number of offspring (i.e., virions, kids, daughter cells) produced each generation, and 3) the variation introduced by replication (i.e., the raw material for **s**election). In fish, the number of offspring is usually the most important dimension. A big mama fish can produce literally millions of eggs, whereas a small one will only produce a few hundred.[3] This phenomenon is called *hyper-allometric scaling*, and what it means is that apex **p**redators like humans and groupers can eat large amounts of immature fish and have very little impact on the final population size. The only thing we should NOT do is eat the sexually mature adult fish.[4] Unfortunately, our intuition and many fishing regulations often work in just the opposite directions and fishing often target the largest individuals. For conservation and to maximize production, it is essential to remember the three dimensions of **e**xpansion.[5]

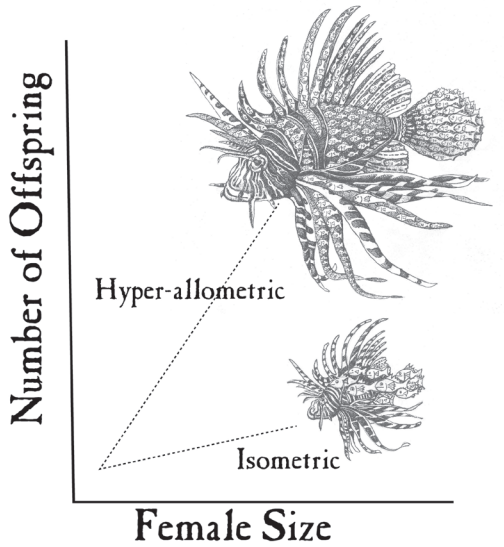


Figure 15.1. Figure 15.1. Eat the children! Big Mama fish produce a disproportionate number of offspring. The eggs and offspring are also more robust. This phenomenon is called hyperallometric scaling and means that we should strongly protect all of the big fish and only predate on the small individuals (Barneche, Diego R., et al. "Fish reproductive-energy output increases disproportionately with body size." Science 360.6389 (2018): *642-645.)*

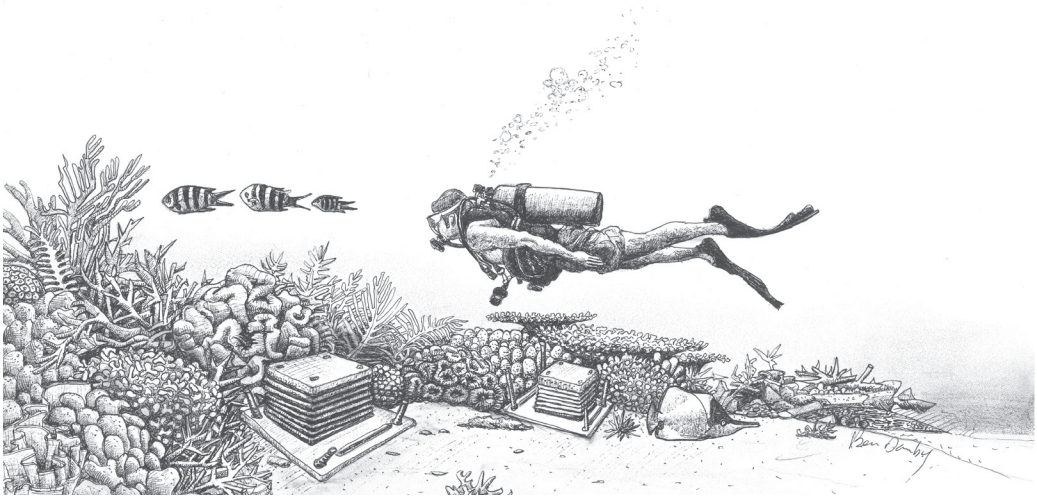
P.H.A.G.E.S. and Rebuilding Coral Reefs

One of the ecosystem services that needs to be re-established on coral reefs is the space-**g**overnor. One effect of coral reef microbialization is the flattening of the reef structure itself. Instead of a highly complex, rugose reef with lots of holes for fish and invertebrates, the degraded reefs are flatter.[6] Reef flattening caused by microbialization has lots of undesirable outcomes, including a loss of habitat and coastal protection. The loss of appropriate spaces for the fish means that, ironically, **p**redator-free reefs do not have many more small fish. Instead of being **g**overned by energy and matter, these reefs are space-limited. In the words of one of my fishy colleagues Dr. Alan Friedlander, "Big holes, big fish."

In the early 2000s, Drs. Nancy Knowlton and Rusty Brainard con-

vened a series of workshops to develop a standard method to measure biodiversity on coral reefs. Autonomous Reef Monitoring Structures, or ARMS for short, were the result. ARMS are basically a settlement surface and hotel for coral reef organisms build out of PVC (i.e., plastic sheets). The idea is that by providing the same habitat for settlement of different organisms it would be possible to compare biodiversity between coral reefs.

Thousands of ARMS have been deployed on coral reefs, as well as



other marine habitats, all over the world. The ARMS are left on the bottom for about 3 years, then they are retrieved and everything on them collected. Using DNA sequencing, we then determine what and how many species have settled. A single ARMS unit can have 10,000 of different species living on it. And collectively, over a million species have been identified on ARMS. Since so much of coral reef biodiversity was recruited to ARMS without any harvesting of coral reef material. Based on this observation, we suggested that ARMS be used to non-destructively collect most of coral reef diversity for conservation and restoration efforts. This became the Coral Reef Arks project.

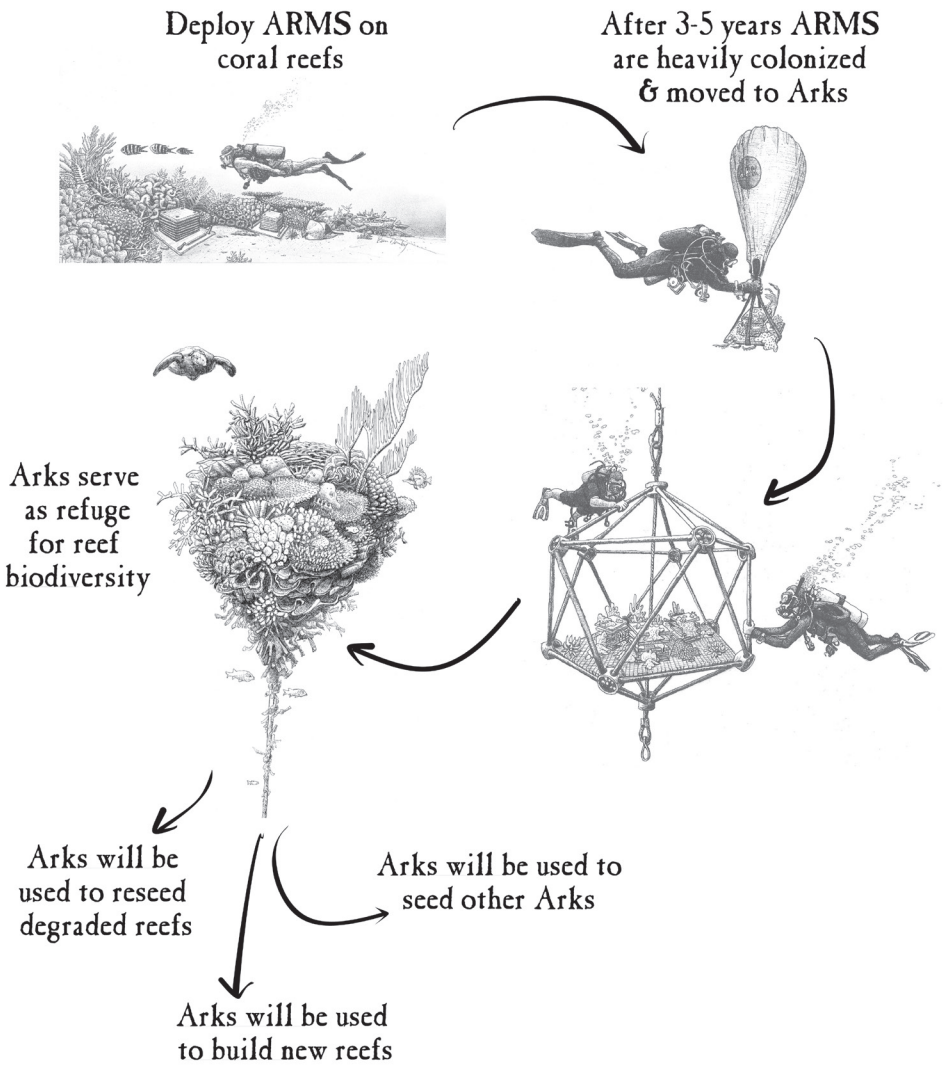
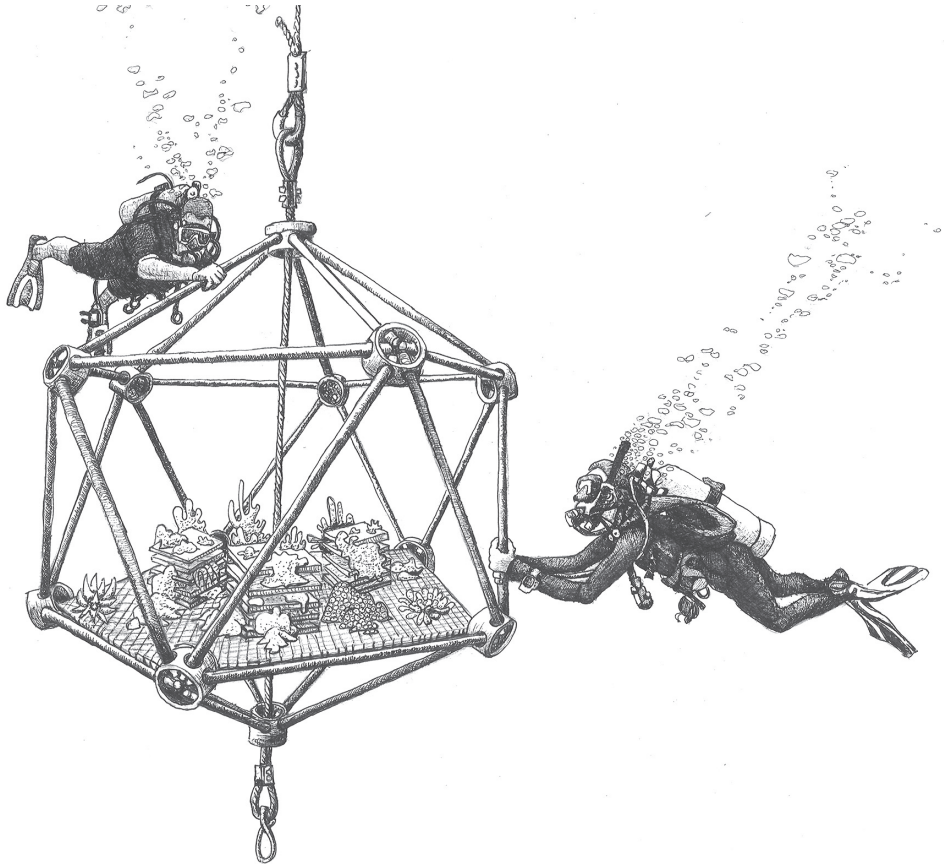


Figure 15.2. The ARMS and Arks development cycle. Coral reef viruses, microbes, and macrobes are recruited to ARMS over a 3–5-year period. This biodiversity will then be transferred to floating superstructures called Coral Reef Arks, which can serve as floating zoos for conservation or the raw materials for restoring coral reefs. Arks may even be used to build new coral reefs as climate change opens up new regions further north and south. Currently, new materials for increasing biodiversity recruitment to the ARMS and prototype Arks are being sea-tested.

Imagine massive floating structures in the open ocean colonized with luminescent corals, anemones, crabs and urchins, and circled by giant schools of fish. These are Coral Reef Arks. Like building blocks, hundreds

of ARMS will be aggregated onto Coral Reef Ark Parks to assemble large reef communities from the ten coral reef regions of the world. These Arks will be placed in sites less vulnerable to climate change and other stressors, ensuring the survival of the entire ecosystem; zoos for coral reefs. The Coral Reef Arks are expected to generate immediate ecosystem and economic benefits in the form of fisheries. By directly manipulating the space **g**overnor, fish populations will increase over time. This has been shown on artificial reefs in different ecosystems.[7] These Coral Reef Ark Parks will also preserve coral reef biodiversity, including natural products, and provide tourism opportunities.

Scientifically, Coral Reef Arks will also create a new way to study how coral reefs are built. As we've seen, overfishing and the subsequent

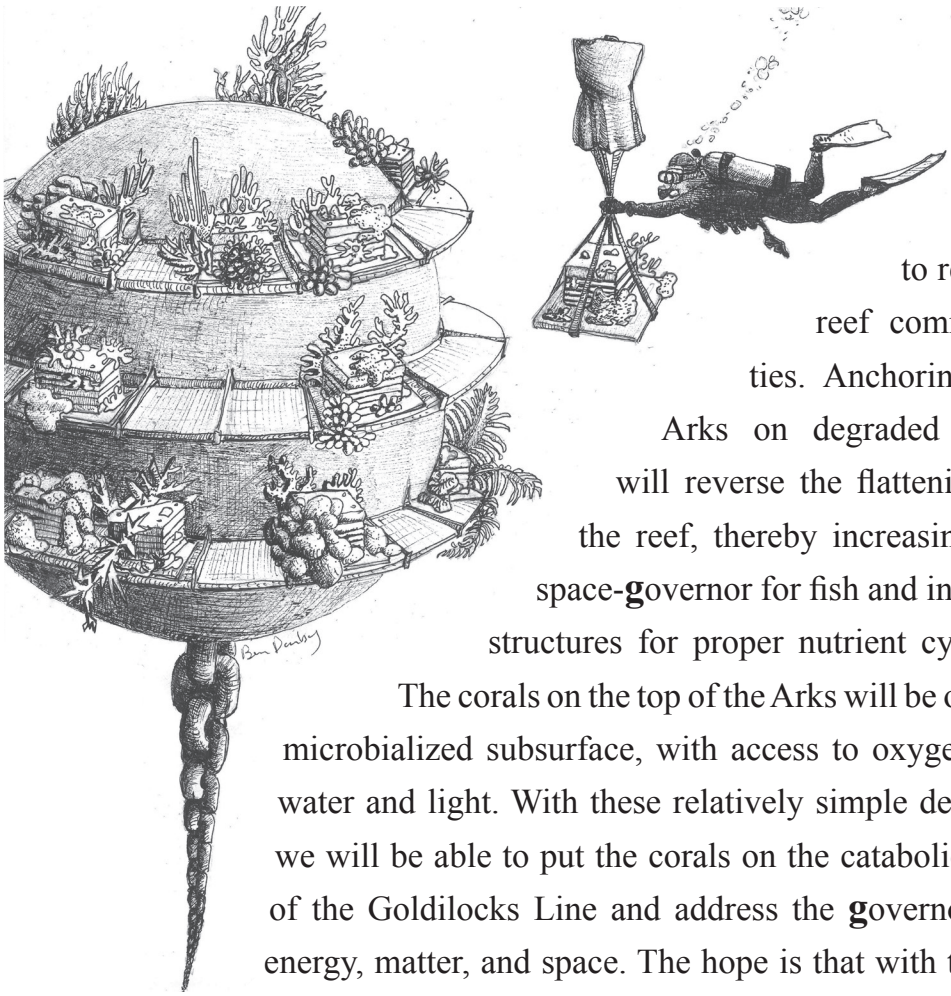


7 Roa-Ureta, Ruben H., Miguel N. Santos, and Francisco Leitão. "Modelling long-term fisheries data to resolve the attraction versus production dilemma of artificial reefs." Ecological Modelling 407 (2019): 108727.

rise of seaweeds shifts coral reefs from the catabolic side of the Goldilocks Line to the anabolic side (primarily through the loss of oxygen through bubbling). As the degraded coral reefs shift further into the anabolic regime, **p**redation on microbes by protists and phage necessitated the rise of lysogens and their associated virulence factors. This causes more coral diseases and the DDAM positive feedback loop. While complicated, these dynamics are reasonably well understood. What we do not understand is how to reverse this process. It is not as simple as removing the stressor to restore an ecosystem. Instead the ecosystems must move through a series of successional states to return to a climax community. Since there is very little knowledge about how to do that in the coral reef environment, building a reef on the Arks offers unique opportunities to document and understand P.H.A.G.E.S. for restoration.[8]

As floating zoos, Coral Reef Arks will be reservoirs of the flora and

8 Current coral reef restoration efforts are mostly focused on the corals themselves. Typically, coral fragments are raised in aquariums or on midwater platforms and then transplanted to degraded reefs. Essentially all of the fragments die within a couple of years because of microbialization and warm water events.



fauna to restore reef communities. Anchoring the Arks on degraded reefs will reverse the flattening of the reef, thereby increasing the space-**g**overnor for fish and internal structures for proper nutrient cycling. The corals on the top of the Arks will be off the microbialized subsurface, with access to oxygenated water and light. With these relatively simple devices, we will be able to put the corals on the catabolic side of the Goldilocks Line and address the **g**overnors of energy, matter, and space. The hope is that with theses essential processes in place, the anchored Coral Reef Arks will give the corals a starting point to **e**xpand and restore the reef.

Restoring degraded reefs with anchored Coral Reef Arks. The internal spaces of the artificial reefs will create spaces for nutrient cycling and fish to hideouts. The corals will initially be off the benthos and able to get enough oxygen. These characters of the anchored Coral Reef Arks will address critical P.H.A.G.E.S. processes and the Goldilocks Line.

Finally, Coral Reef Arks will provide the means to expand coral reefs into new regions of the world's oceans. Rising and warming waters will open new areas for coral reefs. Under natural conditions, it might take hundreds or even hundreds-of-thousands of years for all of the millions of coral

reef species to find a path to these new spaces. Coral Reef Arks will be able to move all of the life forms in one event. These sorts of *assisted migration* schemes have lots of pluses and minuses and it is will be important to consider all P.H.A.G.E.S. as we embark on these new trajectories. Just like the reintroduction of wolves into Yellowstone, there will be unforeseen effects. However, with the rapidly accelerating pace of global climate change we are going to have to be more proactive.

P.H.A.G.E.S. and Global Climate Change

For at least 2.4 billion years, cyanobacteria and other photosynthesizers have been splitting water to release oxygen and putting high energy electrons around carbon dioxide to make sugar.[9] Decoupling between the gaseous oxygen and solid sugar has led to an oxygenated atmosphere almost devoid of carbon dioxide. This means that Earth's atmosphere has switched from the oxygen-poor side of the Goldilocks Line to the oxygen-rich side (~21% atmospheric oxygen at sea level). Not surprisingly, the rise of multicellular wholobionts was closely tied to the increase in atmospheric oxygen.

So, where did all the CO_2 locked up into sugar go? Much of it got buried through different versions of the *biological pump*.[10] Basically, this means that complex compounds made from the sugar accumulated in low-oxygen zones in the ocean and on land (e.g., oil, coal, peat, permafrost, soils and sediments). Over geological time scales, these organic carbon pools were buried and squished at high pressures and temperatures to

9 Kopp, Robert E., et al. "The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis." Proceedings of the National Academy of Sciences 102.32 (2005): 11131-11136. Cardona, Tanai, James W. Murray, and A. William Rutherford. "Origin and evolution of water oxidation before the last common ancestor of the cyanobacteria." Molecular Biology and Evolution 32.5 (2015): 1310-1328.

10 Chris Deutsch from the University of Washington is working on a feedback model of oxygen and particle size that helps explain why macrobes create oxygen conditions good for themselves. The general idea is that more oxygen means that animals can get bigger. In turn, this creates bigger detritus like feces and corpses, which are more resistant to microbial degradation. Over time a positive feedback is created, where more detritus is buried and more oxygen is leftover, thereby favoring bigger animals.

produce fossil fuels like oil and coal. And then humans figured out how to extract all of that stored energy by burning the fossil fuels.

The consequences of burning this really old sugar are pretty amazing. The most publicized is the global increase in temperature that is causing the sea levels to rise.[11] Probably more disturbing, however, is the decrease in oxygen by burning these old sugars. Every time we burn a gallon of gas, oxygen must be part of the mixture. Effectively, we are microbializing the planet by depleting atmospheric oxygen.[12]

11 Sea level will rise because of the melting ice sheets and thermal expansion of the ocean. This could be quite dramatic and flood vast areas of land.

12 Deoxygenation is no joke. It has led to major die offs of macrobes in the past and humans need to be careful to avoid a positive feedback that would turn the ocean into an anoxic soup. Dahl, Tais W., et al. "Atmosphere–ocean oxygen and productivity dynamics during early animal radiations." Proceedings of the National Academy of Sciences 116.39 (2019): 19352-19361.

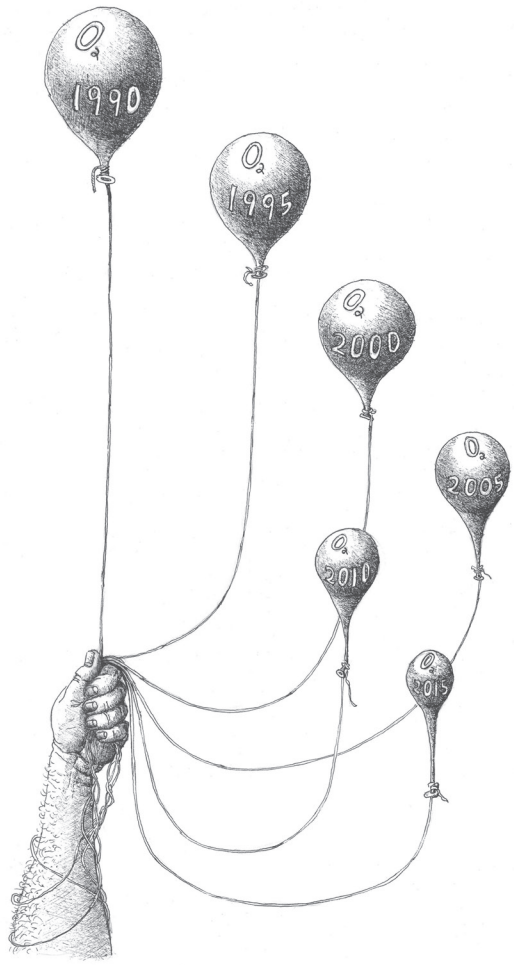


Figure 15.4. Change in oxygen at Scripps Pier. The famous graph of increasing atmospheric carbon dioxide is called the Keeling Curve after Charles David Keeling, a scientist at Scripps Institution of Oceanography (SIO). SIO scientists have also been measuring the decrease in oxygen. Roughly speaking, Earth's atmosphere is losing 19 O_2 molecules out of every million each year due to burning of fossil fuel (https://scrippso2.ucsd.edu/faq.html). This is shifting the planet towards the microbialized side of the Goldilocks Line.

The situation is reasonably dire. As with fishing on coral reefs, our direct and indirect activities are leading to global microbialization. There are many feedbacks, few of them good. The microbial food web speeds up with temperature, which releases more CO_2 thereby creating a positive

feedback loop of much suckiness for animals.[13] All the extra CO_2 lowers the pH, which allows microbes to eat even more of the stored organic carbon. Again, this is bad for animals because the viral and microbial activity depletes even more oxygen. Microbialization is so prevalent that oxygen minimum zones (OMZs), places where microbial activity had reduced oxygen to nearly zero, are becoming common features of the near-shore oceans and lakes. These changes are exacerbated by erosion, caused by all the melting ice, which transports more organic carbon into water bodies. This primes the marine microbial food web to release even more carbon dioxide. Processes analogous to those on degraded coral reefs are turning much of the world's near-shore environments into playgrounds for piggybacking viruses and pathogenic DIVAs. So, what can we do?

In 2050, humans will be directly releasing 50 Gigatonnes of CO_2 into the atmosphere via fossil fuel burning.[14] To alleviate the downside of this massive increase, we need to speed up the biological pump that sequesters CO_2 and reoxygenates our planet.[15] The biological pump works by: 1) photosynthesis producing oxygen and sugar, 2) the oxygen bubbling away and the sugar is transformed into more complex organic carbon structures like cellulose, and 3) the complex organic carbon is exported into areas where degradation is slow (e.g., cold, dark, low oxygen). The most obvi-

13 Azam, Farooq. "Microbial control of oceanic carbon flux: the plot thickens." Science 280.5364 (1998): 694-696. Thingstad, T. F., et al. "Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem." Nature 455.7211 (2008): 387-390. Wilkman, Eric, et al. "Temperature response of respiration across the heterogeneous landscape of the Alaskan Arctic tundra." Journal of Geophysical Research: Biogeosciences 123.7 (2018): 2287-2302.

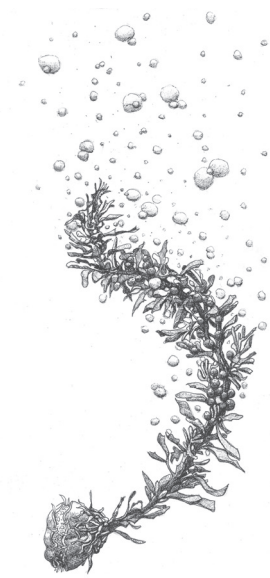
14 A Gigatonne or metric gigaton is equal to 1,000,000,000 (10^9) metric tons. A metric ton is 1,000 kilograms making a gigatonne equal to 1,000,000,000,000 kilograms (10^{12}) kg.

15 Speeding up the biological pump is one of the most common themes in geoengineering, basically engineering the planet. Humans are already geoengineering, just in an uncontrolled way. Our activities influence or even dominate every major biogeochemical cycle on the planet. The original attempt at speeding up the biological pump involved dumping iron into the iron-depleted regions of the ocean to speed up photosynthesis. This did not work because of processes that could have been predicted if the scientists had considered the process qualified in P.H.A.G.E.S.

ous place is the deep ocean. So, what we really need to do is get rid of that 50 Gigatonnes of CO_2 using something like a tree that floats in the ocean while it grows and then sinks into the dark, cold, suboxic waters of the deep ocean. Obviously, a floating tree that self-destructs has not evolved through **e**xpansion and **s**election. But could we engineer one??

Kelps are wonders of the natural world. Giant and Bull kelps creates near-shore forests along the west coasts of the Americas and east coasts of Asia and Australia. Kelps can also grow up to a meter per day. To do this, kelps incorporates sugars into complex organic carbons like alginate. A fully-grown Giant Kelp, *Macrocystis pyrifera*, can be 30 meters long and has incorporated 5 kg of carbon into its body. Kelps float via a bulb filled with gas called a pneumatocyst. A kelp is floating tree. How can we sink it?

Kelps hold themselves in place through *holdfasts*, tangles of kelp material that looks very much like a tree's root system. Holdfasts usually anchor on a rock, but we can convince kelp to hold onto almost anything by using a settlement cue. For the sake of argument, let's engineer the kelp to holdfast to a clam. Over time that clam will get larger and larger, until it weighs so much that it pulls the kelp down. Once this human created entity starts, the sinking will be really fast because the gas filled pneumatocysts will compress until they collapse.[16] This gives us our sinkers. For convenience, let's call these kelp-clam constructs Floaters-Coupled-to-Sinker, or FLoCS for short.



16 Another possibility would be to bioengineer a virus to blow open the kelp pneumonocytes.

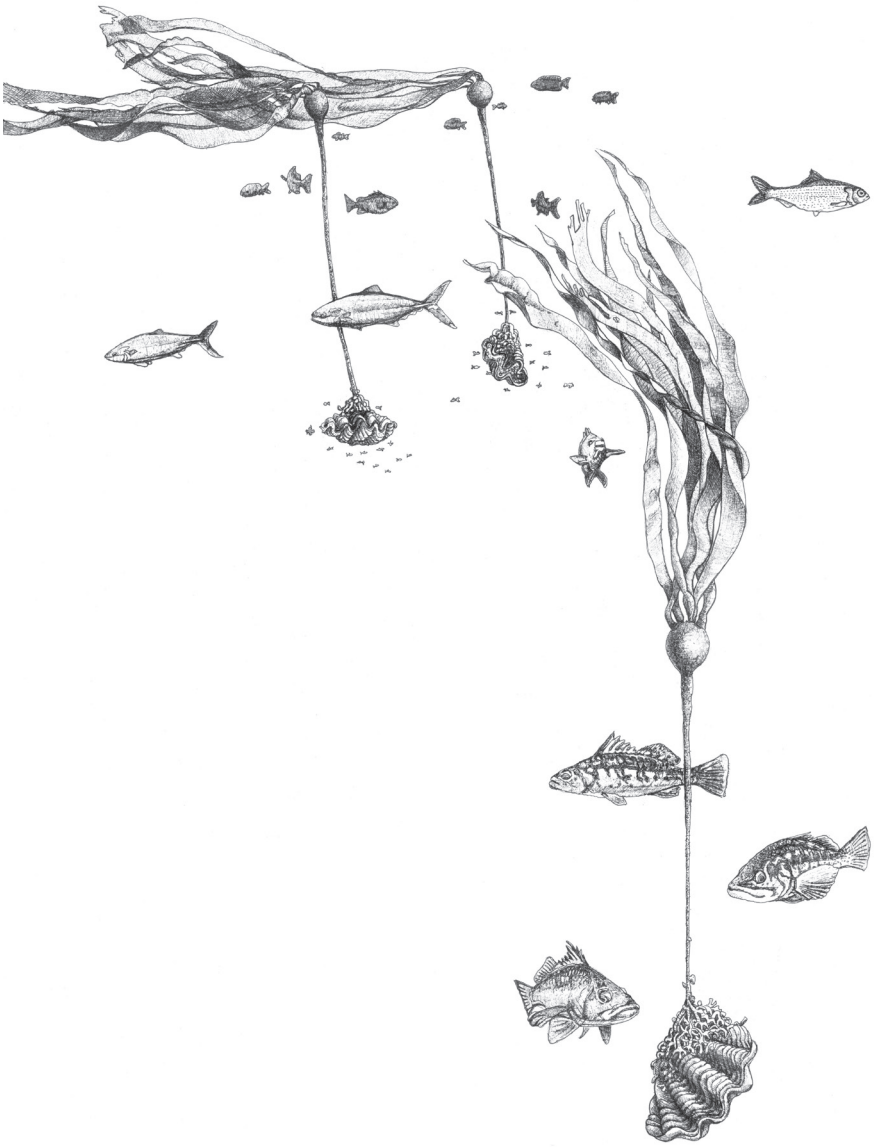


Figure 15.5. Floaters-Coupled-to-Sinkers (FLoCS) are one potential example of ecosystem engineering to lower atmospheric CO_2 and increase oxygen. While many people are hesitant to trying engineering the Earth, humanity will probably need to alleviate some of the changes already initiated by our uncontrolled ecosystem engineering (e.g., fossil fuel burning, nitrogen-loading, habitat destruction, chemical pollution, et cetera). Before we start large-scale ecosystem engineering, we need to consider all of P.H.A.G.E.S. to make sure we do not make the current situation worse.

To get rid of 50 Gigatonnes of CO_2 each year, we would need 10,000,000,000,000 FLoCS.[17] **E**xpansion works on our side in this case. Both kelp and clams are spawners that produce massive numbers of offspring. A single kelp can produce about 100,000 spores per minute and many clam-like organisms can produce over 1,000,000 larvae during a single spawning event. Getting the spores and larvae to stick together, the coupling, is also not too hard. In most cases, we know specific chemicals that recruit both the proposed floater and sinker. The couple could be as simple as a piece of paper with the chemical to recruit kelp spores on one side and another chemical to attack clam larvae on the other. This simplicity means that it is theoretically possible to make FLoCS in vast numbers.

A consideration of P.H.A.G.E.S. brings up a number of potential problems with the FLoCS proposal. The first challenge are the **g**overnors; where is the energy, matter, and space to grow 10,000,000,000,000 FLoCS? Energy is easy, it will be from the sun. Space to harvest that much energy is harder. It would be necessary to use vast swaths of the open ocean. Matter is also problematic, too many FLoCS in one region would seriously deplete nutrients and a fertilization scheme may be necessary. Other considerations include the grazers (i.e., the **p**redators of the kelp), which might eat the kelp before it can sink.

17 Each Bull Kelp dry weight is about 20 kg and about 25% carbon, therefore a mature bull kelp represents 5 kgs of carbon dioxide. To export 50 gigatonnes of carbon dioxide per year would require producing 1013 Bull Kelp FLoCS (total photosynthesis is about 70 gigatonnes per year).

d) About 10^5 container ships cross the major oceans per year; therefore, each container ship needs to deploy 10^8 FLoCS

e) Spawning bull kelp produces 105 spores per minute. Therefore, only need a bull kelp spawning for about 1,000 minutes (less than a day).

f) Mussels produce about 107 larva per individual. So, you need about 10 mussels per ship.

The Phuture

Futile cycles, lionfish fisheries, Coral Reef Arks, and FLoCS are all potential solutions to improve human, animal, and ecosystem health. None of them are perfect. This is why P.H.A.G.E.S. and the Goldilocks Line are so useful, they make you consider the many dimensions of any potential solution and help break simple cause-and-effect thinking.

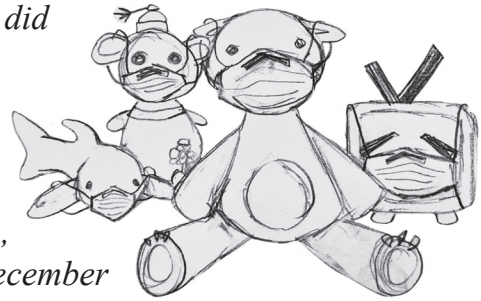
Viromics

"Are we really going to meet to study the virome in disease and health in the middle of a pandemic? This could be a Darwin award:)"

"Ah, finally an award we can win." Mya emailed back.

The first meeting solely dedicated to the virome was organized by Mya Brietbart, Rick Bushman, and David Wang in Lake Tahoe. Mya and I had worked together for almost 20 years and we published the first shotgun sequencing study of a natural viral community in 2002. This approach eventually became known as viromics. Since it is possible to separate the viruses from all the cells through a combination of filtration and centrifugations steps, shotgun sequencing of just the viral community became known as viromics. Hence, the title of the meeting The Global Virome in Health and Disease.[1]

Just before the viromics meeting, my daughter asked me if she should be worried about the newly emerging CoVID-19. I told her that it was a dangerous disease, but it was so uncommon in the USA that there wasn't much of a chance of contacting it. No reason to be especially worried. I did know that the SARS-CoV-2 was starting to spread at a worrisome rate and that we were headed for quarantine. A visiting scientist in my lab, the virologist Dr. Lili Han, had returned to China in early-December 2019; just in time to be put into quarantine. I had regularly got updates from her as China raced to contain the virus.



In mid-February, Lili wrote, "I hope everything is all right!

Now we should stay and work at home all day because of the cunning virus, and don't know when it's over!" She was at home with her super-charming, ultra-energetic daughter Belle doing lots of baking. Belle sent a photo of all her stuffed animals with KN95 masks.

I wrote back, "It couldn't be fun to be stuck at home for long periods of rest. It seems like the quarantine is working in that the number of new cases is steady and/or declining. Be safe, tell Belle "hi" from me."

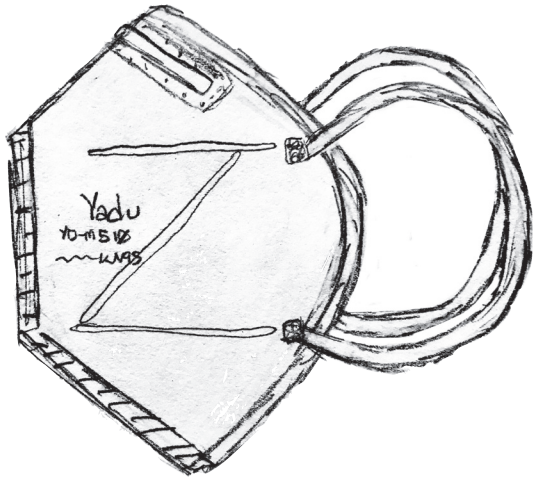
Up to this point, it really looked like SARS2 was going to be a lot like SARS1. Social distancing, limiting travel from outbreak zones, localized quarantining, and testing would be enough to keep the virus in check. At the Viromics meeting it became clear that this wasn't the case. Eddy Holmes, a virologist that investigates spillover viruses, presented his data suggesting a recombination event between two coronaviruses from different host species (bats and pangolins). Other speakers presented data on the spread of the virus, particularly worrying was the ability of SARS2 to produce asymptomatic carriers. But it was at the bar later that night that I first learned how bad the USA's response was proceeding.

Over beers, it became apparent that the Center for Disease Control (CDC) was in disarray. The official testing was flawed. The scientists were pushing for respiratory control measures, but the higher ups were pushing for surface sanitization. Apparently, being higher in the bureaucracy meant the you didn't know that SARS stood for Severe Acute Respiratory Syndrome. There weren't enough masks for first responders, let alone the civilian population. The federal response was in complete disarray because of bureaucratic hurdles and very poor leadership. The discussion was so alarming that I didn't sleep that night. Instead I spent the early morning hours reading the literature coming out of China.

The next day, I sent a flurry of emails to order supplies and equipment to detect the virus. Much of my lab's work was put

on hold as we switched to CoVID-19 studies. I ordered the three drugs that had shown possible effectiveness in early Chinese and French studies.

And I called my daughter and wife early that morning, "We need to start being very careful. This is pandemic is going to be very bad."



Chapter 16.
CoVID-19 and P.H.A.G.E.S.

Epidemics and pandemics caused by viruses and microbes are one of history's great forces.[1] Even though these outbreaks occur frequently, society is mostly surprised when a new disease shows up. HIV, SARS, Ebola, and N1HI, and viruses have all caused significant economic and social disruption over the last 50 years. These spillover viruses are a much bigger threat than any hostile nation or terrorist group and should be our top security priority. To protect ourselves, we need to understand the enemy. So, let's try to better understand CoVID-19 using the Goldilocks Line and P.H.A.G.E.S. First, identify where the Goldilocks Line controls the system.

Where is the Goldilocks Line in CoVID-19? In CoVID-19 the Goldilocks Line is situated in the lungs, where the human and virus compete for oxygen. This struggle determines who lives and necessitates treatments like increased oxygen, ventilation, and perfusion.

P.H.A.G.E.S. Table for CoVID-19: The relationship between P.H.A.G.E.S. and CoVID-19 are based on what is known about the disease early in the pandemic. As more information accumulates, additional relationships will emerge and change this table.

Table 16.1 P.H.A.G.E.S. Table for CoVID-19.

P	P1: SARS-CoV-2 virus is eating humans. P2: Human immune response is fighting the SARS-CoV-2 virus.
H	H1: Billions of potential hosts H2: Comorbidities H3: Origin of SARS-CoV-2 H4: Prior exposure to other coronaviruses
A	A1: Anti-SARS-CoV-2 vaccines would significantly change human wholobiont-virus interactions.
G	G1: SARS-CoV-2 exploits oxygen rich tissue (i.e., aerobic with lots of ATP).
E	E1: **E**xpansion of virus in one individual human. E2: **E**xpansion of virus in human population. E3: **E**xpansion introduces mutations/variations into the virus. E4: **E**xpansion of human immune cells to fight the virus. E5: Human response to limit **e**xpansion (masks, gloves/ handwashing, stay-at-home, social distancing)
S	S1: Strains of SARS-CoV-2 will be selected to be more easily aerosolized (e.g., dry cough, infect through masks and/or eyes). S2: SARS-CoV-2 develops longer lag times to keep carriers asymptomatic. S3: Specific immune responses to kill the SARS-CoV-2 virus will be selected (e.g., antibodies). S4: SARS-CoV-2 will evolve to better evade immune system. S5: SARS-CoV-2 strains will be selected to resist anti-viral drugs and vaccines. S6: Strains of SARS-CoV-2 will be selected to last longer in environmental reservoirs. S7: Strains will be selected to avoid screening by human detection systems (e.g., RT-qPCR and antibody kits). S8: Some strains of SARS-CoV-2 will be selected to be more resistant to disinfectants S9: SARS-CoV-2 will move to novel, non-human reservoirs.

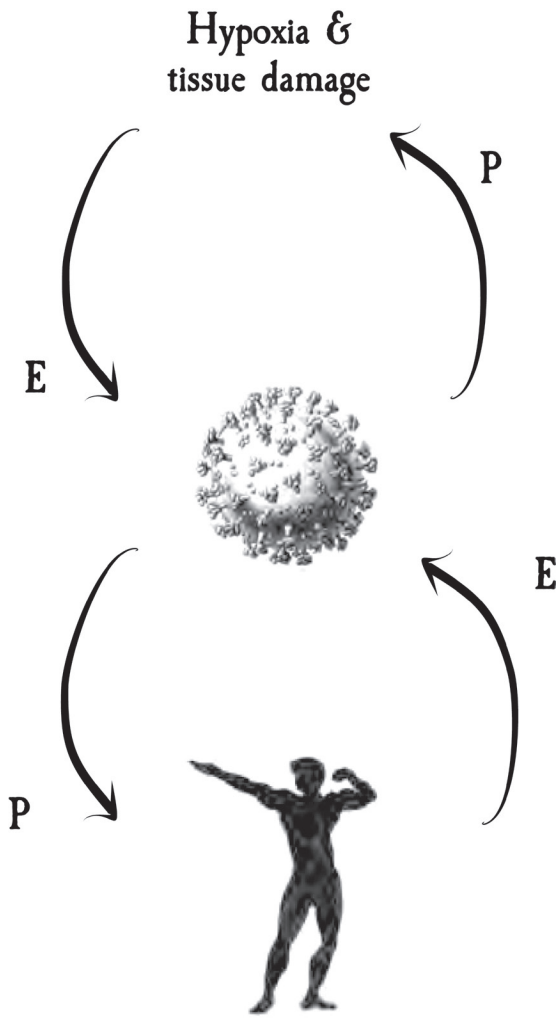


Figure 16.1. P.H.A.G.E.S and CoVID-19. CoVID-19 is currently predating on human tissue. This exponential **e**xpansion of the SARS-CoV-2 virus within the human host increases the total number of viruses and kills off human tissue. If this predation is strong enough, the human losses enough lung tissue and moves to the wrong side of the Goldilocks Line. The increasing pool of SARS-CoV-2 virions enter the environment where they can infect new human hosts and produce more virions.

This simple figure of P.H.A.G.E.S. captures CoVID-19 and the massive exponential **e**xpansion of the virus. E1 is the **e**xpansion of the virus in the individual human host. Each cell that is taken over and lysed by

the virus will produce 1,000s of new viruses. These new viruses will then infect more cells in the person's lungs. This leads to literally millions of viruses attacking the person's lung tissue. All of this damage causes the person to cough and shed viruses into the air, thereby infecting new people.

Within a single patient, this massive SARS-CoV-2 exponential **e**xpansion rapidly pushes up against the Goldilocks Line. All of the tissue damage destroys the lungs, which fill with fluid and limit oxygen diffusion into the blood. Instead of the aerobic-ATP rich metabolisms, the virus must now deal with suboxic/anaerobic conditions (G1). This could be viewed as a form of niche construction, because it causes the human to cough and start spreading the virus to other patients. We would predict that some of the SARS-CoV-2 viruses enter a temperate-like state at this point, which might explain some of the anomalies with the testing (i.e., positive patients become negative and then positive again). Since this is a recent spillover virus, it is also possible that it is strictly virulent and must hop to new oxygenic tissue. In either case, the virulent lifecycle and tissue damage lead to the patient suffocating, producing more viruses, and coughing to spread the virus.

Since humans must always remain on the oxygen-rich side of the Goldilocks Line, doctors treating CoVID-19 patients use enriched oxygen treatments and ventilators to keep the patients alive. Of course, this also feeds oxygen to the SARS-CoV-2 viruses. In practical terms, this becomes a race against **e**xpansion of the virus versus **e**xpansion of the human's immune cells. At least tens of millions of people's immune cells lost this race and they suffocated from CoVID-19.

The P.H.A.G.E.S. and CoVID-19 figure offers some insights into the control and treatment options for dealing with the disease. First, if the SARS-CoV-2 virus needs oxygen-rich environments, then it might be better to treat patients by perfusing their blood with oxygen rather than trying to force oxygen into the lungs via ventilators. Long-term perfusion of large numbers of patients is not currently possible, but this approach could be

used in the future for other respiratory spillovers. More research is needed on how the SARS-CoV-2 virus deals with hypoxic conditions.

The power of exponential **e**xpansion was a shock to many people as CoVID-19 spread through the USA and world. In early 2020, slowing down the **e**xpansion was the only real weapon to combat the disease. Social distancing, quarantining, and masks all slow down the transmission amongst the human population. The most successful strategies used these methods coupled with detection strategies to increase their effectiveness in specific locales. As with any exponential **e**xpansion, stopping the replicator in the early part of the curve is best, and wide-spread detection really helps. The lack of masks and wide-spread detection of SARS-CoV-2 is one of the main reasons that the USA did so poorly stopping CoVID-19.

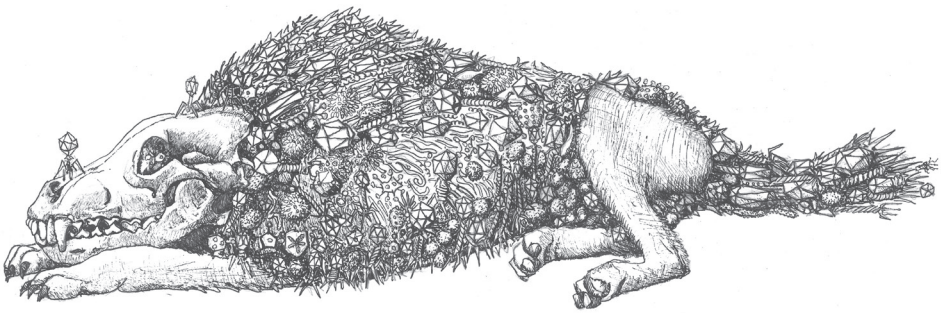
The P.H.A.G.E.S. Future for CoVID-19

In addition to creating a whole bunch of viruses and sick people, exponential **e**xpansion is also the fodder for **s**election. As of March 2021, millions of people (10^6) infected with SARS-CoV-2 and each one of those harbors at least 100,000,000 (10^8) viruses. This means that there are at least 100,000,000,000,000 (10^{14}) SARS-CoV-2 viruses on the planet. These large numbers mean that point mutations (i.e., single base substitution) at every site in the SARS-CoV-2 genome is already here for **s**election. So, what would we expect to happen?

To stop transmission, humans have applied strong selection pressures on SARS-CoV-2. To survive, virus strains that last longer in environmental reservoirs and/or are more easily aerosolized might be selected. Since early detection reduces the spread of the virus, some strains will be selected to avoid screening. To counter this possibility, the SARS-CoV-2 detection protocols use multiple sites, instead of one. The focus on sterilizing surfaces means that some strains of SARS-CoV-2 will be selected to be more resistance to disinfectants if this is an important transmission route. Still

other strains will develop longer lag times to keep carriers asymptomatic to facilitate the spread and some strains will move to non-human reservoirs. There are already numerous reports about SARS-CoV-2 in cats, including a tiger. And, depressingly, other SARS-CoV-2 strains will be selected to resist any antiviral drugs or vaccines that are developed to keep the virus in check. Humans and SARS-CoV-2 are now in a Red Queen Race. While all of this sounds bad, these evolutionary dynamics will almost assuredly attenuate the SARS-CoV-2 virus and it will become less virulent over time. The most likely scenario is that local outbreaks will continue, but the severity will decrease.

Conclusions
The Predator Wars

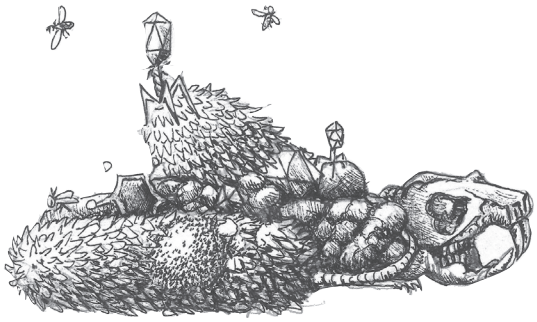


The greatest **p**redators on the Earth are viruses and humans. Both groups have developed ways to transmit pure information and dramatically redirect ecosystems. Currently, human activities ranging from overfishing to overnutrition are facilitating spillover viruses that are inherently pro-virus and anti-human. Underlying the struggle of these two great **p**redator groups are complex feedback processes captured in P.H.A.G.E.S.. This great biological war, delimited by the Goldilocks Line, is being won by the piggybacking viruses and DIVAs at the expense of humans and other animals. Humans do not need to lose this war. Unlike our ancestors, we now know how dangerous these viruses can be. And, unlike the viruses, we can evolve via intention.

Mike Furlan's death from a provirus-encoded virulence factor and Tom Patterson's survival because of bacteriophage therapy points to a future where near, real-time -omics methods, like those used in the Cystic Fibrosis Rapid Response (CFRR), will be combined with treatments like bacteriophage therapy to treat acute diseases.

Stopping the immediately dangerous pathogen will not cure CF and other chronic disease. In fact, this will open a niche for other bacteria to enter. The only way to stop this endless cycle is to fix the underlying causes. Viruses may be a solution to this problem as well. Viruses that infect eukaryotic cells are weapons that humans need to develop to their full potential. Tropism means that these viruses seek out specific cell types in the body. This specificity can be used to target cells that need to die, like those in a tumor. Tropism can also be used to deliver DNA into a specific cell type. This is the hope of gene therapy. When this technology is fully developed, it will be possible to exterminate many genetic diseases.

With some hard work, it is possible to turn viruses into allies. Bacteriophage can cure patients like Tom Patterson and others can kill our cancers. We can utilize horizontal gene transfer by viruses to edit the genetic code. And by watching the viruses through the lens of metagenomics, the Goldilocks Line, and P.H.A.G.E.S., we can start to rationally manipulate ecosystems ranging from wholobionts to Earth itself.



Appendices

Appendix I
Maths and Stats

Most scientists and philosophers agree that the behavior of natural systems should be explainable using mathematics. The practice of building models of complex, biological phenomena is incredibly useful. Models help our brains think past our traditional 3D world; humans are bad at envisioning many moving dimensions. Models help us understand multitudes of moving pieces and even predict what is going to happen in the future.

Roughly speaking, modeling can be divided into statistics (stats) and mathematics (maths). These disciplines have many sub-disciplines that overlap with each other. Here are some of the more important mathy things to consider:

1) *Statistical Modeling:* These models and analysis tools incorporate probability. That is, how often would something happen that would be expected to occur by chance versus what was observed. There has been a revolution in these methods and conventional dice rolling (i.e., frequentist) and Bayesian biased statistics are being replaced by Machine Learning techniques like Random Forests and Artificial Neural Networks (ANNs). These newer statistical tools are well-suited for picking apart the massive datasets being generated by contemporary biologists. Computers often identify patterns not apparent to the human brain. Statistical models often have strong predictive power, even when the ultimate cause is not known. Much of what is presented in this book is based on these approaches.

2) *Analytical Math Models:* These are the more traditional ways of

mathematical modeling; think algebra, calculus, and Newton's Laws. On the plus-side, these approaches are extremely good at reducing complicated interactions to simpler explanations. On the negative-side, these models are often sensitive to initial conditions (i.e., **h**istory) and oversimplification (i.e., the rest of P.H.A.G.E.S.).

3) *Stochastic Models:* Stochastic models combine the probabilistic nature of statistics with more conventional mathematical modeling approaches. These types of models are less susceptible to **h**istory and have been incredibly successful in physics, chemistry, economics, and some areas of biology. These models will be increasingly important in the future.

4) *New Math:* It is possible, and in fact likely, that the math necessary to model biological systems as described by the Goldilocks Line and P.H.A.G.E.S. simply does not exist. Life is incredibly complicated in comparison to other physical systems and our current tools are approximations. Rather than applying old methods to these systems, it may be time to develop approaches explicitly for biology. Remember that Newton had to invent calculus to revolutionize physics. He did not just apply pre-existing math tools. This leaves an open field for the next generation of mathy types.

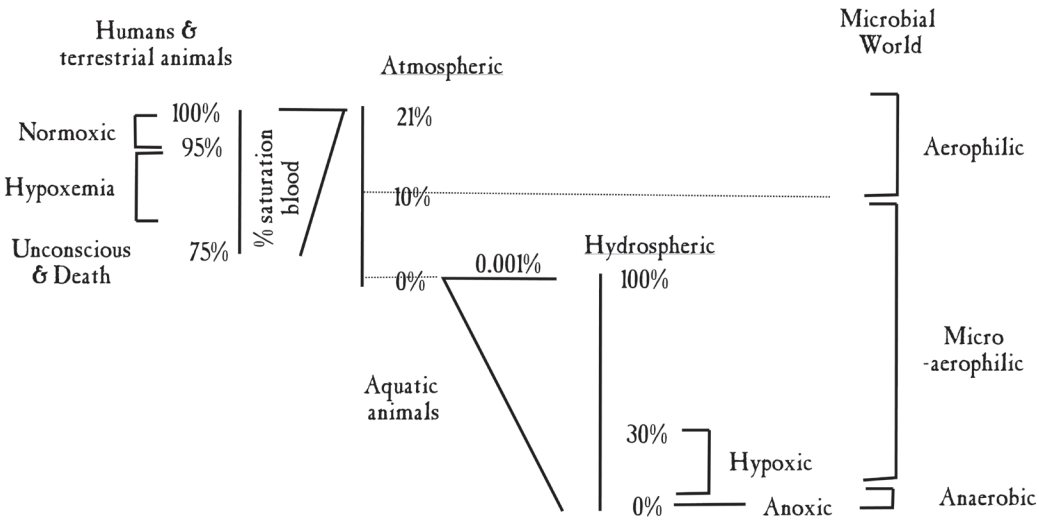
Appendix II.
Oxygen and the Goldilocks Line

Understanding how relative oxygen concentrations work is both important and a little annoying. Gaseous, atmospheric oxygen is ~21% in a volume to volume measurement (vol:vol). In other words, one liter of air at sea level contains 210 mls of molecular oxygen (O_2). When discussing gaseous oxygen this vol:vol percentage is traditionally used.

Oxygen is not exceptionally soluble in water, and well oxygenated water only contains about 0.001% oxygen (vol:vol). Since it is inconvenient to say or write out all those decimals, the strict vol:vol metric is not used when referring to oxygen dissolved into liquids. Instead, the convention is to use "percent saturation", which is the percentage of molecular oxygen in the liquid divided by the total amount of oxygen that would dissolve into the same liquid at equilibrium. This means that a well oxygenated stream water has 100% oxygen saturation, which is equivalent to 0.001% on the vol:vol gaseous scale. Reasonable? Kinda, but it gets much worse.

Oxygen saturation in a liquid is dependent on three main variables: salinity, temperature, and % oxygen in the air (vol:vol) that the liquid is in equilibrium with. This means that 100% oxygen saturation for seawater is different than 100% oxygen saturation for freshwater. And 100% oxygen saturation in cold seawater is different than 100% oxygen saturation in warm seawater. For some place like Yellowstone, the effective concentration of the gaseous oxygen changes because of the altitude (about 15% of

sea level) and in the hospital patients will be given 100% molecular oxygen (vol:vol). Urrghh![1]



Relative Oxygen concentrations for biology. Current atmospheric oxygen concentrations are ~21%. Diffusion and mixing of oxygen from the atmosphere with the hydrosphere, yields an equilibrium of about 0.001% oxygen. For convenience, this 0.001% oxygen is equivalent to 100% saturation. On this scale, 1-30% of oxygen saturation is called hypoxic. And unmeasurable oxygen is called anoxic (~0%). Blood oxygen saturation is really a measure of the amount of oxygen that is being carried by hemoglobulin. Microbes mostly are characterized by the oxygen concentrations in their surrounding atmosphere, which make it even more confusing because they are primarily grown in liquids.

Life is adapted to the whole spectrum of oxygen concentrations, which means that conditions that suffocated one organism are completely normal for another. Fish, for example, are quite happy in environments with oxygen concentrations that rapidly kill humans. For biology, the actual amount of oxygen is much less important than the relative concentrations. This means that the hypothetical Goldilocks Line is also relative.

Life tries very hard to control metabolisms around the Goldilocks Line. Enzymes and subcellular structures like the mitochondria tightly control the flow of electrons from sugar intermediates to oxygen.

In regard to the Goldilocks Line and metabolism *Nicotinamide Ade-*

nine Dinucleotide Phosphate, or *NADP*$^+$ for short, is even more important than ATP. NADP$^+$ shuttles electrons from one energy pool to another. NADP$^+$ has a carbon ring structure where high energy electrons can race around. This ring will attract a high-energy electron, which converts NADP$^+$ to NADP. Another high energy electron can be added to this NADP, creating NADP$^-$. In turn this attracts any free H+ in the area to make NADPH. The NADPH is now a battery carrying two high energy electrons. These high-energy electrons are used in most of the central building/anabolic metabolisms. Importantly, NADPH is made by several metabolic pathways that do not require oxygen (e.g., Pentose Phosphate Pathway, Entner–Doudoroff pathway).

The Goldilocks Line is hypothetical because no natural system will ever have exactly the correct amount of sugar and oxygen to balance on this line. However, it is important to point out that cells need some building and some ATP generation at all times. That means that these pathways run concurrently, and the Goldilocks Line represents an abstraction where building/anabolic metabolisms predominate over ATP-generating/catabolic metabolisms.

Appendix III
Assembly, Succession, and DIVAs

Assemblies of wholobionts and other ecosystems develop through *succession*. The easiest way to think of succession is the reasonably predictable arrival of *pioneers* that are replaced by later arrivals. The early pioneers create conditions that facilitate establishment of the succeeding community. For example, a forest fire in Yellowstone will create open ground that is colonized by fast-growing herbaceous plant species often called "weeds".[2] These pioneers help stabilize the soil. Over time, the pioneers will be replaced by grasses, bushes, and seedling trees. These intermediate ecological communities are called *sere* (the adjective is seral). Seral communities do things like aerate the soil, fix nitrogen, and release phosphorous from rocks. Eventually full-sized trees will gain back the ground and shade out the earlier pioneer and seral communities. This last stage is called a *climax* community.[3]

Microbial succession is also reasonably predictable. The equivalent of a forest fire in the microbial world would be elk remains from a wolf kill. The fast-growing, pioneer microbes are often facultative anaerobes. These microbes are speedy, but very messy, eaters and include some of the most prevalent pathogens like *Escherichia coli*, *Streptococcus* spp., and *Staphylococcus* spp. The yeast in beer and wine fermentations, *Saccharomyces cerevisiae*, is also a pioneer species.

With so much energy, matter, and space open in the elk corpse, the pioneering microbes use the fastest metabolic pathways possible. When

microbes do this really rapid eating, central metabolism backs up and produces fermentation products like short-chain fatty acids. The physiological response is called the Crabtree-Warburg effect.[4] This metabolism is functionally the same as anabolic metabolisms, which you will hopefully remember means a lot of primary metabolites for building biomass. Since the facultative anaerobic microbes can also use oxygen to make ATP through catabolic metabolisms, these early pioneers **e**xpand extremely rapidly.

The pioneer species, and their corpses from viral **p**redation, produce a whole bunch of waste products that feed the seral communities. These seral microbes eat leftover food, waste products of the pioneering community, and usually use up the last of any oxygen. Many of these seral microbes actively construct barriers to oxygen diffusion like alginate (e.g., cystic fibrosis) and bacterial cellulose (e.g., Kombucha) to create and stabilize anaerobic conditions. In some cases, the oxygen diffusion barrier is set up by physical factors (e.g., wet soil, sediments, and the thermo/chemocline in the ocean). As the last of the oxygen disappears, microbes that can use alternative electron acceptors take over and setup Winogradsky communities. Finally, as the last of any inorganic electron acceptors are used up, an obligate anaerobic community is established.

Classically, successional processes are envisioned as taking place over time and most of the literature deals with plant communities. Microbial succession is a little different because the pioneer, seral, and climax communities usually co-occur in time and are instead spatially **a**ssembled. In our colon, the pioneer species like *E. coli* colonized the small zone where food and oxygen enter from the small intestine. Seral communities of Winogradsky microbes are established and the majority of the gut community is the obligate anaerobes. These spatially **a**ssembled communities are long-lived and more like the climax communities of plant communities.

4 Crabtree and Warburg studied the same physiological phenomena in yeast and cancer cells, respectively.

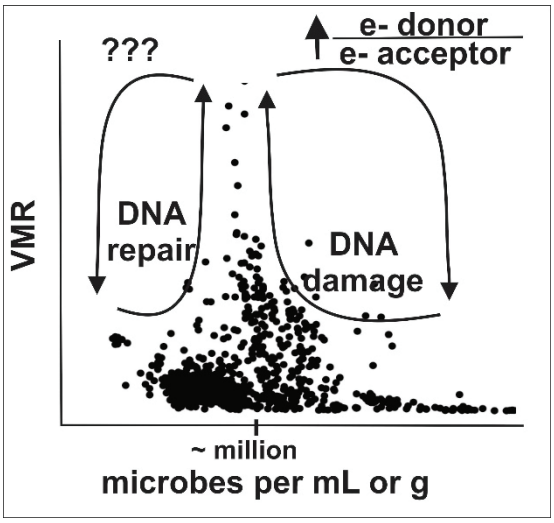
This makes the **a**ssembly of P.H.A.G.E.S. subtly different than traditional succession.

Once spatially assembled microbial communities are **a**ssembled, they can remain in place for very long times. As an illustration, let's consider the open ocean. Unlike most communities we've talked about in this book, the upper part of the open ocean has plenty of oxygen and other electron acceptors; the oxygen-rich side of the Goldilocks Line. There is plenty of energy in the form of sunlight, but the matter-**g**overnor is restrictive. In particular, iron, phosphate, and organic nitrogen can be limiting. Under these conditions, microbial autotrophs, instead of large plants and marcroalgae, are the primary producers making the sugar. In turn, this sugar supports a community of heterotrophic microbes. In the oxygen-rich, matter-**g**overned upper ocean, the rest of P.H.A.G.E.S. have been playing out over eons. And some of the detritus from this system rains down to the deeper, non-lit ocean. Below about 1000 meters there is a thermal-chemocline, where oxygen becomes more limiting. While not completely anaerobic, there is competition for electron acceptors and the deep ocean functions much like a very long Winogradsky column. This oceanic **a**ssembly has been reasonably stable for billions of years.

In some parts of the near-shore ocean, the matter-**g**overnors are not limiting. These regions can be naturally occurring, where deep, nutrient water upwells to the surface because of geology. These regions can also be created by humans dumping lots of nutrients into the near-shore environment in the form of run-off from agriculture lands or our sewage. When the autotrophs are released from the matter-**g**overnor, they produce a lot of sugar, which becomes biomass. As this increased biomass rains into the deep ocean, the oxygen below the thermal-chemocline is used up. These are Oxygen Minimum Zones (OMZs), where Winogradsky metabolisms and obligate anaerobes thrive, but most animals die. This is why we have the great dead zone extending from the mouth of the Mississippi River.

The OMZs are important because they are much like our colon and colonized by Dinner Is Very Available, or DIVA, microbes. The DIVA communities are nearly continuously fed high-energy, high-matter containing food and are electron acceptor limited. These DIVA communities are **a**ssembled from facultative anaerobes to seral Winogradsky communities to obligate autotrophs, and they can be very long lived. Human activity is increasing the prevalence of DIVA communities, often to the detriment of ourselves and other animals.

Appendix IV
Piggyback-the-Winner



Piggyback-the-Winner (PtW) was published in 2016 to help explain the decrease in virus-to microbe- ratios (VMR) with increasing microbial abundances observed on coral reefs and in several other environments.[5,6] When the data from every available study that counted both viruses and microbes were plotted as shown in the figure below, it became clear that at higher abundances the number of viruses per microbial cell decreased from over 10 viruses per 1 cell (>10:1) to one virus per cell (1:1). For fun, we called this the narwhal curve because the peak in the middle kind of looks like the narwhal tusk and somehow that nomenclature survived the peer-review process.

To generate this figure, viruses and microbes were counted under

the microscope by staining their DNA with a fluorescent dye. In general, higher microbial abundances (right side of the curve) occur in environments with lots of organic carbon and little oxygen like feces, sediments, and wet soils. This is the oxygen-poor side of the Goldilocks Line. The narwhal's tusk in the middle of the plot suggests that this region is where interesting dynamics are taking place. Initially, we thought this was the Kill-the-Winner (KtW) region. However, we now think that the much higher VMR numbers are caused by provirus induction.[7]

The first mathematical models of bacteria-virus interactions were adaptions of a classical ecological model called Lotka-Volterra. Basically, **p**redator-prey cycling is based on density. So, the winning bacteria that reaches the highest concentration is the most likely to die, hence KtW. The nice thing about models is testing their predictions against observations. In this case, we found that most variations of KtW models did not reproduce the narwhal curve, suggesting that some other dynamic was occurring.[8]

Beyond the narwhal curve, more temperate viruses were observed on coral reef systems with higher microbial numbers and the lower VMRs. While there are real bioinformatic challenges to identifying temperate viral DNA texts in metagenomes, this is still the best approach available for identifying temperate behavior. Based on the narwhal curve and increased number of temperate viruses, we proposed PtW. In PtW, proviruses protect their host cells from viral lysis (as well as protist predation) and that is why

7 You might think that no-one would look at the narwhal curve and argue that there is nothing different about the tusk region versus the far right and left. And you would be wrong. By framing the statistical test in certain ways, it is possible to argue that there are no differences in these regions. This is called "p-hacking" and "anti-p-hacking", where analyses are chosen based on the desired statistical outcome, rather than based on best fit to the data being analyzed. And this is why everyone hates stats:)

8 Another KtW model by Thingstad et al. (2014) predicted much of the observations. This model is very similar to PtW, but predicts the rise of immunity via mechanisms like restriction enzymes, CRISPRS, and other defense systems, rather than lysogeny. Thingstad, T. Frede, et al. "A theoretical analysis of how strain-specific viruses can control microbial species diversity." Proceedings of the National Academy of Sciences 111.21 (2014): 7813-7818.

fewer free virions are observed. At this point, it was important to test the PtW hypothesis with experiments.

Establishment of lysogeny by viruses is one of the oldest fields of study in molecular biology. Studies of the bacteriophage lambda switch were central to understanding the connections between gene regulation, RNA processing, protein functions, and phenotype. The lambda switch is also one of the most modeled systems in biology. Given this background, what do we expect in terms of KtW versus PtW? First, one of the common types of studies in virology is Multiplicity of Infection (MOI). Basically, different concentrations of viruses are added to a host to see the effects. In these types of experiments, adding more temperate bacteriophages leads to more lysogeny. At a MOI of 10 bacteriophage per cell, lysogeny are formed in 100% of the cases. So, increasing density will favor lysogeny, which is consistent with PtW. In contrast, KtW predicts that more hosts will be infected and killed. In the case of the lambda switch we even know the mechanism, that when more than one bacteriophage infects the host cell, more copies of the cI repressor are transcribed and favor lysogeny.

The lambda switch also predicts the physiological underpinning of PtW. As cells grow up to high densities, they use up both electron donors and acceptors. In a donor rich environment (i.e., oxygen-poor side of the Goldilocks Line) cells utilize metabolisms that generate less ATP and more building blocks. That is, anabolic metabolisms. And when ATP drops, lysogeny is favored. Again, this is consistent with PtW (high cell abundance equal high lysogeny) and not KtW (high cell abundances equal high lytic behavior).

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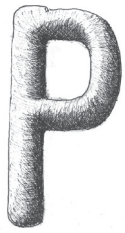
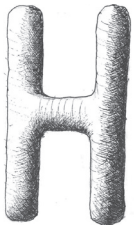
Organizing P.H.A.G.E.S.

Icons of things like viruses, cells, molecules, organisms, et cetera.

Arrows connect things and are labelled with the relevant P.H.A.G.E.S. processes

- use numbers to indicate process in supporting table (e.g., P1, P2, etc.)
- use capital letter subscripts for subdivisions of major processes

P.H.A.G.E.S. process	Variations of process & subscript
	V = Virulent virus C = Carnivory G = Grazing/herbivory P = Parasitic F = Failed predation event *In general, predation has a negative effect on the prey and positive effect on the **p**redator. Therefore, predation is labelled with a minus sign (-) when pointed towards a prey pool.*
	

A	L = Lysogeny w/ temperate virus I = Induction of provirus; re-entry into lytic cycle M = Mutualistic P = Parasitic
G	A = Anabolic **G**overnors mean that bigger molecules are built, and electrons are moved to higher energy states C = Catabolic **G**overnors mean that larger molecules are broken into smaller molecules and electrons are moved to lower energy states to generate ATP. *Note that Anabolic and Catabolic **G**overnors are tightly linked and the designation refers to the net effect of the phenomena of interest, which side of the Goldilocks Line.* E = energy M = matter S = space
E	#^# = base and exponent; in the simplest case of bacterial cells the base is 2 and the exponent is the number of generations; viruses & other organisms can have different bases - the exponent can be positive (+) or negative (-); toxic poisoning of protists by lysogens with provirus-encoded virulence factors
S	I = inheritable N = negative **s**election P = positive **s**election S = stabilizing **s**election