Simulating Behavioral Microcystin Impairment in Fish

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SIMULATING BEHAVIORAL MICROCYSTIN IMPAIRMENT IN FISH

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

by
Nicholas Richard Keeney
B.A., Boston University 2010
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ABSTRACT

Fish experiencing blooms of the cyanobacteria genera *Microcystis* and *Anabaena* acquire microcystin and saxitoxin through ingestion of contaminated food and absorption of dissolved toxin. Even low chronic doses induce sensory and motor impairment—the impact of which is unquantified in wild populations. Here, I introduce Lagrangian particle models for cyanobacteria and fish which test the hypotheses that impairment symptoms suppress movement and growth. This is implemented within the Finite-Volume Coastal Ocean Model (FVCOM). Cyanobacteria particles move vertically according to mixing and buoyancy (a function of cellular reservoirs). Fish navigate the horizontal domain, foraging in high growth areas, and fleeing when toxin increases. The framework is demonstrated here for the case of juvenile fish encountering *Microcystis aeruginosa* in an idealized Louisiana estuary. Self-shading reduces bloom growth, and causes algae to collect at the surface. Turbulent diffusivity is insufficient to break up this layer, so dissolved toxin becomes surface-intensified. Fish seek high growth areas in this environment, and dietary uptake increases. This triggers flight and swimming impairment. As cyanobacteria excrete microcystin, absorption forces fish to become intoxicated even in areas of lower toxic risk. Repeated flight means fish spend more time in suboptimal areas, with final growth reduced up to 6.6%. *In vivo*, this would be exacerbated by physiological stress and the metabolic cost of toxin removal. Collective movement (group diffusivity) is suppressed nearly 50% during wide-spread intoxication. Simulations show that within a certain parameter space, both movement and growth are suppressed relative to the control case as expected. However, additional experiments resulted in higher growth, indicating the methods are sensitive to model parameterization. Ultimately, these are sandbox cases, which will require carefully-designed lab and field experiments before predictive capability can be assumed.
CHAPTER 1. BACKGROUND

Harmful algal blooms (HABs) are an overgrowth of phytoplankton to the detriment of ecology, economy or public health. Severe blooms kill wildlife *en masse*, and can cost millions of dollars in lost fishery and aquaculture revenue (Landsberg, Dolah, and Doucette 2005; Samson, Shumway, and Weis 2008). Some fish mortality is attributable to anoxia following bacterial respiration of bloom biomass—exacerbated by mechanical irritation or damage to gills by algal spines (Landsberg, Dolah, and Doucette 2005; Granéli and Hansen 2006; Ibelings and Havens 2008). However, toxic phytoplankton are a greater threat. Their toxins have physiological functions—organelle precursors, nitrogen stores, allelopathic inhibitors (Turner and Tester 1997). When ingested or absorbed by fish, these poisonous metabolites decrease fitness through the alteration of behavior and physiology. The comparative severity of these two impairment pathways depends on timing and bloom toxicity. Behavior responds instantaneously to harmful conditions, while physiological adaptation requires longer. Ultimately, potency can be such that the consumption of as few as six contaminated prey constitutes a lethal dose (Samson, Shumway, and Weis 2008). Sublethal behavioral change manifests at less than five percent of that level (Kane, Salierno, and Brewer 2005), suggesting that the occasional ingestion of toxin-laden food can deliver an acute dose. Between feeding events, continuous absorption of dissolved toxins—offset by active physiological removal (depuration)—contributes to a time-varying chronic dose.

Toxic blooms occur worldwide, but are particularly devastating to estuaries, which act as a refuge for juveniles of commercially and ecologically important species. Fish in these systems navigate interconnected lakes and channels, alternately utilizing open water and inundated nursery habitat to forage and avoid predators. The young are especially susceptible to absorption of dissolved toxins, because of a fast metabolism, thin epithelium, and high surface-area-to-volume ratio (Lefebvre, Trainer, and Scholz 2004). Ontogeny of diet and habitat obfuscates toxin dynamics (Rose et al. 2003). Absorption takes back seat to ingestion, and total exposure depends on life history and the ability to avoid noxious algae by sense or chance (Dekshenieks et al. 2001; Kane, Salierno, and Brewer 2005). Specific behaviors—defined as reactions to biological, physical or chemical cues—control the delivery of toxin. Information obtained about the environment, paired with innate physiological awareness, forms an internal model of state and domain; and impairment in sensing, remembering, or reacting to these stimuli counteracts evolutionary optimization (i.e., decreases fitness) because there exists a fundamental difference between reality and the perception thereof. Scaling this type of individual impairment to the group level is a significant
challenge of marine toxicology (Murphy et al. 2008). The dearth of fine-scale data appropriate for parametrization of such a model is an obstacle. The effects of some algal toxins have been described for a few fish species in controlled laboratory cases. However, these have not been translated to the group scale, nor examined from a simplified theoretical point of view to date. I propose that during blooms, behavioral change (impairment) is ubiquitous, and has emergent consequences for the vitality and movement of fish. Specifically, I hypothesize that according to described phytotoxin symptoms,

Hypothesis 1—Sublethal behavioral impairment reduces growth

Hypothesis 2—Sublethal behavior impairment suppresses collective movement

To prove these, I describe the growth and movement of individuals exposed to phenomenologically distinct phases of a theoretical bloom, and summarize movement as a collective diffusion process (Flierl et al. 1999). Identifying and quantifying intoxicated movement in the field is no easy task (due to environmental noise), but will eventually be necessary to predict how the increasing frequency and severity of blooms worldwide may alter fish communities (Landsberg, Dolah, and Doucette 2005; Berger et al. 2008). The practical approach, at this stage of understanding, is to use a series of coupled models representing necessary components of the system: domain, toxin-producers, and fish.

There is a rich literature on marine pollutant modeling from which to draw inspiration, though many have limitations when applied to algal toxins. For example, Jaworska et al (1997) simulated polychlorinated biphenyl (PCB) effects on hatching and growth in juvenile largemouth bass. However, their approach did not include uptake or depuration—necessary for dynamics of more transient water soluble phytotoxins (Tester, Turner, and Shea 2000). Fecundity is the model's measure of success. However, reproduction can depend on years of survival in some species, during which fish may experience multiple blooms. My interest is in the physiological tax imposed on routine actions by periodic exposure. Virtual fish must be able to move and interact with both a dissolved toxin field and the toxic organisms of interest: introducing the need for a spatially explicit framework. In this case, I implement a Lagrangian particle method embedded in an unstructured-grid hydrodynamics model—the Finite-Volume Coastal Ocean Model (FVCOM). FVCOM is ideal for coastal regions with a complex estuarine morphology, and has been applied extensively to the Louisiana coast (Huang et al. 2011).

Louisiana estuaries experience blooms of the cyanobacteria genera *Anabaena, Microcystis, Cylindrospermopsis, Raphidiopsis, and Aphanizomenon* (Ren et al. 2009; Garcia et al. 2010; Das et al. 2012). These
cyanobacteria are found in fresh to brackish salinity regimes the world over (Redden and Rukminasari 2008). Blooms occur during calm periods of stratification, and may persist for months. Some fresh and oligohaline water bodies (e.g., Lac des Allemands) in the upper estuary are dominated year round by *Anabaena flos-aquae*, *Anabaena circinalis*, and *Microcystis aeruginosa* (Berger et al. 2008). All three species produce a shared suite of substances featuring microcystin (MC) and saxitoxin (STX). These are the toxins of interest in the following simulations, and are grouped under a single numerical proxy tracer. Light, temperature, and nutrients effect algal growth and toxicity (Wiedner et al. 2003; Tonk et al. 2009).

Temperate waters typically have a spring nitrogen crash favoring summer blooms of nitrogen-fixing *Anabaena* (Beversdorf, Miller, and McMahon 2013). However, some Louisiana lakes—such as those composing the upper Barataria estuary—have ample nutrients supplied by freshwater inputs (and urban runoff from the New Orleans metro area). The estuary was cut off from seasonal flooding by the channelization of the Mississippi River until the operation of the Davis Pond Diversion (DPD) in July 2002 (Ren et al. 2009). Periodic releases of river water into Lake Cataouatche are intended to combat salinity intrusion, alleviate flood risk, replenish sediment, and increase fisheries production (Das et al. 2012). This introduces nutrients, and pushes the salinity gradient down estuary: expanding the range of the pH- and salinity-tolerant cyanobacteria. Without other impediments to growth, temperature becomes limiting. Although both genera thrive around 20–30 ºC (Jones, Baltz, and Allen 2002; Garcia et al. 2010), a summer temperature increase accelerates *Microcystis aeruginosa* growth, while slowing *Anabaena* (Tonk et al. 2009). Dissolved toxin has multiple peaks, first March–May (following early *Anabaena* blooms) and again in July (only days after a bloom of *Microcystis aeruginosa*). Toxin concentrations ranged 0.17–1.42 mg/m^3^. It was found in catfish carcasses and blue crabs associated with the blooms (Garcia et al. 2010). High dissolved toxin is expected in shallow systems because cyanobacteria cells are prone to photolysis when exposed to high temperatures or depleted inorganic carbon (Ibelings and Havens 2008).

The focus of this thesis is fish movement, so I choose to model only the dynamics of *Microcystis aeruginosa* (which I reference further as simply *Microcystis*) in order to generate a time varying toxin environment for the virtual fish. However, there exist individual-based models for each of the genera suitable for implementation as Lagrangian particles (Rabouille, Thébault, and Salençon 2003; Hellweger et al. 2008). *Microcystis*—to its advantage—is frequently studied. Its growth rate increases monotonically with ambient light up to a saturation level (Wiedner et al. 2003). Additionally, temperature and orthophosphate abundance increase toxin production; while
temperature also increases excretion (which can at times exceed production). Simply put, a Louisiana summer encourages potent blooms, from which toxins leak quickly.

*Microcystis* is colonial, meaning that while errant cells do exist, most are found in spherical assemblages (Costas et al. 2008). Gas vesicles and an enveloping mucus layer give colonies nearly neutral buoyancy.

Carbohydrate storage during the fixation phase of photosynthesis increases cell density. Less dense proteinaceous cellular material is then synthesized from this ballast. Cell respiration and excretion result in dynamic buoyancy, which forces vertical movement (Rabouille, Salençon, and Thébault 2005). Colonies at the surface can form a persistent scum when the magnitude of their daily fluctuation is less than the difference in density between water and colony. Actually, thin layers of this type can form at any pycnocline or thermocline with a steep enough gradient (Dekshenieks et al. 2001). Depending on the strain, between 40% and 93% of the cells in a bloom are toxin producing (Sabart et al. 2010)—although, here I assume in the model that all toxin-producers, for simplicity. The threat of dietary toxins to fish is then a function of colony abundance, and the MC each contains. High abundance instigates grazer encounters, and of course increases total toxin excretion, facilitating ingestive and absorptive accumulation. This news is not dire to all species, whose relative ingestion varies by feeding style. For example: large colonies block filter feeding, and selective grazers can target non-toxic morsels or avoid blooms entirely (Visser et al. 2005). Further, the intracellular toxin of *Microcystis* colonies can be inversely related to bloom density, so that at very high abundance, ingested cells deliver a lower dose (Kardinaal and Visser 2005). When fish are present, but do not graze, colonies may react by accelerating toxin production (Jang et al. 2004). The dissolved phase then increases, and absorption becomes dominate, since there is no ingestion (Sabart et al. 2010; Rouco et al. 2011). The concentration of toxin-excreters near areas of low diffusivity, such as stratified lakes (Sweers 1970), results in depth-partitioning, so that fish utilizing distinct ranges of the water column will not have the same exposure.

Bioactive MC alters osmoregulation and serum chemistry (Malbrouck and Kestemont 2006). It also causes oxidative stress in the hippocampus, which impairs spatial memory (Li et al. 2011), and has similar neurotoxic symptoms to domoic acid (DA), which disorients mammals, birds, and fish (Lefebvre, Trainer, and Scholz 2004; Landsberg, Dolah, and Doucette 2005)—causing spinning and inability to coordinate social movement (Lefebvre, Dovel, and Silver 2001; Kane, Salierno, and Brewer 2005). The lethal injection dose in juvenile loach is a solution of about 600 mg/m³. This is not a natural method for delivery, but gives an idea of how much toxin a fish can
tolerate. Dissolved MC has low membrane crossing potential that limits uptake, but it nevertheless inhibits ATPase activity in gill ion pumps (Ibelings and Havens 2008). In other words—there is evidence that it hampers respiration and homeostasis, with effects potentially cascading to many physiological functions. Immersion in a solution of 5 mg/m³ triggers increased activity (interpreted as attempted flight, but limited by tank size) which is replaced with lethargy at 50 mg/m³. The timing of peak activity is species dependent. For example, freshwater belica shift to nocturnal activity, while zebrafish become more mobile in daylight. In both cases the total activity over the day decreased (Baganz, Staaks, and Steinberg 1998; Baganz et al. 2004). Metabolization takes place in the liver through conjugation with the antioxidant glutathione (GSH), catalyzed by glutathione S-transferase (GST). Increased GST levels following exposure of zebrafish to 1 mg/m³ show that some species have adaptive tolerance (Wiegand et al. 1999). However, depuration depletes GSH and can cause oxidative damage (Ibelings and Havens 2008). This slows development and alters metabolism: glycogen production during anaerobic activity stops, and protective proteins no longer shield against damaging temperatures (Moon, Walsh, and Mommsen 1985). In short, beyond the drunken swimming, there is a real physiological cost associated with the removal of bodily toxins (as opposed to the opportunity cost imposed by suboptimal behavioral responses).

Microcystis and Anabaena also produce saxitoxin (STX) and anatoxin-a (Oberemm et al. 1999). STX is commonly associated with dinoflagellates (Chen and Chou 2001; Samson, Shumway, and Weis 2008). It blocks sodium-ion channels—resulting in paralysis (Turner and Tester 1997)—and has hypoxia-like effects due to chemical damage of gill tissue (Chen and Chou 2001). It concentrates in shellfish, which are a vector for humans (Kwong et al. 2006) and benthic feeding fish. Dissolved STX is a copepod feeding deterrent (Kwong et al. 2006) and fish repellant (Samson, Shumway, and Weis 2008). A solution around 300 mg/m³ causes deformities of the eye and swim bladder in embryos, and irreversibly reduces tactile response in larvae after exposure for two days (Lefebvre, Trainer, and Scholz 2004). Sheepshead and mummichog feeding on contaminated copepods attempted to capture fewer prey at 1.0-2.0 μg g⁻¹ (Samson, Shumway, and Weis 2008). Lethal injected doses are 0.4 μg and 0.75 μg per 100 grams of tissue for anchovy and black sea bream (Kwong et al. 2006). This delivery method is suspect when applied to ingested or absorbed toxins, but the study did induce production of depuration enzymes before death, showing physiological adaptation to acute exposure.

Based on these laboratory experiments, I expect that intoxication with STX and MC have shared motor, sensory and metabolic symptoms. These need to be incorporated into future models of behavioral ecology, when the
species of interest may be exposed to regular toxic events. As far as parametric movement is concerned, the swimming speed decreases and directional randomness increases. *In silico* this translates to tortuosity and greater residence time. This will effect how much time an individual spends in profitable places (Barraquand and Benhamou 2008). In cases with point or probability-field representations of predators and food, changing these swimming parameters decreases encounter rates. For example, methyl-mercury intoxication suppresses speed, and should decrease captures by passive predators. But, these simulations also suggest that mortality can increase, because there are fewer successful escapes from fast swimming predators (Murphy et al. 2008). Although there is evidence of sight-based predators failing to notice slow fish (Samson, Shumway, and Weis 2008), generally, fish with delayed reaction or truncated sensing volumes—in addition to impaired movement—are more likely to approach predators, and will capture fewer prey. There is also the aforementioned cost of toxin removal by the liver and, potentially, diminished spatial understanding due to memory or cognition impairment (Li et al. 2011).

Anderson (2002) presented a probabilistic (event-based) scheme well suited to these modifications, which has recently been applied to comparative movement ecology (Watkins and Rose 2013). In it, fish forage and grow in length and mass, using directed random-walk movement rules, parametrically decomposed into speed and random angle. Individual behavior is determined at a fine temporal scale, based on sensing local conditions (event detection) and remembering the recently traversed conditions (memory). The original study was, at its core, an examination of the disruption of foraging bouts by predator encounters. I instead use movement rules that change with intoxication, as a flexible approximation of sensorimotor impairment. Virtual fish possess equations describing toxin dynamics, which introduce the concept of time-varying impairment—absent in many ecological toxin models. When fish reach a low toxin threshold, avoidance is triggered, following laboratory observations of agitation and increased activity at low does. When a second threshold is surpassed, swimming speed decreases and directional randomness increases. The impact of impairment on movement is discussed in terms of growth optimization (Watkins and Rose 2013), and group diffusivity (Flierl et al. 1999).

The architects of the toxic environment are *Microcystis* colony particles. Or super-individuals, in that they account for all colonies with common traits under the umbrella of a single tracked particle which may grow beyond the natural limit of a single colony (Scheffer et al. 1995). Cases with large physical domains and realistic forcing conditions are well suited for an Eulerian model, in which primary production and algal toxicity are evaluated as differential equations for stationary points on a simulation mesh. These exist. However, Visser (1997) and
subsequently Rabouille et al. (2001, 2005), modeled *Microcystis* in a fashion that can applied to either Eulerian or Lagrangian methods. When both fish and cyanobacteria are implemented as Lagrangian particles with shared generic procedures, computational methods for each biological model are similar, and easier to scale up later. For this reason, I adopt Lagrangian methods in this study. The introduction of extensible particle classes to the existing FVCOM code is one of the contributions of this research to the ecosystem modeling community.

In the following chapters, I describe the use of these methods to simulate a shallow domain reminiscent of a Louisiana oligohaline lake under summer temperature and light conditions. Application can be extended to any salinity or morphological regime, and I include suggestions for scaling and modifying the methods. These physical conditions are used as a jumping off point to test the cyanobacteria model presented by Rabouille et al. (2005) in a novel domain. This case is much shallower (5 m compared to 40 m), and offers a compromise between the 2 m depth of the upper Barataria Estuary, and the 10 m depth of Lake Pontchartrain. Forcing *Microcystis* growth with simplified temperature and light conditions gives rise to dense surface blooms, as is common in temperate lakes. The deeper European reservoirs for which the model was originally formulated are optically clear, which is not the case in turbid Louisiana estuaries, meaning that light levels at depth are over-estimated in these simulations. The toxicity of the algal biomass, and the concentration of dissolved toxin in the simulation domain, determine uptake by—and intoxication of—the virtual fish. The models are only partially-coupled, in that fish behavior depends on the state of cyanobacteria, but no feedback exists. The total toxin and carbon of the bloom is not reduced, so mass and stoichiometry are not conserved. Toxin dynamics of different fish species vary greatly. Rather than trying to predict the movement of a species, I emphasize critical points where the dominant toxin pathway changes for a non-specific theoretical fish. Results are therefore an example of what might occur in *some* fish, based on the general principles of the field as understood at this time. I discuss data needed to validate and refine the model, and how qualitative and quantitative aspects of the results can be summarized with collective movement metrics. I hope this methods paper will inform the practice of large-scale, process-based investigations of fish intoxication, and how sublethal impairment manifests at the group level.
CHAPTER 2. BEHAVIORAL IMPAIRMENT EXPERIMENTS

2.1 Introduction

Beginning with the first principles of ichthyotoxic behavioral impairment, I present a framework for translating the individual symptoms of microcystin intoxication in laboratory studies to an ecologically significant endpoint. The method is by no means predictive, but demonstrates the coupling of two existing models to produce phenomenologically relevant results. Computational modeling of biological and physical processes is an affair of constant refinement, and I hope these experiments are of use to the developing theory of the sublethal effects of harmful blooms on early-stage fish. Estimates of group effects are particularly valuable to sympatric feedback in the fields of ecological modeling, movement ecology, and animal behavior. At the highest level, it is nearly impossible to tease out the quantitative effects of a single variable. This is true in modeling and field observation. Across the spectrum, the controlled laboratory experiment—whose conditions, relative to nature, can be questionable—empirically judges the significance of those factors. Following the tradition of the thought experiment, I will frame a method (among many) that bridges the gap between these natural scales. In the previous chapter I hypothesized that,

Hypothesis 1—Sublethal behavioral impairment reduces growth

Hypothesis 2—Sublethal behavioral impairment suppresses collective movement

These introduce a number of requirements, which tinge the discussion and formulation of the models. The following rules and comments are based on existing research. Fish must sense and respond to the environment and their internal state. Toxicity and intoxication must be dynamic (governed by a system of differential equations). The fundamental nature of behavioral impairment is that during some periods an individual may think itself performing an optimized search, while in fact doing quite poorly. Therefore, toxin must trigger both intentional and forced responses. Movement parameters must change with intoxication such that paths become more random. And finally, growth must be explicit, and should probably be the measure of self worth. That is, an individual knows its physiological state, and takes action to improve growth until interrupted by higher value behavior (e.g. predator/toxin avoidance, reproduction)

These rules apply broadly to any pairing of fish and cyanobacteria. The vital rates for species of interest will need to be rigorously defined, but the processes effecting individuals should be similar. This is the outcome of evolutionary optimization, and is an assumption central to theoretical biological modeling (Sousa et al. 2010). The cyanobacteria genera most common in Louisiana estuaries are *Microcystis* and *Anabaena*. Their blooms overlap, but
the delay in peak abundance is enough to consider separate events. I only include *Microcystis*. Ideally, a simulation would include their competition, but the source of the toxin is, actually, irrelevant to the stated hypotheses. So, while the choice of *Microcystis* over *Anabaena* is arbitrary, it does pose some additional questions.

For instance, there is a temptation in biological-physical modeling to vertically integrate the domain, and resolve particle movement only in the horizontal to reduce computation. *Microcystis*, however, has dynamic buoyancy and will, in theory, grow at any depth so long as there is sufficient light. During simulations of migration in deep reservoirs, the particles accumulate carbohydrate near the surface as they fix dissolved inorganic carbon during photosynthesis (Rabouille, Thébault, and Salençon 2003). Like an over-weighted diver, they sink. *In situ* irradiance attenuates at depth, carbohydrate ballast is cast off in the conversion to cellular material, and buoyancy increases. A daily oscillation should result, but Louisiana lakes are about one twentieth the depth of the domain described by Rabouille et al., (2005). Cyanobacteria therefore cannot sink out of the euphotic zone (although, as previously mentioned, these methods under-estimate attenuation due to turbidity). Where will they accumulate, and under what conditions? How might trends in regional temperature change bloom development? Does a two-dimensional simulation domain cut it? Do field sampling methods possibly over- or underestimate abundance when there are only surface collections? If colonies are concentrated in a thin layer at some depth, does the local production of toxin outpace mixing by turbulent diffusion? Fish exposure depends on all these factors. If dissolved and particulate toxins are not uniform, how is impairment partitioned between fish utilizing different domains (benthos, neuston, et cetera)? Does said impairment lead to positive or negative feedback in toxin uptake?

To answer these questions, I implement two existing models (*Microcystis*, and a small generic fish) which I believe "got it right" as far as abstracting and emphasizing the processes involved. I expand on the deterministic cyanobacteria model (Rabouille, Salençon, and Thébault 2005) by adding toxin dynamics, and introduce turbulent diffusion of both dissolved toxin and algal particles. Growth and movement result in a spatially resolved toxin field, to which I subject small generic fish (Watkins and Rose 2013). A simplified schematic of the system is shown below in Figure 2.1, and the methods will be discussed at length in the following section. Results show strong surface intensification of the dissolved toxin profile over time, as cyanobacteria collect at the air-water interface. Fish exhibit dynamic intoxication, which drives afflicted individuals into low growth areas, where they end up spending most of the experiment. Intoxicated groups had lower behavioral diffusivity than the sober control group. The pattern of bloom toxicity and exposure dictates final fish growth reduction, and timing of minimum effective
diffusivity. The considerations and methods by which I reach those statement are described in the following. The models are numerically precise, but not necessarily very accurate. My intention has been to create a generalized framework for hypothesis testing, that can be applied to a variety of organism pairings. With time and continued development, I hope these methods will be validated and better constrained using field data and laboratory investigations.

2.2 Methods

2.2.1 Model Overview

I modify existing methods for individual-based Microcystis (Rabouille, Salençon, and Thébault 2005) and fish movement (Watkins and Rose 2013) by incorporating toxin dynamics, and immersing them in a shared—and idealized—environment modeled after the shallow oligohaline lakes of Barataria Estuary, Louisiana, USA. The

Figure 2.1 Model System—A simplified schematic of the model system. Circles are state variables for carbon and toxin. Italics indicate a process, some of which are sources (+) and sinks (-). Red text is environmental variables; red lines are linkages to processes; grey regions are biological sub-model scope.
continued testing and refining of these algorithms is one contribution of the work. These biological models are implemented within the FVCOM Lagrangian particle tracking framework. The existing Fortran 90 code base was modernized to Fortran 2003 standards as necessary. Coding the new (used) models for FVCOM, and updating the existing code base for heterogeneous particle swarms is another contribution. Lagrangian particles are point representations of individuals or groups, which move by active (biological) and passive (physical) processes. Passive transport, including advection and turbulent diffusion, counteract the behavioral optimization of foraging and migration—so these processes are suppressed to focus on biology. The variables and units used throughout are presented in Table 2.1.

Table 2.1 Key Variable Definitions and Units

<table>
<thead>
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<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>$A_{XY}$</td>
<td>Domain Area</td>
<td>m$^2$</td>
</tr>
<tr>
<td>$B_i$</td>
<td>Overlying Biomass</td>
<td>g/m$^2$</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Carbohydrate</td>
<td>g</td>
</tr>
<tr>
<td>$D$</td>
<td>Mean Squared Distance</td>
<td>m$^2$</td>
</tr>
<tr>
<td>$E_i$</td>
<td>Encounters</td>
<td>—</td>
</tr>
<tr>
<td>$I_0$, $I_i$</td>
<td>Irradiance (Surface, Depth)</td>
<td>W/m$^2$</td>
</tr>
<tr>
<td>$K$, $K'$, $K''$</td>
<td>Vertical Diffusivity and Derivatives</td>
<td>m$^2$/s, m/s, s$^{-1}$</td>
</tr>
<tr>
<td>$L_i$, $^eL_i$</td>
<td>Body Length, Effective Length</td>
<td>m</td>
</tr>
<tr>
<td>$M_i$</td>
<td>Cell Protein / Fish Mass</td>
<td>g</td>
</tr>
<tr>
<td>$P_{i,j,k}$</td>
<td>Event Probability</td>
<td>—</td>
</tr>
<tr>
<td>$R_i$</td>
<td>Particle Radius</td>
<td>m</td>
</tr>
<tr>
<td>$S_R$</td>
<td>Salinity</td>
<td>—</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
<td>hr</td>
</tr>
<tr>
<td>$U_{i,j,k}$</td>
<td>Behavior Utility</td>
<td>—</td>
</tr>
<tr>
<td>$V_i$, $V_{i,j,k}$</td>
<td>Velocity (Colony, Fish)</td>
<td>m/s</td>
</tr>
<tr>
<td>$X_i$, $Y_i$, $Z_i$</td>
<td>Position Component</td>
<td>m</td>
</tr>
<tr>
<td>$\gamma_i$, $\gamma_N$</td>
<td>Internal / Grid Toxin</td>
<td>g, g/m</td>
</tr>
<tr>
<td>$e_{i,j}$</td>
<td>Event Logical</td>
<td>—</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Cartesian Angle</td>
<td>rad</td>
</tr>
<tr>
<td>$\eta_{i,s,t}$</td>
<td>Dynamic Viscosity</td>
<td>g/s/m</td>
</tr>
<tr>
<td>$\rho_{i,s,t}$</td>
<td>Water Density</td>
<td>g/m$^3$</td>
</tr>
</tbody>
</table>
Microcystis colony particles grow, move, and produce a toxin tracer. Growth is controlled by carbon mass transfer between the environment (a source/sink) and cellular reservoirs, forced by temperature and light. Here I emphasize temperature, since it is a key factor in bloom timing and severity. The balance of the cellular carbon pools determines the density of cyanobacteria colonies, forces buoyant vertical movement. While they move, colonies transfer toxin into the environment—the computational mesh. Colony movement, and the excretion and diffusion of toxin, are resolved only in the vertical dimension. This means that biomass, intracellular toxin, and dissolved toxin are uniformly distributed across the horizontal domain. Colony particles can therefore be said to represent infinitesimally thin layers of cyanobacteria with finite areal extent.

Fish movement, instead, is constrained to a horizontal plane. I base behavior on an existing stochastic foraging model (Anderson 2002), in which behaviors consist of predefined movement rules arising from interactions of points, boundaries, environmental fields and physiological state (collectively referred to as agents). I avoid particle-particle interactions because computation time doesn't scale well with a large number of individuals. Although there are bookkeeping tricks which can reduce particle queries (Akat and Gazi 2008; Rose et al. 2015), I focus on iterative costs which do not require these kind of N-body calculations. I also use a doubly-periodic domain, to eschew boundary interactions. Instead, there are two agents which cue behavior: environmental growth potential and the internal toxin pool. Fish growth is a function of the maximum possible growth rate, modulated by the suitability of the environment at the particle's current position. The choice of movement rules should act to optimize growth, and performance should be better than truly random motion. I do not consider the effect of temperature on the growth rate of the fish, as is standard in most bioenergetic models. This is done specifically so that identical groups can be used in each of the experiments (which each have a unique temperature regime), with confidence that the only factor modulating growth is their movement and foraging success.

Fish acquire toxin as they grow (implicit ingestion), and/or through absorption from the dissolved pool. Accumulating a low dose triggers a switch in behavior, akin to agitation and flight. Accumulating a high dose induces impairment, which is not a separate behavior, but instead a decrease in swimming speed and a more random distribution of turning angles. Four toxin scenarios—consecutive in time—are evaluated for their impact on the final growth of individuals. These correspond to growth and toxicity trends and stages in naturally occurring blooms, and include: a zero toxin control case (Control, A); a case with increasing intracellular toxin, but no dissolved toxin (Formation, B); a case with constant intracellular toxin, and increasing dissolved toxin (Intensification, C); and
finally a case with increasing bloom toxicity and increasing dissolved toxin (Decline, D). Although toxicity increases in D, this is because the overall biomass is decreasing, hence the name decline. The scenarios also cover all combinations of binary states for toxin production and excretion (i.e. A: off-off, B: on-off, C: on-on, D: off-on). The movement of the group is described as a collective behavioral diffusivity coefficient ($K_F$). Individual fish movement already has a stochastic component, so I omit advection and eddy diffusion from their movement, and therefore $K_F$ describes only behavioral motion.

So, temperature and light force cyanobacteria growth and toxicity. In some simulations, toxin leaks into the environment, where constant turbulent diffusivity forces the evolution of the vertical toxin profile. This non-uniform profile results in depth-varying absorption by individual fish, which time shifts behavioral triggers. The suitability field presents fish with a horizontally-varying feeding cue, which also triggers avoidance if the biomass is toxic. The point of this study is to begin examining theoretical trade offs inherent to these behaviors, and how individual decisions propagate to the group level. My intention is that such microcosm experiments (when validated with real movement data) be used to parameterize Eulerian fish movement in finite-volume or finite-element simulations.

2.2.2 Spatial and Temporal Scales

The choice of scale in this hybrid Lagrangian-Eulerian method is especially important, as the processes involved span seconds to months, and micrometers to kilometers. Physiochemical processes imply microscales of time and space, while behavior is only meaningful where the analytical window is sufficiently large to capture drift in the statistical description of group movement. In this case, the instantaneous qualities of movement are as important as the end result: growth as a measure of fitness. Integration step, duration, particle count and spatial resolution need to be balanced to suit the limits of computation while providing results free of discretization artifacts. I will justify—throughout the methods—the assumptions I use to simplify the model and maintain generality. The experiments use small quantities of particles, and are intended to inform further refinement.

The framework linearly interpolates precomputed physical fields ($dt = 1.0$ hr) to 50 intermediate intervals ($dt = 0.02$ hr). This serves as the default step size for fourth-order explicit Runge-Kutta solutions to cyanobacteria movement and mass transfer. The same step is used for Euler solutions to dissolved toxin and particle diffusion, as well as the movement and growth of Lagrangian fish. The chosen time step satisfies the numerical stability
requirements for all processes. Particle position and state are output for analysis and visualization every 5 steps (\(dt = 0.1\ hr\)). This smooths the time series, and reduces data storage, without compromising interpretability.

Each of the four scenarios mentioned in the previous section (control, A; formation, B; intensification, C; decline, D) last 720 hours, equivalently 30 days, and are sequential—with some conditions carrying over between runs. The effective simulation length is therefore 120 days: corresponding approximately with the April–July season during which Microcystis blooms and declines in upper Barataria lakes (Garcia et al. 2010). Two of these water bodies, Lac Des Allemands and Lake Cataouatche, have an average depth of about two meters and areas of 37 and 49 km\(^2\) (Hopkinson, Day, and Kjerfve 1985; Dash et al. 2011). The FVCOM unstructured mesh can accommodate any morphology, but I eschew complex cases in favor of a square experimental domain with uniform bathymetry (\(\sim 5.0\ m\)). The horizontal domain has 500 m side length, for a total area of 0.25 km\(^2\). This encourages encounters with a single spatially resolved feature (the suitability agent, discussed later) while keeping particle count low. Approximately a bathtub—though deeper than most I've known—it offers a generalized case compared to shallower water bodies in Louisiana. It is closer in depth to Lake Ponchartrain, which also experiences seasonal toxic blooms, which can be triggered by the opening of flood control structures. Functionally, shallowness limits the vertical oscillation of Microcystis colonies, and changes the dominant light limitation factor from attenuation to self-shading, as compared to the original cyanobacteria model (Rabouille, Salençon, and Thébault 2005). In this regime, the uppermost bloom biomass induces light limitation in the lower portion. This modification extends the application of the original model by Rabouille et al. (2005) from relatively deep reservoirs to a much shallower system. I maintain their parameterization, due to the lack of empirical physiological rates for local strains. Realistically, the cyanobacteria in Louisiana have optimal growth conditions tuned to a more tropical climate than those of temperate Europe. Again, the experiment is demonstrative, and not meant to accurately predict bloom timing and severity, only to supply a dynamic toxic environment to test the fish hypotheses.

### 2.2.3 Random Deviates

The model uses three types of random deviate: uniform, Gaussian, and truncated Gaussian. The latter two are similar, except that values for the truncated distribution are rejected if outside \([-\sigma, \sigma]\). Because of this constraint, the uniform deviates and truncated Gaussian deviates have the same range and mean. Gaussian deviates are generated using a transform of two uniform pseudorandom variates (Box and Muller 1958). As the number of
samples increases, the Gaussian distribution converges toward the PDF for zero expected value (μ=0) and unity variance (σ²=1) denoted N(0,1). When there are only 50 samples, μ=0.24 and σ²=0.74; at 500 samples μ=0.02 and σ²=1.01; and at 5,000 samples μ=0.01 and σ²=0.99. Several million random deviates are calculated during each experiment.

2.2.4 Computational Grid

The square horizontal domain is subdivided into right-isosceles triangular elements with 10 m base length and alternating orientation. The horizontal grid is only used for bookkeeping during program execution, and is not shown. The horizontal domain is doubly-periodic, so that particles crossing a boundary re-enter the mesh at the opposing edge. Twenty-six sigma layers divide the vertical dimension into even 0.2 m intervals. The vertical dissolved toxin profile (g/m) is resolved at these depths. The vertical grid elements are used for finite-difference dissolved toxin diffusion. The number of layers is determined by the time step necessary for stability of the diffusion calculations. The method is conditionally stable while dt ≤ 12.5 · (N−1)⁻², where N is the number of sigma layers (with a fixed five-meter depth). Solving the quadratic gives the maximum number of sigma layers possible for a specified time interval. For instance, with 11 layers the time step must be shorter than 0.125 hr. The original time step for fish movement was one second (Anderson 2002), at which the method is stable up to 213 sigma layers. Intermediate values that do not require a nested loop are desirable. The choice of dt=0.02 hr (just over one minute) has an exact root of 26 sigma layers.

2.2.5 Environmental Variables

The model uses a number of physical variables (salinity, temperature, turbulent diffusivity, irradiance) and a single biological proxy variable (growth potential, also referred to as suitability)—see Figure 2.1. Irradiance follows a twelve-hour photoperiod with fixed amplitude in each simulation. Because it is not grid-based, I present irradiance within the biological algorithms (see Cyanobacteria Dynamics below). Salinity and temperature fields (and therefore density) are spatially uniform. Salinity is time invariant (Sₐ=3), and used only as a parameter to calculate density and dynamic viscosity, although a spatially varying Sₐ field could limit growth or cue behaviors such as gradient following (Rose, Huang, and Justic 2014). The salinity value is the upper range of those found in the oligohaline water bodies. Each experiment has initial and final temperature values (T) similar to Lac des
Allemands at –0.3 m depth during April–July, 2009 (Fox 2010). Temperature is a constant 20°C for the control experiment (A), then increases linearly between 20–25°C and 25–30°C during the next two experiments (B and C respectively). It remains a constant 30°C in the final experiment (D). This piecewise linear temperature function has the advantage of being symmetric about the 25°C growth optimum used by Rabouille and Salençon (2005), and it never exceeds the 36°C lethal maximum. During the periods of rising temperature, \( T = 6.94 \cdot 10^{-3} t + T_0 \), where \( T \) is the instantaneous temperature, \( t \) is the elapsed time in the experiment (hours) and \( T_0 \) is the initial temperature (20°C or 25°C). These temperature trends will be revisited in the experiment descriptions later in this section.

The density of water at atmospheric pressure is a polynomial equation of temperature and salinity (Millero et al. 2008). The conditions in these experiments satisfy \( T < 40°C \) and \( S_R < 42 \) within which the calculated water density \( (\rho_{S,T}) \) has 0.01% error. This replaces the temperature method of Rabouille et al. (2005) and applies to particle velocity in a salinity gradient—were there one (e.g., a channel transport scenario). See Sharqway et al. (2010) for the equations.

Horizontal eddy diffusivity is zero. Cyanobacteria movement in X and Y is ignored, and fish simply move too fast to be meaningfully displaced in a low-flow scenario. Vertical diffusivity on the other hand is uniform, \( K = 0.036 \, \text{m}^2/\text{hr} \). The first derivative \( (K') \) is everywhere natural (zero), including at the boundaries. The value is chosen based on the vertical eddy diffusivity observed in lakes during summer stratification (Sweers 1970). In fact, the entire water column in Louisiana estuarine lakes is fully mixed every three days by high winds or summer thunderstorms (Hopkinson, Day, and Kjerfve 1985). For this reason \( K \) and \( K' \), which control particle random walks and diffusion of dissolved toxin, are underestimated. They apply realistically only to the periods when wind mixing is low enough that a strong thermocline can develop—conditions which may, also, lead to pronounced anoxia below the thermocline. As fish suffocate, they are forced upward into oxygenated water, where *Microcystis* is concentrated. If toxin excretion outpaces diffusion, exposure to dissolved toxin concentration increases for fish constrained at the surface. To test whether this scenario can arise, dissolved toxin at each sigma layer \( (\gamma_N) \) disperses according to vertical diffusivity \( (K) \). Since layers are evenly spaced I use a centered-difference, second-order accurate method, with no flux boundaries \( (d\gamma/dz = 0) \) at the surface and bottom,

\[
d\gamma_N/dt = K \left( \gamma_{N+1} + \gamma_{N-1} - 2\gamma_N \right) dz^{-2} \quad [2.1]
\]

The selection criteria of a stable time step for this equation \( (dt=0.02 \, \text{hr}) \) was addressed above in reference to vertical
grid spacing. The source term for dissolved toxin—excretion by *Microcystis* colony particles—is presented within the section on bloom toxicity. No sinks for dissolved toxin are considered.

Impairment resulting from toxin exposure should reduce growth. Without explicitly including bioenergetics, this is still possible by assuming that growth is mostly an indication of the amount of food the animal can obtain. It, of course, has physiological limits. The simplest method is to give growth a range between zero and some maximum, established—perhaps—by force-feeding small generic fish in a laboratory somewhere. If a fish obtains only enough food for basic metabolic maintenance, its growth potential is zero ($G_X = 0$). If it is given free reign at the buffet, then growth potential is one ($G_X = 1$). A region of high growth potential, which will be referred to as suitability, crosses the horizontal domain,

$$G_X = 0.5 \cdot [1 + \sin(0.004 \cdot \pi \cdot [X−125])]$$

The band is centered in the middle of the $X$ domain (250 m), where $G_X = 1$. This function is continuous and differentiable, including at the boundaries, since the domain is doubly periodic. The minimum is zero, which occurs along the east and west boundaries. Over the domain, $\bar{G}_X = 0.5$.

2.2.6 Cyanobacteria Movement

*Microcystis* move vertically, based on buoyancy relative to the surrounding water. The natural hydrodynamic unit for *Microcystis* is the colony, for which the Stoke's velocity is,

$$\frac{dZ_i}{dt} = 1962 \cdot R_i^2 \cdot \eta_{S,T}^{-1} (\rho_{S,T} − 1072.1 + e^{4.644−0.7(C_i)})$$

The change in particle position ($dZ_i$, meters) is based on empirical cell density data, and depends on colony radius in meters ($R_i$), and cellular proteinaceous material ($M_i$) and carbohydrate stores ($C_i$) in grams of carbon (Rabouille, Salençon, and Thébault 2005; Howard 1993). The greeks are dynamic viscosity ($\eta_{S,T}$) and density ($\rho_{S,T}$), which in turn are functions of *in situ* salinity and temperature. I assign random colony radii (meters) at the beginning of each experiment, $R_i = [5N(0,1) + 75] \cdot 10^{-6}$. This is in the lower range of natural colony diameter, which increases time in the water column away from boundaries. A more robust approach would include time-varying diameter, which is beyond the scope of this work. Colony particles within a single are initialized with fixed $M_i$ and a $C_i$ value that results in $dZ_i/dt = 0$ during the first time step. Since the water density is a function of $T$ and $S_R$, which are uniform across the physical domain, intra-experiment colony carbon pools are initially identical. The initial values are prescribed for each experiment, and will be introduced below. This component of vertical movement is mechanistic,
since density and viscosity depend ultimately on physical parameters (Sharqawy, Lienhard V, and Zubair 2011). Appendix B lists the full parameterization of Equation [2.3].

Equation [2.3] is valid only where Reynolds' number \( \text{Re}_i = 2 \rho_s T dZ_i / dt \cdot R_i \cdot \eta_s T i^{-1} \) is very small. Error in \( dZ_i / dt \) decreases as \( \text{Re}_i \) approaches zero, and is within 10% of the true value while \( \text{Re}_i < 0.5 \) (Rabouille, Thébault, and Salençon 2003). \( \text{Re}_i \) remains below this threshold during the numerical experiments. \( C_i / M_i \) is initialized such that particles are in a temporary state of neutral buoyancy (\( dZ_i / dt = 0.0 \) m/hr).

Boundaries are non-reflecting so that particles collect at the free surface and bottom—also true for stage position updates during Runge-Kutta integration. This ensures that the current light conditions are related to particle history, whereas reflecting boundaries can cause particles with upward velocity to move below their previous position (Ross and Sharples 2004). A particle with relatively constant upward velocity at a reflective boundary can reach an artificial equilibrium below the surface: purely an artifact of adopting a convenient boundary interaction scheme. Cyanobacteria instead experience vertical turbulent diffusion, implemented as a random walk, to prevent boundary accumulation. This randomizes the order of neighbor particles and, therefore, the shading effect they have on themselves and each other. The magnitude of the random jump depends on the shape of the vertical diffusivity profile (Ross and Sharples 2004) Here the diffusivity profile is uniform and natural, and the equation is simplified to,

\[
dZ_i = N(0,1) \cdot [2K \cdot dt]^{0.5} \quad [2.4]
\]

This uses a random deviates drawn from a Gaussian probability distribution \( N(0,1) \) with zero expected value and unit variance. The time step must be much less than the minimum of the inverse of the second derivative (Ross and Sharples 2004). The second derivative of a uniform profile approaches zero, the inverse approaches infinity, and therefore \( dt=0.02 \) hr is appropriate for this application.

2.2.7 Cyanobacteria Dynamics

*Microcystis* colonies have three cellular reservoirs—proteinaceous cellular material (\( M_i \)) and carbohydrate ballast (\( C_i \)) for carbon, and a single toxin proxy (\( \gamma_i \)) representing MC and STX (which are considered to be in constant proportion and exert a combined effect on fish). Colonies draw carbon from the environment, transfer it between reservoirs, and lose it to the water column. Colonies produce toxin, and excrete it to the surrounding water. Carbon and toxin mass conservation is not considered (there are sources and sinks). The transfer equations are
named and enumerated, and expressed in a format that emphasizes internal state variables. Table 2.2 summarizes how these individual processes are combined into differential equations for each reservoir. Limiting equations drawn from the literature are presented as in-line equations, which are sometimes used in multiple transfer equations. These represent environmental forcing, and require interactions with other agents.

Table 2.2 Carbon and Toxin Mass Transfer Equations

<table>
<thead>
<tr>
<th>State Variable</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate ($C_i$)</td>
<td>$\frac{dC_i}{dt} = \text{fixation} - \text{synthesis} - \text{respiration}$</td>
</tr>
<tr>
<td>Protein ($M_i$)</td>
<td>$\frac{dM_i}{dt} = \text{synthesis} - \text{excretion}$</td>
</tr>
<tr>
<td>Toxin ($\gamma_i$)</td>
<td>$\frac{d\gamma_i}{dt} = \text{production} - \text{excretion(toxin)}$</td>
</tr>
</tbody>
</table>

$\text{CO}_2$ fixation is the only source of carbon, drawn from an inexhaustible pool (Table 2.2). Carbohydrate is stored at a fixation rate ($g/hr$) limited by carbon ratio and a function of the irradiance ($I$) at the particle's position,

$$\text{fixation} = 1.873 \cdot 10^{-3} \cdot f(I) \cdot (4M_i - C_i) \cdot [-1 + e^{-14(C_i+M_i)/A}] \cdot A \cdot (C_i + M_i)^{-1}$$  \[2.5\]

The domain area ($A$) is $2.5 \cdot 10^5$ m$^2$. The expression $[-1 + e^{-14(C_i+M_i)/A}] \cdot A \cdot (C_i + M_i)^{-1}$ is introduced to shade the particle by the average of the logarithmic attenuation through the particle's own biomass divided by that area. Short version, a particle with a lot of carbon shades itself even at the surface. The limiter $f(I)$ is a function of actual and optimal irradiance, and the amount of algal biomass in the interceding water. By adopting the optimal light value of 250 W/m$^2$ from Rabouille et al. (2005) this reduces to $f(I) = 7.92 \cdot I, (0.016 \cdot I^2 - 0.08 \cdot I + 10^3)^{-1}$ (Rabouille, Salençon, and Thébault 2005; Eilers and Peeters 1988). Irradiance is uniform over the horizontal domain, but time-varying and attenuated at depth. There is a twelve-hour photoperiod, centered at noon. This is a valid representation near the beginning of the experiment, since in nature the Spring Equinox would have just passed in late March. As the simulation progresses, the photoperiod should lengthen, but for now I keep it consistent throughout to highlight temperature effects. Between dawn and dusk (6:00–18:00 each day), irradiance (W/m$^2$) at depth is,

$$I_d = [325 + 325 \cdot \cos(0.522t)] \cdot e^{0.15Z_i - 14B_i}$$  \[2.6\]

where $B_i$ is the integrated overlying biomass ($g/m^2$). I present an efficient method for calculating this below. For now, consider the diurnal signal. At night, irradiance is zero (0:00–6:00 and 18:00–0:00 each day). Maximum surface irradiance is 650 W/m$^2$ at noon, as per Rabouille et al. (2005). The piecewise light curve transitions smoothly between light and dark periods; it is continuous and differentiable. The average of surface irradiance during the light
interval (06:00–18:00) is 325 W/m^2, which is greater than the optimal 250 W/m^2 for *Microcystis* (Rabouille and Salençon 2005).

Light available for carbon fixation by cyanobacteria depends on the depth of all particles in the simulation. Starting at the surface, each successive particle acts as a filter which attenuates the light passing through it and reaching particles below. As the particles accumulate biomass, the attenuation of light becomes greater and limits carbon fixation. Euphotic depth (where light is 1% surface irradiance) can be derived from Equation [2.6] with a little algebra, \( Z_E = 93.333B - 30.701 \). This point will be at bottom where \( B < 0.275 \) g/m^2 and at surface where \( B > 0.329 \) g/m^2. When \( Z_E \) approaches zero, photosynthesis is possible only at the surface. Light surfaces for attenuation vs. time, and depth vs. biomass, are shown in Figure 2.2. These demonstrate the effect of attenuation through *water only* over the daily light cycle, and second, the combined effect of attenuation and shading on noon irradiance at depth. The overlying biomass is updated during each calculation of irradiance at a particle position.

Overlying biomass for the \( i \)th *Microcystis* colony is \( B_i = 1.0 \cdot \Sigma(C_j + M_j) \cdot A^{-1} \), where \( C \) and \( M \) are colony carbon pools, and \( j \) is the set of all colonies satisfying \( Z_j > Z_i \) (remember that \( Z \) is negative). I introduce a stand-in upscaling factor (1.0) to indicate that this scheme should be modified for larger scale simulations where colonies are not distributed across the entire horizontal domain. Increasing this value causes colonies to act as if in a smaller effective area, and therefore strengthens shading. Integrating overlying biomass is computationally expensive but important, because collective optimization leads to dense scum with little light penetration (Flynn and Fashamz 2002). I use a recursive binary partitioning method to efficiently sort and integrate the biomasses of overlying colonies.

Figure 2.2 Light Attenuation—the left plot (A) shows the time evolution of the depth-attenuated irradiance profile (W/m^2) with \( B_i = 0.0 \) g/m^2. The t-axis is one period of the 24-hour light cycle, and the Z-axis is the depth range (m). The right plot (B) shows the attenuated irradiance at noon as a function of depth and the natural log of \( B_i \).
Fixation becomes a surface function of $I_i$ and $C_i/M_i$ with a maximum of 10.5 g/hr if $M_i=100$ g (Figure 2.3A). Fixation cannot be negative, so it is set to 0.0 g/hr in cases where $C_i/M_i > 4$ (approximately the maximum empirical ratio observed by Visser et al. 1997). Cyanobacteria protein synthesis (g/hr) is instead temperature-limited, and proportional to $C_i$ (Rabouille, Salençon, and Thébault 2005),

$$\text{synthesis} = 5 \cdot 10^{-2} f_i(T) \cdot C_i \quad [2.7]$$

Limiting function $f_i(T)$ depends on *in situ*, optimal and lethal temperatures. I adopt the parameters used by Rabouille et al. (2005) to obtain $f_i(T) = T^4 (2.379 - 0.068 \cdot T)^0.48$. This reappears in respiration, the sum metabolic cost (g/hr) of cell maintenance and protein synthesis (Rabouille, Salençon, and Thébault 2005),

$$\text{respiration} = -10^{-2} f_i(T) \cdot C_i + 4 \cdot 10^{-2} f_2(T) \cdot M_i \quad [2.8]$$

Respiration shares, with excretion, the exponential dependence equation $f_2(T) = 0.286 \cdot e^{0.05T - 0.15}$ (Rabouille, Salençon, and Thébault 2005). *Microcystis* excrete carbon through their cell membrane at a base rate (g/hr) proportional to $C_i$ plus an activity rate proportional to $M_i$ (Rabouille, Salençon, and Thébault 2005),

$$\text{excretion} = f_2(T) \cdot (0.004 \cdot C_i + 0.05 \cdot M_i) \quad [2.9]$$

Carbon excretion is always greater than zero. Both respiration and excretion are carbon sinks. Solutions to each environmentally-dependent process are shown in Figure 2.3 for a range of $C_i/M_i$ ratios, normalized to $M_i=100$ g.

I assume *Microcystis* a dominant monoculture, unaffected by aggregate grazing. This would not be sound for cases of high grazer density, or where *Microcystis* is only a minor player in the phytoplankton. Instead, direct grazing should be implemented. The overhead cost of determining fish-colony distance can be defrayed with good dynamic neighborhood topology (Akat and Gazi 2008; Rose et al. 2015). During migration, cells produce toxin, which leaks to the dissolved phase or is ingested by foragers.

I use a single tracer ($\gamma$) to represent the bioactive fractions of MC, STX and ATX. Lagrangian particle toxin (whether fish or cyanobacteria) is denoted $\gamma_i$ while Eulerian dissolved toxin is $\gamma_N$. The subscript N is the sigma layer index, starting with $N=1$ at the surface. Toxin production by cyanobacteria (g/hr) is a constant proportion of protein ($10^{-4} \cdot M_i$) during formation (B) and intensification (C) experiments. I estimate this by converting whole cell data (Wiedner et al. 2003) to a per protein value, and assuming cellular material consists of (as far as this study is concerned) 50% carbon and 50% junk (Spitzer et al. 1996). It is zero in the control (A) and decline (D) experiments. Excretion (g/hr) is a constant rate that is set to zero during control (A) and formation (B) experiments. During intensification (C) and decline (D) it is $-10^{-4} \cdot M_i$. The differential equation for cellular toxin ($d\gamma_i/dt$) therefore
depends on the experiment (see Experiment Descriptions below). Mass (g) is transferred to the vertical diffusion mesh (g/m) by adding weighted amounts at sigma layers between which the colony is located (γ_N and γ_{N+1}). The contribution to each sigma layer varies linearly with distance from the particle. This increases the area under the depth-toxin curve, adding to total mass (g) in the dissolved system by the trapezoidal area \( \Delta \gamma_i = 0.2 (\Delta \gamma_N + \Delta \gamma_{N+1}) \). When the particle is near the surface or the bottom, the trapezoid is truncated and the area of the missing triangle is added instead between the layers bracketing the particle's position.

Figure 2.3 *Microcystis* Carbon Transfer Functions—Plots show a range of carbon transfer solutions for a colony with M_i=100 g. Fixation (A) as f(I, C_i/M_i). Synthesis (B) as f(T, C_i/M_i). Respiration (C) as f(T, C_i/M_i). Excretion (D) as f(T, C_i/M_i). In all cases the vertical axis is mass transfer (g/hr). The vertical scale changes between plots. Note that the irradiance axis of (A) is inverted w.r.t. (B–D).

2.2.8 Fish Behavior

Fish behavior is event-based. There are two types of agents (j) which elicit a behavioral response, called an event (Boolean, \( \epsilon_j \)). This scheme is based on the interpretation of Anderson (2002) by Watkins and Rose (2013). Fish monitor their own internal toxin pool: this is the intoxication agent (j=1). When the toxin level of an individual
exceeds 0.005 μg/g (micrograms of microcystin per gram body weight), an intoxication detection event occurs ($\varepsilon_1=1$). The environmental growth potential (suitability agent, $j=2$) is spatially explicit. A default behavior—random walk—will also be defined ($j=0$). During movement, individuals experience a gradient of suitable habitat, with value ranging $0 \leq G_X \leq 1$. When $G_X > 0.5$ at a particle’s position, a high growth event occurs ($\varepsilon_2=1$). This will occur any time the fish enters the region defined by $125 < X < 375$ m. Fish possess a probabilistic internal model (memory), which abstracts event history over multiple timescales,

$$\Delta P_{j,k} = P_{j,k}(m_k-1) + \varepsilon_k(1-m_k) \quad [2.10]$$

The probability of an agent encounter $P_{j,k}$ is calculated with two memory coefficients ($m_k$) specified: $m_0=0.50$, $m_1=0.96$ (Anderson, 2002). Greater memory value allows an event signal to persist. Behavior is classified as a tactic (short-term, $k=0$) or strategy (relying on memory to improve fitness over time, $k=1$). While $\varepsilon_j=1$, probability increases until saturation at $P_{j,k}=1$, and elsewhere decreases monotonically toward zero. From this picture of the environment, individuals select the set of movement rules which should result in growth. The utility of a specific behavior is the product of the probability of an encounter with that agent and the intrinsic utility of that occurrence,

$$U_{j,k} = u_j \cdot P_{j,k} \quad [2.11]$$

Movement therefore depends on the history of intoxication, and encounters with high growth rate. The intrinsic utility ($u_j$) is used to weight the potential behaviors. Foraging is the central behavior of the model, and since there is not a trade off (such as mortality), feeding behavior is fully weighted ($u_2=1.0$). Internal toxin detection triggers avoidance, the movement rules for which are based on reducing mortality in a spatially-explicit field. I assume that the basic function of the two types of avoidance (non-specific versus directional) are evolutionarily similar: the fish is responding as best it knows to novel conditions. Because of this, and to encourage feeding during intoxication, the intrinsic utility of avoidance is scaled down to 10% of the feeding value ($u_1=0.1$). The highest utility value among the $j$-$k$ combinations determines swimming speed and angle (Table 2.3). If all members of $U_{j,k}$ are less than 0.01, the fish defaults to a pure random walk. Fish particles always move; sometimes they just do so faster or more randomly. Swimming speed, $|V|$, is given in body-lengths per time step. When updating position, this is scaled by effective length (*L) which will be used as an impairment proxy. The direction of movement ($\theta$, radians) is the previous angle plus a random component. During active avoidance triggered by internal toxin detection ($j=1$, $k=0$), $\pi$ radians are added to the new swimming direction, unless the fish switched direction during the last time step. This caveat is added to prevent extreme residence times which result from the star-shaped trajectories of a fish traversing the same
region repeatedly. Such artifact patterns do no fulfill the evolutionary role of avoidance, and need to be accounted for (Watkins and Rose 2013).

The movement rules result in distinct bouts. Tactical growth swimming is slow, with a wide random turn angle, which should keep the fish in a profitable area. When the fish transitions to the strategic growth seeking behavior, speed increases and the angle range decreases. This causes a fish leaving a profitable patch to make a quick search bout, which is likely to loop the fish back toward the patch. Tactical intoxication avoidance has the highest swimming speed, and a narrower turning angle than growth behaviors. This results in a fish detecting intoxication to reverse direction and quickly flee the area. In the discussion this will be referred to as the flight behavior. The transition to strategic avoidance decreases speed by half, but does not change the angle. These values are adopted from Watkins and Rose (2013), except that the angle scalar for tactical foraging has been increased from 0.1 to 0.25 to prevent individuals from getting stuck due to constantly reversing direction. I believe this is valid, since the avoidance cue is not explicitly directional and the fish are less likely to know the best angle at which to flee. I also use truncated Gaussian deviates for turning angle during sober periods, while Watkins and Rose (2013) use uniform deviates. During impairment, the random deviates switch to a uniform distribution to increase randomness of movement without altering the range of possible angles. For this reason, and because fish slightly larger, the behavior during intoxicated periods should be more similar to the results of their experiments than under sober conditions.

Particle position changes according to $\Delta X/\Delta t = *L \cdot |V| \cdot \cos(\theta)$ and $\Delta Y/\Delta t = *L \cdot |V| \cdot \sin(\theta)$. Particles which cross one boundary reenter the domain from the opposite boundary with the same velocity. The original fish movement models were two-dimensional (Anderson 2002; Watkins and Rose 2013), so here the Z position is fixed, to force the relative exposure to dissolved and particulate toxins to vary in fish operating on different horizontal planes. In surface intensified toxin profiles, a benthic feeding fish will experience far lower concentrations of microcystin than would a fish feeding at air-water interface. In some cases, fish may be forced into oxygenated upper layers by anoxic summer conditions. This will show that vertical resource use by physiologically identical fish can change uptake, without explicitly modeling their vertical distribution and movement. When an individual fish's internal toxin level reaches a second higher threshold, 0.015 μg/g, impairment is activated. This is not modeled as a selectable behavior, because it should alter how fish already act instead of introducing new rules. While intoxication remains above this threshold, the body-length is scaled to effective length (*L=0.9·L), which decreases swimming speed by 10%.
Furthermore, the truncated Gaussian deviates in movement rules are replaced with uniform random deviates. The range of both is [-1, 1], but probability of values near the limits is greater for uniform deviates. Swimming is slower and more random. The distribution for the default behavior is unchanged.

Table 2.3 Movement Rules—The direction and speed of movement is specified for each combination of agent and timescale, and an alternate default behavior. The default behavior is a non-inertial random walk-like method from Watkins and Rose (2013). Agent-specific behaviors are inertial: swimming angle (radians) is equal to the previous direction, plus a random component. \( N_T \) indicates a truncated Gaussian distribution with zero expected value and unit variance. This is replaced with the uniform deviate, \( U(-1,1) \), during impairment. Swimming speed (body-lengths per time step) is a constant for each behavior.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Tactical (k=0)</th>
<th>Strategic (k=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity (j=1)</td>
<td>( \Delta \theta = \pi + 0.25\pi \cdot N_T(0,1) )</td>
<td>( \Delta \theta = 0.25\pi \cdot N_T(0,1) )</td>
</tr>
<tr>
<td></td>
<td>(</td>
<td>V</td>
</tr>
<tr>
<td>Growth (j=2)</td>
<td>( \Delta \theta = \pi \cdot N_T(0,1) )</td>
<td>( \Delta \theta = 0.5\pi \cdot N_T(0,1) )</td>
</tr>
<tr>
<td></td>
<td>(</td>
<td>V</td>
</tr>
<tr>
<td>Default</td>
<td>( \theta = 2\pi \cdot U(-1,1) )</td>
<td>(</td>
</tr>
</tbody>
</table>

2.2.9 Fish Growth

Fish increase in length (L) over time depending on the quality of the domain they occupy. That is,

\[
\frac{\Delta L}{\Delta t} = G_{\text{max}} \cdot G_X \quad [2.12]
\]

where \( G_{\text{max}} \) is the maximum growth rate (0.03 mm/hr) and \( G_X \) is the suitability value at the particle's X location (Equation [2.2]). The initial body-length for all fish particles is 0.10 m. Over the course of a 30 day experiment with ideal growth, this would increase to 0.12 m. The final length of a fish moving randomly, given mean \( G_X=0.5 \), should be less than 0.11 m. As the fish grows in length, the mass (g) increases according to (Watkins and Rose 2013),

\[
M_i = 2 \cdot 10^{6} \cdot (1000 \cdot L_i)^{3.38} \quad [2.13]
\]

Fish start with \( M_i = 11.51 \) g, and will have a final mass no greater than \( M_i = 21.31 \) g. The mean mass after 30 days for a random swimmer (\( G_X=0.5 \)) should be around 16.28 g. The difference in fish mass between time steps will be referred to as \( \Delta M_i \) in the calculation of toxin ingestion. The value depends on length history, and is greatest if the
individual achieves optimal growth every time step, in which case the final steps of the simulation would have 
\[ \Delta M_i < 3.6 \cdot 10^{-4} \text{ g}. \]

2.2.10 Ichthyotoxicity

Toxin accumulation in fish has two sources and one sink—uptake by ingestion and absorption, and removal by depuration (Figure 2.1). The amount sequestered in the fish is assumed to be a small fraction of the total available toxin, and so there is no explicit transfer between the environment and individual fish. Ingestion (g/hr) is equal to the total toxin load of consumed prey items, which I assume are in equilibrium with the set of all cyanobacteria particles. This averaging scheme sustains the precedent of no fish–colony position queries. Without explicitly identifying a prey, the mean toxin load of the colony particles is a good estimate, that hedges bets between biomagnification and bloom avoidance. If respiration is consistent, then the magnitude of \( \Delta M_i \) (change in fish mass, calculated from change in length) is an indication of food consumption and assimilation. Toxin is assimilated proportionally to the amount of consumed biomass. The following cyanobacteria experiments will show that the maximum instantaneous mean toxin load of particles within a single experiment, \( \bar{\gamma}_j \left( C_j + M_j \right)^{-1} \), is about \( 2 \cdot 10^5 \mu g/g \). Subscript \( j \) indicates the set of state variable values for all cyanobacteria particles. Depending on the spatial scheme, the extent of the cyanobacteria set \( j \) could be any neighborhood topology. For fish feeding directly on bloom biomass this is a sound condition. However, fish feeding on zooplankton may be exposed to either concentrated or diluted toxin loads. This will depend on whether the food source can in turn avoid, or is ecologically incompatible with, \textit{Microcystis}.

Since \( \Delta M_i < 3.6 \cdot 10^{-4} \text{ g} \), the maximum possible rate of toxin ingestion is \( 3.6 \cdot 10^3 \mu g/hr \). If this were sustained over 30 days—with no other sources or sinks considered—a fish particle could accumulate \( 2.6 \cdot 10^3 \text{ g} \) of microcystin (were there no removal). The actual uptake will be less, since growth varies with position. \( \Delta M_i \) increment increases as individuals grow in length and is greatest where the environment promotes growth.

Absorption is, instead, proportional to body-length (\( L_i \)) and \textit{in situ} dissolved toxin concentration (\( \gamma_z \)). This is also size dependent, but scales differently than \( \Delta M \), so that the relative contributions from diet and absorption change with length. There are characteristic periods when each pathway is dominant, although the exact timing and magnitude of mass transfer are not sufficiently parameterized to be predictive. Depuration is proportional to toxin
The coefficients can be changed such that depuration is equal to maximum absorption or ingestion when the tissue toxin is near the greatest observed whole body values—e.g., Soares et al., (2004)—about 1 μg for fish this size. Here they are chosen *a posteriori* to produce distinct toxin signal across all experiments. The first coefficient (ingestion scalar, $2 \cdot 10^{-5}$) is the least to account for the fact that while the ratio of toxin to carbon may be high, adjusting for cell stoichiometry would result in a much lower value. This term must also be discounted for low transfer of toxin from the gut to the tissue. The absorption coefficient ($4.675 \cdot 10^{-3}$) is set so that the process outweighs ingestion only at high dissolved concentrations (w.r.t. values in the four experiments discussed in the study). I choose a depuration coefficient ($10^{-2}$ g/g) in concert with these to produce realistic toxin levels in fish. The general behavior of Equation [2.14] is to increase intoxication while the first derivative of the combined uptake rate is positive, and to decrease intoxication when it is negative. If uptake (ingestion plus absorption) is constant, then depuration will eventually reach an equilibrium state. This accounts for some species' ability to quickly adapt to ambient toxin levels by increasing production of depuration enzymes. Because the values are hypothetical, I will discuss toxin dynamics qualitatively relative to critical points, where model behavior changes.

If bioenergetics were included, the depuration term of equation should be converted to a real metabolic cost and subtracted from biomass as part of respiration. The absorption term is a deferred cost: toxin that will have to be removed at some future time. The real cost of a behavior might be known to the individual, and could be used to adjust utility.

2.2.11 Outputs and Analysis

Cyanobacteria carbon results are discussed in terms of cumulative (bloom) and mean (individual) biomass. Long term trends in bloom growth are evaluated based on the increase in total carbon (g) sequestered across all proteinaceous cellular material pools, $\Sigma(M_i)$. Mean particle biomass, $\Sigma(C_i + M_i) \cdot N^{-1}$, is instead the sum of all carbohydrate and protein masses divided by the number of particles in the simulation ($N=100$). This metric exhibits a daily signal in response to the light cycle—a daylight increase in $C_i$ from fixation, followed by a dark period when colonies convert $C_i$ to $M_i$, and lose carbon to the water column. At times, this will be presented with standard deviation, so show the variability among colonies. High standard deviation in mean particle biomass is an indication
that the combination of random mixing and heterogeneous colony radii causes drift in the state variables of individuals. The daily carbon transfer signal is examined from the point of view of the mean carbon ratio (g/g), \( \Sigma(C_i/M_i) \cdot N^{-1} \). Colony trajectories are evaluated only in the Z dimension. The mean and standard deviation of particle position (m) together describe the vertical distribution of Microcystis in the water column. Greater standard deviation indicates the influence of radius on the magnitude of migratory oscillations, and/or a strong mixing effect when buoyancy is nearly neutral.

The total mass of intracellular and dissolved toxin within the domain is carried over between experiments (B to C, C to D). Since toxin ingestion is a function of the average ratio of microcystin to bloom biomass, time series toxicity is presented as the mean ratio, \( \Sigma(\gamma/(C_i+M_i)) \cdot N^{-1} \). This is given with the standard deviation—a high value of which indicates non-uniform toxin loads, due especially to a decline in the biomass because of shading. Dissolved toxin lives on the computational grid as a one dimensional diffusion concentration (g/m), but results are divided by simulation area to give the volumetric unit (g/m³) for comparison with concentrations used in laboratory investigations.

Fish intoxication state (instantaneous mean of all fish particles in the experiment) is the sum of internal toxin mass over body weight, divided by the number of fish particles, \( \Sigma(\gamma/M_i) \cdot N^{-1} \). Growth suppression within toxin experiments (B–D) is calculated by normalizing total fish weight as a percentage of the control experiment (A). From fish X positions I calculate the instantaneous mean suitability (\( \overline{G}_X \)) time series for the group of fish. When this appears in figures, I use a two-hour, centered averaging window for clarity. The X,Y position is used to calculate individual mean square displacement \( D_i = 0.25 \cdot \Sigma(\Delta X_i^2 + \Delta Y_i^2) \). The mean value of \( D \) for the group is the behavioral diffusivity, \( D_i = \Sigma(D_i) \cdot N^{-1} \). This is averaged over 5 time intervals (10 day overlapping durations) to obtain the mean behavioral diffusivity \( \overline{K}_d \) during unique phases of the simulated bloom. Phase windows (I–V) are 0–10, 5–15, 10–20, 15–25 and 20–30 days respectively.
I do not test significance of final summary values, due to uncertainty regarding their expected distribution, and because I want to de-emphasize empirical validity. I believe that at this point simply demonstrating a qualitative trend is sufficient. Future use of the model should expand on this. For instance, the averaging windows here were chosen \textit{a posteriori}. There will need to be a systematic exploration of what signals show up in the data when different windows are used.

### 2.2.12 Experiment Descriptions

I use four 30 day experiments which occur in sequence. Final values of temperature, total toxin and total protein are used to initialize successive experiments (e.g. the results of intensification become the initial conditions of decline). For dissolved and particulate pools, the total mass is re-distributed evenly among all particles or sigma layers (they are set to the mean) to minimize the effect of randomly anomalous particles on the processes of others. This step would not be necessary for a very large number of cyanobacteria colonies, but here each experiment has only 100 particles randomly distributed within the mesh. The horizontal domain is ignored for colony particles, so the effect is that particles with variable radius and identical state variables are randomly placed within the vertical domain. The initial position does not affect longterm trends. The variation of parameters and initial conditions between experiments is summarized below (Table 2.4).

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<tbody>
<tr>
<td>Control (A)</td>
<td>20°C</td>
<td>—</td>
<td>—</td>
<td>2.5 g</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Formation (B)</td>
<td>20–25°C</td>
<td>$10^{-4}$ g/g/hr</td>
<td>—</td>
<td>2.90·$10^6$ g</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Intensification (C)</td>
<td>25–30°C</td>
<td>$10^{-4}$ g/g/hr</td>
<td>$10^{-4}$ g/g/hr</td>
<td>2.50·$10^7$ g</td>
<td>9.8·$10^5$ g</td>
<td>—</td>
</tr>
<tr>
<td>Decline (D)</td>
<td>30°C</td>
<td>—</td>
<td>$10^{-4}$ g/g/hr</td>
<td>2.46·$10^7$ g</td>
<td>9.8·$10^5$ g</td>
<td>3.2·$10^5$ g</td>
</tr>
</tbody>
</table>

In the control experiment (A), toxin state variables are all zero. Cyanobacteria do not produce or release toxin and, therefore, fish behavior cannot be impaired. Temperature is a constant 20°C, and the initial algal biomass is small, $\Sigma(M_i) = 2.5$ g. In (B) temperature increases over thirty days from 20 to 25°C. This final value is equal to the optimal growth temperature of the cyanobacteria (Rabouille, Salençon, and Thébault 2005). Toxin production is active, but the cells are healthy and do not excrete toxin. During (C) cyanobacteria both produce and excrete toxin...
(10^{-4} \text{ g/g/hr}). Temperature linearly increases from the optimal value at the end of (B) to a high treatment of 30^\circ C. Finally, in late blooms (D) toxin production stops, while toxin excretion remains 10^{-4} \text{ g/g/hr} to account for cell lysis. Temperature remains a constant 30^\circ C. Fish particles across all experiments use random initial horizontal positions and angles. In each experiment there are 200 fish particles divided between two groups. Half the particles have a fixed depth of −0.1 m, and the other half −4.9 m. Fish state variables at the start of all experiments are identical.

\section*{2.3 Results}

\subsection*{2.3.1 \textit{Microcystis} Growth and Buoyancy}

The discussion chapter will focus on the interaction of fish and cyanobacteria. Here I comment on the behavior of the sub-models, when it helps interpret the results. The following result summary section covers short and long term growth, carbon ratio trends, and the resulting movement of cyanobacteria. During the control experiment (A) the total cyanobacteria protein mass in the lake, Σ(Mi), increases from an initial value of 2.5 g to 2.898\cdot10^6 g (Table 2.5). This sixth order increase in magnitude agrees with the anecdote that cyanobacteria can quickly choke a water body, seeded from very low standing stocks. Mean particle mass per unit lake volume increases exponentially (Figure 2.4), to a final value of 0.044±0.016 g m$^{-3}$. This consists of a diel fixation signal superimposed on the long term protein growth curve. Initially all intra-experiment particles have identical state variables, but their radii and random diffusion introduce differential net growth, resulting in increasing standard deviation.

| Table 2.5 Tracer Masses—The initial and final mass of relevant chemical pools for each type of experiment. These values are used to calculate the chemical concentrations (g/m$^3$) listed elsewhere. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                  | Total Protein (g) | Total Cell Toxin (g) | Total Dissolved Toxin (g) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Control (A)                     | Initial | Final        | Initial | Final        | Initial | Final        | Initial | Final        |
| Growth (B)                      | 2.898\cdot10^6 | 2.502\cdot10^7 | 0.0     | 9.826\cdot10^5 | —       | —             | —       | —             |
| Active (C)                      | 2.502\cdot10^7 | 2.461\cdot10^7 | 9.826\cdot10^5 | 9.826\cdot10^5 | 0.0     | 3.162\cdot10^5 |
| Crash (D)                       | 2.461\cdot10^7 | 2.351\cdot10^7 | 9.826\cdot10^5 | 7.967\cdot10^4 | 3.162\cdot10^5 | 4.401\cdot10^5 |

The carbohydrate to protein ratio in the control experiment has low standard deviation over the first 15 days (Figure 2.5) because the particles are constrained at the sediment, in a favorable light field, with too little
biomass to shade each other. If attenuation due to turbidity were increased, growth would be slower, but resuspension would occur more quickly because the limited light would not allow the accumulation of carbohydrate ballast. The initial jump indicates that neutral buoyancy within the limited vertical domain is not a stable condition—unlimited by light, the only stable position is at the sediment. This period is the spin up period for the biological model. As resuspension and self-shading kick in toward the end of the control (see Figure 2.6), variance grows and the oscillation of mean ratio attenuates (Figure 2.5). The final mean carbon ratio is 0.917±0.283, with a maximum of 1.063 during the final day of the simulation.

In experiment (B), total initial protein equals that at the end of the control (A), 2.898·10^6 g. Essentially, the bloom is allowed to continue from the previous state. Cyanobacteria grow over 30 days to a total protein mass of 2.502·10^7 g, about a factor of ten greater (Table 2.5). The mean particle biomass increases toward a saturation level around 0.235±0.105 g/m^3; the standard deviation again widens based on particle radius and mixing (Figure 2.4). Carbon ratio has high variance in the first 15 days of the growth experiment (Figure 2.5) as particles begin the migration to the surface (Figure 2.6). When movement is unconstrained by boundaries, the vertical distribution of cyanobacteria particles is more random (e.g. near t=10 days). Carbon ratio variance decreases around day 17 as

---

**Figure 2.4 Mean Colony Biomass**—Each cyanobacteria particle starts with the same carbon pool, so the initial standard deviation is in all cases zero. Diel oscillations in carbohydrate are superimposed over a continuous protein growth curve. Lines show the trend in the mean sum of particle carbohydrate and protein, divided over the simulation domain, for the corresponding experiment type. The colored regions are plus/minus one standard deviation. The initial and final values do not line up because protein, Σ(M), is preserved between experiments, while total carbon, Σ(C+M), is not.
particles begin to collect at the surface, then increases slightly and remains nearly constant as particles find an equilibrium state. The mean ratio decreases over time, inverse to the temperature trend, to a final midnight value of 0.146±0.086, with a maximum of 0.215 in the final day of the experiment.

Rising temperature in experiment (C) encourages carbon excretion and respiration, which over time reduces biomass. From an initial value of $2.502 \times 10^7$ g, the total cyanobacteria protein declines by 1.6% to $2.461 \times 10^7$ g (Table 2.5). The mean particle biomass decreases to $0.206 \pm 0.165$ g/m$^3$ (Figure 2.4). Were temperature to remain at the 25°C optimum of the B-C transition, the mean and total biomass should remain constant (after removing carbohydrate from the curve). The instantiation of particles with neutral buoyancy causes an opposite response to that seen in the control experiment (A). Because biomass is high, strong shading causes particle to collectively approach the surface as they convert initial carbohydrate to protein (Figure 2.6). Their only stable position is at the surface, which requires a midnight carbon ratio around $0.040 \pm 0.028$ (Figure 2.5). The maximum daytime carbon ratio in the last day of the experiment was 0.124.

Experiment (D) has a constant temperature of 30°C which, after a conditioning period, allows mean particle biomass to reach a stable daily oscillation near $0.198 \pm 0.158$ g/m$^3$ (Figure 2.4). Cyanobacteria protein declines 4.5%
from $2.461 \cdot 10^7$ to $2.351 \cdot 10^7$ g (Table 2.5). The high standard deviation of the mean particle value is due to larger colonies having greater buoyant velocities, which overcomes the downward mixing of turbulence. At the surface the random walk direction can only be negative. If a particle's upward velocity is not great enough it will lose its place at the surface, and be shaded by others. As colonies reach the surface during the eighteenth day (Figure 2.6), and the carbon ratio quickly stabilizes near $0.042 \pm 0.029$ (Figure 2.5). This is nearly equal to the state at the end of (C) since the temperature is a constant and equal to that at the end of the previous experiment.

![Figure 2.6 Mean Colony Trajectory](image)

**Figure 2.6 Mean Colony Trajectory**—Shows the mean particle trajectory for each experiment and the standard deviation range. Trajectory (A) shows the gradual resuspension of particles from the sediment during the control. The red line (B) shows the turbulent distribution of a growing and unlimited group. Lines (C-D) show fully developed surface scums in late stage experiments, with only very small oscillations.

### 2.3.2 Bloom Toxicity

No toxin is produced or excreted during the control experiment (A). Production becomes active during (B) but excretion remains zero. The total intracellular toxin in the domain at the (B-C) transition reaches $9.826 \cdot 10^5$ g (Table 2.5), with a mean particle toxin concentration of $8 \pm 2$ mg/m$^3$. Since toxin production is constant, variation is due to the differences in the protein mass of each particle. Ultimately, since *Microcystis* particles within a single experiment are initially identical but for radius, toxicity is a function of size. There are two distinct phases of toxin mass accumulation: exponential while biomass grows; transitioning to linear as growth saturates. The mean toxin to
protein ratio ($\gamma/M$) is more biologically relevant than total toxin per colony, this increases exponentially to $0.047 \pm 0.024$ g/g (Figure 2.7).

Production and excretion are equal for experiment (C), so particles have constant intracellular toxin concentration equal to 8 mg/m$^3$, and total intracellular toxin mass of $9.826 \cdot 10^5$ g (Table 2.5). Total biomass is in decline due to the temperature ramping, so the potency of cyanobacteria cells—expressed as the toxin to protein ratio—increases from 0.047 to a final state of $0.202 \pm 0.322$ g/g (Figure 2.7). The standard deviation here is larger than the mean because toxicity is positively skewed. Continuously light-limited colonies will eventually run out of carbohydrate and burn their protein. As this happens, initial toxin carried over from (B) drives up the ratio. Toxin excretion by established scum outpaces diffusion, so that dissolved toxin accumulates at the surface (Figure 2.8). The value at the surface is 0.232 g/m$^3$ at the end of experiment (C). The final vertical mean concentration of dissolved toxin is 0.025 g/m$^3$—about one tenth of the final surface value.

![Figure 2.7 Mean Colony Toxicity—Plot shows the mean ratio of toxin to total carbon, plus and minus one standard deviation. This value determines the dietary uptake of toxins by fish. Particles start with identical intra-experiment toxin pools, so standard deviation in all cases is initially zero.](image)

Toxin is excreted but not produced in the final experiment (D). Total intracellular toxin declines from $9.826 \cdot 10^5$ to $7.967 \cdot 10^4$ g (Table 2.5), more than one order of magnitude less. However, the toxin to protein ratio increases, as in (C), but at a reduced rate since the excretion rates of toxin and carbon are dueling (Figure 2.7). The average dissolved toxin concentration increases from 0.025 to 0.035 g/m$^3$ by the end of the simulation (Figure 2.8). Surface intensification is reduced—the mean is 32.2% of the surface value of 0.108 g/m$^3$.  

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2.3.3 Fish Intoxication

Each experiment, based on the formation and senescence of a toxic bloom, results in a characteristic fish group intoxication curve (Figure 2.9). The maximum mean toxin load, in proportion to body weight, is about 0.032 μg/g. The control experiment (A) curve is obviously flat zero. The mean toxin load for the formation experiment (B) increases monotonically over the entire thirty days—because there is no dissolved toxin, surface and demersal groups are not differentiated. The mean value surpasses the intoxication detection threshold (0.005 μg/g) after 10 days. It then continues to rise, with a continuously widening standard deviation envelope. The mean exceeds the impairment threshold (0.015 μg/g) after 25 days. The final mean value is the maximum, at about 0.02 μg/g. The mean plus the standard deviation crosses the intoxication detection and impairment thresholds at 7 days and 18 days respectively. At these critical points, most individuals exhibit the related behavior.

During the intensification experiment (C) the mean fish toxin load increases in both surface and demersal particle groups for the first 10 days, crossing the lower threshold after the first 3 days (Figure 2.9). After this point,
bottom dwellers approach depuration equilibrium, while the increasing surface concentration drives up the toxin load of those fish feeding at the surface. The uptake through ingestion gradually pushes the mean toxin load up over time against the competing depuration, though this occurs more slowly than in (B). The final value for surface fish is 0.032 μg/g, while the mean for the lower group of particles is 0.030 μg/g. The standard deviation is large, indicating that intoxication is prevalent, but does not effect all fish at all times.

The decline experiment (D) is characterized by early intoxication, tracking with the intensification experiment (C) for the first 3 days, followed by a decay in mean toxin load that falls below the detection threshold by the end of the experiment (Figure 2.9). Mean toxin load crosses the lower threshold in the first day, and the second threshold at the end of the third day. The mean toxin load of the surface group in this case lags behind that of the demersal by about a day. Both reach a maximum mean value of 0.017 μg/g, during days 6 and 7. This period has a large standard deviation, which converges with time as toxin loads decrease and fish recover. The mean for demersal fish drops below impairment level after 8 days, with those near the surface lagging behind at 11 days due to absorption in a high dissolved toxin regime. Because the dissolved toxin is not as surface intensified (Figure 2.8),
the difference in groups is not as pronounced as in (C). The two groups drop below the detection threshold on days 21 and 24, respectively. The final mean values are 0.003 and 0.004 μg/g. The mean plus one standard deviation drops below the lower threshold by the end of the experiment, which indicates most fish have returned to sobriety.

2.3.4 Fish Growth

Mean body weight at the end of all experiments was lower than that of the control (Figure 2.10). The fish in the control experiment (A) were the only group which successfully optimized foraging, indicated by an above average mean suitability ($G_x > 0.5$) during most of the simulation (Figure 2.11). During bloom formation (B) suitability was less then average for the entire experiment. Intensification (C) and decline (D) experiments had generally suboptimal suitability, with several successful periods. The outcome is a reduction in growth for (B–D) relative to that of the control (A). Early reductions in growth contribute to lower performance ($G_x < 0.5$) which persists through the experiment. This is a positive feedback loop—smaller fish swim slower, therefore they can't reach high growth areas as effectively, so they grow more slowly, therefore they can't reach high growth areas as

![Figure 2.10 Mean Fish Mass](image)

Figure 2.10 Mean Fish Mass—Fish in all experiments have the same initial mass. Growth in the control (A) experiment is greatest. During formation (B), toxin exposure is the same in both groups, so the mean is for all fish (N=200). In the intensification (C) and decline (D) phases, the fish constrained to the surface (dotted lines, N=100) had greater mean biomass than those near the bottom (solid lines, N=100). The yellow envelope is the range of possible growth curves, indicating a maximum relative growth suppression of about 50% (minimum over maximum).
effectively, et cetera. I will refer to this as structural impairment, because propagation is not through behavior or memory, but instead stored in the physical form of the organism. Fish performed poorly during bloom formation (B), with a 5.8% decrease in final mean body weight. During intensification (C)—which has similar conditions as the end of formation—the fish perform better than in (B). The final growth reduction for demersal individuals is 4.1%, while surface individuals only decrease 2.4%. Demersal fish in the decline experiment (D) have the lowest mean growth over the 30 days, at a 6.6% reduction in final mass, while the surface individuals have 5%. The greatest theoretical growth suppression in the domain is 51.7%—for fish which have no net growth, compared to fish with maximum net growth.

![Graph showing Mean Group Suitability over time.](image)

Figure 2.11 Mean Group Suitability—The mean suitability of the control (A) group of fish is above average (G_x=0.5, indicated by dashed black line) for most of the simulation. This demonstrates that sober movement rules lead to profitable places. During periods of extended intoxication in (C) and (D), fish in toxin enriched surface layer (dotted lines) experienced higher mean suitability than those near the bottom (solid lines). Fish in (B) had consistently below average suitability, which decreased after the onset of majority intoxication. Data, as depicted, are smoothed using a two hour, centered averaging window.

The spatial distribution of fish in the control experiment is noticeably different from that of fish experiencing avoidance or intoxication en masse. Figure 2.12 shows a subset of the fish particles in each experiment at t=22 and t=30 days. The former is a time when fish in the control (A) and those in the intensification experiment (C) are performing above average (G_x>0.5), while all other groups do poorly (G_x<0.5). By the end of the simulation, the fish in experiments A–C are performing at or below average (G_x≤0.5), while those in the decline case (D) are noticeably clustered in low-growth areas (Figure 2.12).
2.3.5 Residence Time

I've shown that the model produces periods of collective fish intoxication, and that the impairment this triggers reduces the final weight of individuals by up to 6.6%. Since I do not explicitly include metabolic costs associated with movement, or the depuration of toxin, the reduced growth potential results from spending more time in suboptimal regions due to impairment and avoidance. Residence time ($\tau$) describes how long, on average, a fish can be expected to remain in a patch (defined as a circle of pre-defined radius, centered at some reference point). Residence time and mean square displacement are inversely related, but serve distinct roles. Here I use the former when discussing the trajectories of individual fish. Figure 2.13 shows example combinations of growth, avoidance and impairment for several individuals, as well as the fraction of particles exhibiting those cues. Figure 2.14 focuses on a single individual during a one day window in each experiment. Overlaid is $\tau$ for that fish. Since data is output every 5 time steps, already 80% of the trajectory data is missing. Basically, each step is the total displacement after 6 minutes. I will show that transient cues occurring during lost steps can be identified in the residence time signal. Though movement has a stochastic component, averaging movement metrics over time and space obfuscates the

Figure 2.12 Fish Particle Positions—The left plot (A) shows a subset (N=50) of particle positions at $t=22$ days. Control particles (black) are slightly concentrated in the high-growth region defined by $125<X<375$, while the other groups occupy primarily the low-growth subdomain. The right plot (B) shows final particle positions. Decline experiment particles (blue) remain concentrated in the low-growth region, while the other particles are randomly distributed.
randomness—this produces a noisy square wave, the amplitude and timing of which indicate bouts associated with specific behaviors. This method is used, rather than explicitly plotting the discrete behaviors \((j,k)\), because direct information about selected behavior is not available in a natural system. On the other hand, it is possible to extract residence time signals from empirical telemetry data. Since these kind of models will need to be validated with movement metrics of real animals in the wild, I discuss how knowledge of position and movement relative to known environmental features can be used to interpret summary statistics like residence time.

Consider the example cues for the control experiment (A) shown in Figure 2.14 for the interval \(19.5 < t < 20.5\) days. The one day time window is normalized to \(0 < t < 1\) days. Corresponding trajectories are shown in Figure 2.15. These figures are cross referenced to show how the feedback between movement and behavioral cues. Notice that the entire path segment is located near the edge of the high suitability region, so the fish has a fast switching signal. The initial regime indicates selection of the default random walk \((j=0)\) with a relatively high \(\tau\), interrupted by transient encounters with high suitability, which trigger brief uses of the strategic search behavior \((j=2, k=1)\). This is axiomatic, since neither cue is present until the fish crosses into the region of high suitability. A several hour long searching bout results in the fish entering the profitable patch at \(t=0.4\). The feeding probability \((j=2, k=0)\) saturates as the particle skips back and forth across the threshold. Residence time increases and the individual stabilizes in the patch since the movement associated with feeding is slower and more random than that of searching. The maximum residence time for feeding (0.42 days) is only slightly greater than the default random walk (0.40 days). The minimum is 0.03 days. Residence time decreases as the particle moves back out of the patch \((t=0.8)\). This is evident in the trajectory, which loops away and then back toward the suitability threshold \((X=375)\). On the second encounter, \(\tau\) reacts more quickly because strategic event probabilities are already conditioned \((P>0)\). The lower plateau of the value during this foraging bout (and at the end of the previous) is due to movement along the axis of high suitability \((\Delta Y>\Delta X)\), during which random displacement may be near it's maxima for feeding behavior, without leaving the patch (Figure 2.15). During the period of greatest \(\tau\), the variance of the random deviates for the swimming angle happen to be greater, which keeps fish in the same area (seen in the sinuosity and local intensity of the trajectory). These intense periods of localized activity are punctuated by excursion bouts, during which the mean of the random deviates temporarily decreases, resulting in greater displacement as fish take several steps in the same direction before making a turn (Figure 2.15).
Figure 2.13 Example Cues for Four Individuals—Plots show behavioral cues over the length of each experiment. The uppermost plot of each color is the fraction of individuals detecting toxin (solid lines), and the fraction of individuals experiencing high growth, $G_x > 0.5$ (shaded regions). The four plots that follow show combinations of internal toxin cue ($\varepsilon_1$, solid lines) and suitability cue ($\varepsilon_2$, shaded) for four arbitrary individuals over 30 days. Cues take a binary state, one when an event occurs and zero when it does not. The vertical scale of each subplot is 0.0-1.0, and is unitless.
This pattern also occurs at the end of the (B) cue signal (Figure 2.14), with additional effects due to the presence of an intoxication cue. This interval, $28 < t < 29$ days, is selected from the second experiment to show how behavior changes during the onset of intoxication. The (B) trajectory during the first 0.5 days of the cue signal is entirely outside the area of high suitability (Figure 2.15). The multiple, noisy, levels of residence time are caused in this case by receiving transient intoxication cues—the fish surpasses the first threshold during lost time steps, which triggers avoidance, and a jump to lower suitability area where they immediately recover. Because of this, the intoxication cue does not show the event. As fish move up the suitability gradient, they ingest more toxin. Cyanobacteria toxicity also increases over time, so fish headed quickly in the wrong direction accumulate plenty of toxin. The switch in direction, accompanying the initiation of toxin avoidance, causes the fish to head down the suitability gradient. Fish swimming down the suitability gradient depurate toxin, unless the rate of increase of cyanobacteria toxin load outpaces the change in X position. Fish swimming along a path of isotoxic risk will have a constant level of intoxication if dissolved toxin and toxin load are also constant. Otherwise, it will depend on the

Figure 2.14 Example Residence Time—Plots show behavioral cues and residence time for one day in life of four individuals across the experiment types. The dashed lines show the toxin detection/avoidance cue ($\varepsilon_2$). The shaded regions show the habitat suitability cue ($\varepsilon_1$), which is one when $G_X > 0.5$, and otherwise zero. Solid lines are residence time, with a maximum of 0.87 days during the decline case (D). The vertical scale of all signals is 0.0–1.0. Cues are unitless, and the residence time unit is days.
contribution of absorption and ingestion. This is essentially a steering effect, causing fish to move toward lower toxin risk, until the baseline in the new area exceeds their detection, at which point another flight is attempted. This effectively moves a particle out of the affected area, which unfortunately for the small generic fish, is also the area of highest growth. Because of the small, doubly periodic domain, the total area existing down gradient decreases.

![Figure 2.15 Example Trajectories](image)

The shift to more consistent flight attempts in (B) is visible at \( t=0.2 \). The maximum and minimum residence times occur here—at 0.49 and 0.02 days respectively. At this point the intoxication event probabilities are conditioned, so that even transient toxin detection begins to trigger tactical avoidance \((j=1, k=0)\), followed by strategic avoidance behavior \((j=1, k=1)\). This period is characterized by sudden switches in direction at high speed, followed by slow movement away from the location of the last known event. At about \( t=0.55 \) days the fish encounters the suitability threshold (a transient encounter, so it does not appear in the cure signal). This cue is weighted ten times higher than avoidance, so the fish initiates search behavior and eventually enters the patch around \( t=0.7 \) days. This of course causes an increase in toxin ingestion, which triggers a persistent intoxication cue,
and the onset of impaired feeding. This fish is climbing the suitability gradient, so has mostly increasing intoxification. The peaks in residence time correspond to periods of impairment, where the turning angle is more random, and the speed decreases. If the fish begins to move down gradient it can recover briefly, leading to faster directed movement (lower τ), as seen at about t=0.95 days. Here avoidance successfully removes fish from the threat (toxin uptake). This is a naive response to an internal cue—fish which cannot detect toxin in the water or in their food can use the inherent knowledge of their own state to trigger avoidance. Knowing that something is wrong, but not exactly how to escape, an animal chooses the best option available: get away. If the risk is still detected, the fish tries again, until something changes. The result, in both this simulation and in laboratory studies, is periods of agitation and chaotic movement, which—overall—is ineffective at removing the individual from a generalized threat. As impairment kicks in, avoidance becomes less effective, since the initial jump is shorter, and the subsequent strategic behavior will be slower and more random.

The interval displayed for the intensification experiment (C) is \(3 < t < 4\) days. The toxin load of the fish during this period is always greater than the detection threshold (Figure 2.14). The initial behavior, until \(t=0.1\), represents an avoidance response. After this point, the fish enters the high suitability region (Figure 2.15), but fails to increase its residence time. This is caused by the de-optimization of the movement rules for patch finding. After leaving the patch, the fish moves down gradient, so returns to a switching state: alternating between impaired and sober avoidance behaviors \((j=1)\), and search behavior \((j=2, k=1)\). During the patch encounter after \(t=0.7\) days, residence time drops suddenly. This is due to the combined effects of fleeing and searching in sequence—as these are the only movement rules that result in bouts of fast directed movement. The fish successful increases it residence time during the next encounter, because impairment sets in during profitable feeding \((j=2, k=1)\). This try is more successful, and leads to a short search bout followed by prolonged feeding. The maximum residence time (0.42 days) corresponds to patch entry. The minimum is 0.02 days.

The interval for the final experiment (D) is \(6 < t < 7\) days. The event signal for toxin detection is one over the full 24 hours (Figure 2.14). The fish spends most of the time in the suitability patch (Figure 2.15). Initial encounters with high growth cues before \(t=0.2\) cause a switch to impaired feeding which, combined with impairment, induces higher residence time, following by a return to avoidance and search when the particle leaves the patch. During this period, minimum residence time occurs. The value, 0.06 days, is higher than minima in any of the other experiments, due to impaired movement rules. The fish successfully enters the patch during a period when the
toxicity of cyanobacteria colonies is in decline. There is a bout of sober foraging (since tactical foraging is weighted higher than avoidance), before impaired tactical foraging sets in after $t=0.8$ days. Residence time increases, and motion is more erratic. When the fish moves back down the suitability gradient, it shows noisy residence time indicating frequent switches between impaired and sober states. The maximum here is 0.87 days. Even in the case with the lowest mean growth (D) some fish—like the blue dot in Figure 2.15—are able to reach high suitability areas through the right combination of early movement and toxin uptake. Here, impairment kicks in and suppresses their movement.

Overall, growth is reduced in experiments where toxin is present, because successful search behavior leads to high ingestion, and triggers avoidance. The associated movement rules push the fish down the suitability gradient, so intoxication decreases, as does growth. Because intracellular and dissolved toxin are dynamic, the location of impairment (high or low suitability regions) will vary by individual, and the timing of growth suppression in the whole population shifts in response to bloom toxicity.

2.3.6 Behavioral Diffusivity

The mean square displacement for a single fish particle is a measure of how far it can be expected to disperse during a given interval, measured in length units squared divided by time ($m^2/hr$). This metric summarizes many output steps as a single expected distance ($N=40$) with an averaging window of 4 hours. The mean across all individuals in the group is the behavioral diffusivity ($K_F$). The mean of $K_F$ over some interval of time ($\bar{K}_F$) describes the collective motion during that period, and can be used to differentiate phases of group impairment. For this reason, each experiment (A-D) is broken into overlapping 10 day intervals, which ensures that collective threshold transitions appear in at least 2 of the 5 phases (I-V). Again, phase windows are, in order: 0–10, 5–15, 10–20, 15–25, and 20–30 days. The fish particles in experiments (C) and (D) are broken into two groups ($N=100$) based on their fixed depth (surface or demersal). $\bar{K}_F$ is calculated for each particle group during each phase.

Over the course of the control experiment (A), the mean value of $\bar{K}_F$ increases monotonically from $421\pm65$ m$^2/hr$ in Phase I to $511\pm52$ m$^2/hr$ in Phase V (Figure 2.16). The trend is due to more individual particles entering the profitable patch, growing in length, and therefore swimming faster. The standard deviation ranges 42–65 m$^2/hr$. Statistically selecting phase windows to minimize the standard deviation would be one method for identifying bouts of a particular behavior. However, standard deviation is only included to show the range of values
within the phase (not the variation among individuals). During the formation experiment (B) the behavioral diffusivity decreases monotonically from a Phase I value of $318 \pm 84 \text{ m}^2/\text{hr}$ to a Phase V value of $255 \pm 20 \text{ m}^2/\text{hr}$. The mean and deviation in Phase I are greatest, while the rest of the experiment is nearly uniform, since toxin load increases during the rest of the experiment, triggering avoidance and impairment behavior *en masse*. The initial values of $K_F$ in the intensification (C) and decline (D) experiments are similar, due the similarity of the intoxication curves (Figure 2.9). The surface and demersal categories in (C) have intoxication onset means of $229 \pm 48 \text{ m}^2/\text{hr}$ and $228 \pm 51 \text{ m}^2/\text{hr}$ respectively. The onset values for experiment (D) are $238 \pm 53 \text{ m}^2/\text{hr}$ and $234 \pm 46 \text{ m}^2/\text{hr}$. The slight intensification in $K_F$ for the surface particles in (D) is due to the 1 day lag in mean intoxication mentioned previously (Figure 2.16). However, the demersal particles recover more quickly from intoxication, and therefore

Figure 2.16 Mean Phase Diffusivity—Mean behavioral diffusivity is shown for each of five ten-day phases. The leftmost black bars are the control experiment (A). Red bars are the formation experiment (B). Intensification (C) is divided into surface and demersal (lighter bars) categories, and is shown in green. The same is true for bloom decline (D), which is blue.
have slightly greater behavioral diffusivity in Phases II and III. The maximum partitioning in (D) occurs in Phase II, where surface particles have $\bar{K}_f=230\pm25 \text{ m}^2/\text{hr}$ and the demersal particles have $\bar{K}_f=241\pm31 \text{ m}^2/\text{hr}$ (about a 4.5% lower at the surface). This pattern is true for (C), in which the depth partitioning begins instead in phases IV and V. The most severe case for (C) is in Phase V, where the surface value is $\bar{K}_f=231\pm27 \text{ m}^2/\text{hr}$ and the demersal value is $\bar{K}_f=253\pm27 \text{ m}^2/\text{hr}$ (8.0% lower at the surface). For both (C) and (D), the means generally increase with time—again due to length growth. The decline case (D) eventually surpasses the diffusivity value of (B) in Phase IV, as the fish recover from early onset intoxication. The final values for particle groups in (D) are 300±33 (surface) and 297±33 m$^2$/hr (bottom).

2.4 Conclusions

In the control (A) example (which only supplies suitability cues), an initial sober encounter with high suitability causes an increase in residence time during feeding, while transient detection of high suitability triggers an initial low $\tau$ search period (Figure 2.14). This behavior effectively steers the individual back toward the patch it just left. During subsequent encounters, the event probabilities are preconditioned, and the behavioral response occurs more quickly. It is also worth noting that the residence times of random walk and feeding are approximately equal, although motion during feeding will be more directional. These observations relate to Hypothesis 2 (sublethal behavioral impairment suppresses collective motion). Suppressing group motion increases residence time. The result is beneficial if a fish is already in a high growth area, but also decreases the efficacy of search bouts—meaning fish should experience lower overall suitability and therefore reduced growth (Hypothesis 1).

Experiments which do include toxin show that impairment—as I believe it should be represented—negatively impacts an individual's ability to maximize growth (Hypothesis 1). Transient detection of the toxin cue induces flight, which pushes the particle down gradient, away from high growth potential. When the fish has recovered, it exhibits persistent strategic avoidance ($j=1$, $k=1$), which increases residence time. This agrees with Hypothesis 2. Fish experiencing this phenomena will spend most of their time in low growth areas, and are continually pushed down gradient by the increasing toxicity of the bloom. Non-specific avoidance, originally intended to respond to a spatially-explicit mortality field, can successfully reduce intoxication state. This indicates that it is based on sound evolutionary principles. This is more likely in situ than true gradient following, since most fish cannot detect the concentration of dissolved toxins.
Fish which happen to enter the high suitability region (avoidance failure) also experience increased residence time, as movement becomes impaired. This results in high growth, and high toxicity, but these fish are outliers. Compared to the control, fish perform best under conditions that do not promote frequently switching behaviors. In other words, if intoxication is unavoidable, it is better for fish to get drunk and stay drunk. This is supported by additional experiments which show increased growth when the algorithms are changed to enforce greater residence time and faster intoxication (Appendix B). These cases also underlined the sensitivity of the model to the selection of behavioral parameters. Within the framework of the first four experiments, both hypotheses were shown to be conditionally true. That is, the simulation produced the expected phenomena of suppressed movement and growth. Supplementary experiments which modified the methods and parameters of the model often produced higher growth, indicating that the results were specific to the model formulation. Determining where exactly within the parameter space these hypotheses are proved or refuted will require further investigation.

Generally, swimming impairment and agitation cause fish to spend less time in high growth areas. This results in smaller fish in the primary cases (Hypothesis 1), which have a reduced swimming speed, and can not traverse a gradient as efficiently as healthier members of the cohort. This feedback persists through each experiment. It impacts the relationship of absorption and ingestion, because the volume-area ratio does not change as quickly. This is called structural impairment, because it is permanently stored in the maintenance and locomotion variables of the individual. The existence of lasting size-partitioning invites the inclusion of both predators and reproductive success in the model. Both would be easy additions, and would extend the model description in such a way that toxin could be transferred to higher trophic levels, and behavioral parameterization accomplished through the application of genetic algorithms (Watkins and Rose 2013). Translating these basic principles from a theoretical exercise to a predictive model will require a systematic study of movement metrics for fish in afflicted areas. Based on currently available knowledge, this framework is a tool for conducting sandbox experiments intended to inform large-scale modeling and experimental design for field studies.
CHAPTER 3. DISCUSSION AND SUMMARY

3.1 Multiple Trophic Levels

I’ve described the spatial fate of toxins, by deriving an effective diffusivity for a group of moving fish particles. Some of these individuals will be eaten, and pass on toxin to the predator. Although MC is not fat soluble, it can concentrate in predator livers and propagate up the food web (Ibelings and Havens 2008). Protein-bound toxin has longer residence time, and will disperse to new locations during migrations. Toxic blooms occur in productive areas, where marine and estuarine fish thrive (Lefebvre, Trainer, and Scholz 2004; Samson, Shumway, and Weis 2008). This invariably results in the transfer of toxin to high trophic levels, including targeted commercial fisheries, which can be vectors for human poisonings. While the behavior of a small generic fish seems in some ways insignificant, it is the foundation for a richer virtual ecosystem. Feedback between growth and toxin uptake could lead to size partitioning in the population, as smaller individuals are preferentially consumed by mobile predators. Of course, the predators are expending energy to catch the smallest, most toxic morsels, and could accrue toxins quite rapidly. Transfer relies on behavior, of both the predator and prey, which may be impaired.

The methods here are flexible enough to be adapted to include any number of unique species, so long as the number of individuals is kept within computational restraints. Unfortunately, the particle tracking code is sequential, meaning that there is no advantage to using modern high-performance computing resources which leverage parallelization and hybrid GPU systems to speed up computation. The framework itself can be adapted for parallelization if a particle topology system were in place. So, there are shortcomings and strengths to the approach, and the following is a discussion of how it will need to be improved before further application.

3.2 Cyanobacteria Methods

The control experiment is used to test convergence of the differential equations of cyanobacteria motion and mass transfer as the integration step approaches 0.02 hours. Since carbon transfer depends on the light field at the particle position, accurately representing the trajectories is imperative. These additional test cases were also run for 30 days. Doubling or quadrupling the number time steps by setting the interval to 0.01 or 0.005 hours does not change the solutions. In all cases the cyanobacteria converge at the bottom, indicating the model is not sensitive to the initial position of individual particles. Particles are constrained at the bottom until near the end of the experiment, when they resuspend. Solutions for each time step during this phase track closely, demonstrating further
that 0.02 hours is a reasonable value. Neither are the solutions sensitive to the changes of the initial biomass or position. Initial depth was either uniform through the water column, or set at the sediment or surface. Total biomass ($\Sigma M/A$) was initialized with values of $2 \cdot 10^{-2}$, $2 \cdot 10^{-4}$, and $2 \cdot 10^{-6}$ g/m$^3$ for each positioning scheme. The initial carbon ratio is always set to make the particle neutrally buoyant, which enforces a first derivative of zero for the equation of motion. With low biomass per volume the water column is relatively clear and the cyanobacteria are able to fix carbohydrate. When the initial distribution is random particles initially float toward the surface as they synthesize cell material, then crash to the bottom when light levels are sufficient for fixation. As biomass increases self-shading causes carbon fixation to drop off. The conversion of the existing stock to protein decreases the density of the colonies and they are gradually resuspended. When they become neutrally buoyant turbulent mixing has its greatest effect so the vertical distribution becomes more random. Greater initial biomass increases shading and instigates earlier resuspension. The growth is of course greater when the initial biomass is high, but the process will eventually saturate with constant forcing. The instantiation of particles has no effect on the overall behavior. The difference in the mean depth trend for the first 6 hours of darkness is due to boundary interactions and is predicted by the diffusion model. Particles at the bottom have constrained positive motion, so their mean increase in depth must be greater than for a random distribution. When the particles begin at the surface, their motion is constrained to the negative direction. The total carbohydrate and protein per volume increases exponentially. Growth does not saturate for any initial biomass tested, which is desired in bloom formation phase as rising temperatures in the following experiment will speed growth drastically.

Alternate particle rules should be defined for *Anabaena*. The buoyancy mechanism will be different, and hydrodynamic properties of the filamentous colonies will result in periods of competition for light and nutrients. Speaking of nutrients, the vital rates of organisms should depend on nutrient availability. The cycles should be explicit, and conserve mass and stoichiometry. A sound empirical toxin excretion rate must also be established. I could not find a study which expressed confidence in the ability to track and conserve total toxin across intracellular and dissolved pools at regular enough intervals to estimate an instantaneous rate. Cyanobacteria could be true super-individuals, with an additional variable representing the number of colonies. A consistent particle count would be maintained by removing individuals that dropped below a biomass threshold, or which left the simulation domain. If particle descriptions also include age and mortality rate, they could die off based on empirical cell longevity. The intracellular toxin of dying cells would be added to the dissolved phase, and result in catastrophic surface
intensification. The decomposition of their biomass may also lead to hypoxia or anoxia. The intermediate organic matter and dissolved toxins should be subject to advection and diffusion. Except of course if they are sequestered in the sediment, which may adversely impact benthic invertebrates. Toxic cells may also fall out of suspension, as active cells or in dormant stage known as an *akinete*. Blooms in shallow systems may be the result of benthic inoculation, or growth of a small pelagic stock (Schöne et al. 2010). Shellfish, Louisiana oysters for example, may accrue a potent toxic load. Carcasses of the unlucky denizens of an afflicted waterway will feed the scavengers, and propagate toxins to higher trophic levels. I believe that heterogeneous particle simulations which include diverse taxa covering basic ecological niches are a logical next step.

### 3.3 Physics and Hydrodynamics

Realistic forcing conditions and a meaningful hydrodynamic scheme are necessary before the model can be said to have any predictive capability. All physical fields and processes might benefit from embellishment, but at this point additional physical complexity only serves to obfuscate the mechanistic movement of cyanobacteria particles and the stochastic movement of fish particles. Real world velocity is not uniformly zero. In fact, species in Louisiana are in the hands of civil engineers. Mississippi River flood control structures, like at Davis Pond, release water into estuaries to combat salinity intrusion, replenish sediment and increase fishery production (Ren et al. 2009; Das et al. 2012). Diversions force the salinity gradient seaward, evoking an ecosystem response on the scale of weeks (Piazza and La Peyre 2007; Rose, Huang, and Justic 2014). Salinity modulates toxin dynamics, and limits the range of both fish and cyanobacteria. Its inclusion will be required to test exposure of fish species favoring overlapping salinity regimes. High volume discharge shortens residence time and mixes the water column. This can interrupt a bloom and flush toxic biomass from the system; however, it also transports toxins to otherwise unaffected regions (Redden and Rukminasari 2008; Das et al. 2012). Additionally, the photoperiod should shift with time, and cloud cover will contribute a random and seasonal signal. Temperature and salinity (and density, as a result) should not be spatially uniform. A thermocline will certainly exist. The stratification will be interrupted regularly by thunderstorms and increased flow. Diffusivity too will matter.
3.4 Dynamic Energy Budget

The physical and chemical components of the model presents a signal rich environment for mobile organisms—in this case fish. Some factors will effect metabolism, some behavior. The best way to determine the relative importance of responding to a certain cue will be to expand the particle description to include the reproductive worth, and repeat experiments over many generations, each of which inherits optimized parameters from the upper echelon of individuals in the previous iteration (e.g. a genetic algorithm). Fish should also starve, die, and meet all manner of horrible end. They should also have explicit motivation to their behavior. A bioenergetic budget would give each action a metabolic cost, and behavior selection could be based on the balance of carbon intake and toxin risk. Toxin that does enter the organism should be partitioned between the gut, tissue, liver, brain, et cetera—as fine a scheme as our current understanding allows. A non-lethal laboratory method for estimating instantaneous depuration and absorption will be extremely valuable in this endeavor. Intoxication should impair sensing, which might change detect thresholds for both concentration cues and distance cues. Predator and prey encounters will change, as will a group's ability to coordinate collective movements like swarms and schools and bait balls.

Moving forward, it is imperative to adhere to the principles of dynamic energy budget (DEB) theory. DEB theory, as described in the seminal paper by Sousa et al. (2010), advises how biological models ought to be formulated. It emphasizes reducing the model system to as few parameters and equations as possible, and basing these on generic measures of metabolism in the individual. I want to evaluate the strength of this model so far, based on several statements from the article. First: "model assumptions should be consistent with empirical patterns". The cyanobacteria buoyancy model is based on empirical data. The toxin model is parameterized with constants based on very limited data. This conforms with the order of preference for types of functions: constant, linear, nonlinear. For lack of a systematic study of toxin excretion, I chose the first of that sequence. Fish growth is based on empirical data for forage fish, and used as generic place holder awaiting more advanced representation. Virtual fish movement and impairment is qualitatively related to the real thing, as seen in laboratories and the wild. Fish toxin dynamics are hypothetical values that produce expected phenomena, which should not satisfy the discerning scientist. This shortcut is okay at the current stage of model development, because the hypotheses only rely on the qualitative effect of impairment, not the how and when of its occurrence. Any quantitative application will require either some daring assumptions or rigorous laboratory trials.
Second: "predictions should be testable in practice, which typically constrains... maximum complexity". This is the current limitation of the field. Fish behavior in the wild is still cryptic, but acoustic tracking and fine-scale sonar imaging are contributing more and more to empirical movement metrics. Quantifying fish movement on multiple scales during a toxic bloom would give the necessary data for validation of fish trajectories. Third: "a model applicable to particular taxa... should... be consistent with an explicit evolutionary scenario" and "explanatory models have to be lean, capturing taxon-specific phenomena in modules that extend the non-taxon-specific core". Fish are cases of mixed structural-acquisition homeostasis. They are isomorphic, meaning that they have a consistent shape, but that their length-to-mass ratio changes over time (and influences the uptake of toxin). However, growth more closely resembles acquisition homeostasis, in that fish exhibit a variety of behaviors, while growth rate itself is constrained to a narrow range of values. These assumptions should true for most fish, and the model can be re-parameterized for unique species. The buffet of explicit behaviors, of course, may be different in each species. Fourth: "The set of parameter values... [is] individual specific". My model does not satisfy this condition, which is rooted in the drift in inherited traits between generations. Microcystis do have particle-specific radii, but otherwise all individual rates are equal. Including inherited (genetic) parameter selection would satisfy this.

3.5 Summary

The sublethal behavioral effects of algal toxins like microcystin on wild populations of fish are poorly understood. This research synthesizes existing field, lab and modeling studies to build a framework for examining how fish and cyanobacteria interact. These experiments accomplish several important tasks. First—they show that in shallow, eutrophic water bodies, with low mixing, Microcystis self-shading leads to dense buoyant scums. Toxin becomes surface intensified, meaning that fish at different levels in the water column will have varying exposure to particulate and dissolved toxins. In choosing between behaviors, fish must evaluate risk. That is, high growth areas may result in greater toxin uptake. Non-directional avoidance may drive fish out of good foraging areas, which can result in lower growth. The detection threshold, and weight of behavior utility, will determine exactly how much time is spent on each action. Frequently switching between behavior classes is detrimental to the fish. Finally, the combination of directional switching and more random movement increases residence time for intoxicated fish. In cases without flight, increased residence time in highly profitable places instead produces increased growth. This effect on collective movement can be summarized as an effective diffusivity, translatable to Eulerian distributions.
REFERENCES


Fox, Cynthia Nichole. 2010. “Seasonal Abundance, Age Structure, Gonadosomatic Index, and Gonad Histology of Yellow Bass Morone Mississipiensis in the Upper Barataria Estuary, Louisiana.” Nicholls State University.


APPENDIX A. EQUATION FORMULATION

Stokes Velocity Substitutions (Equation 2.3)

\[ \rho_{\text{cell},i} \text{ kg m}^{-3} = \rho_{\text{cell},\text{min}} \text{ kg m}^{-3} + (\rho_{\text{cell},\text{max}} \text{ kg m}^{-3} - \rho_{\text{cell},\text{min}} \text{ kg m}^{-3}) (1 - e^{-0.7 \text{ Ci/Mi}}) \]

\[ \rho_{\text{cell},i} = 1037 + (1150 - 1037) (1 - e^{-0.7 \text{ Ci/Mi}}) \]

\[ \rho_{\text{cell},i} = 1150 - 113 e^{-0.7 \text{ Ci/Mi}} \]

\[ \rho_{i} \text{ kg m}^{-3} = (1 - \%_{\text{cell}}) (\rho_{S,T} \text{ kg m}^{-3} + 0.7 \text{ kg m}^{-3}) + \%_{\text{cell}} ((1 - \%_{\text{vesc}}) \rho_{\text{cell},i} \text{ kg m}^{-3} + \%_{\text{vesc}} \rho_{\text{vesc}} \text{ kg m}^{-3}) \]

\[ \rho_{i} = (1.0 - 0.25) (\rho_{S,T} + 0.7) + 0.25 ((1.0 - 0.08) \rho_{\text{cell},i} + 0.08 * 150.0) \]

\[ \rho_{i} = 0.75 \rho_{S,T} + 0.525 + 0.23 \rho_{\text{cell},i} + 3.0 \]

\[ \rho_{i} = 3.525 + 0.75 \rho_{S,T} + 0.23 (1150 - 113 e^{-0.7 \text{ Ci/Mi}}) \]

\[ \rho_{i} = 268.025 + 0.75 \rho_{S,T} - 25.99 e^{-0.7 \text{ Ci/Mi}} \]

\[ \delta Z/\delta t \text{ m h}^{-1} = (3600 \text{ s h}^{-1}) (2/9) (9.81 \text{ m s}^{-2}) (R_{i}^{2} \text{ m}^{2}) (\rho_{S,T} - \rho_{i} \text{ kg m}^{-3}) (\eta_{S,T}^{-1} \text{ kg}^{-1} \text{ m}^{2} \text{ s}^{2} \text{ m}^{-1}) \]

\[ \delta Z/\delta t = (3600) (2/9) (9.81) (R_{i}^{2}) (\rho_{S,T} - \rho_{i}) (\eta_{S,T}^{-1}) \]

\[ \delta Z/\delta t = (800) (9.81) (R_{i}^{2}) (\eta_{S,T}^{-1}) (\rho_{S,T} - 268.025 - 0.75 \rho_{S,T} + 25.99 e^{-0.7 \text{ Ci/Mi}}) \]

\[ \delta Z/\delta t = 7848 R_{i}^{2} \eta_{S,T}^{-1} (0.25 \rho_{S,T} - 268.025 + 25.99 e^{-0.7 \text{ Ci/Mi}}) \]

\[ \delta Z/\delta t = 1962 R_{i}^{2} \eta_{S,T}^{-1} (\rho_{S,T} - 1072.1 + e^{4.644 - 0.7 \text{ Ci/Mi}}) \]

\[ \delta Z/\delta t = 1.103625E-5 \eta_{S,T}^{-1} (\rho_{S,T} - 1072.1 + e^{4.644 - 0.7 \text{ Ci/Mi}}) \text{ for } R_{i} = 7.5 \times 10^{-5} \]
APPENDIX B. SENSITIVITY OF GROWTH

Although the mean mass of fish was reduced for the four main simulations, I tested several modifications to the fish algorithms which resulted in increased growth. The mean final mass for these additional cases is shown below in Table B.1. These were all run as altered instances of the Formation (B) experiment, meaning only ingestion was involved. In four experiments, fish particles were instantiated with $G_X=0$ (meaning also that $X=0$). Likewise 50% lower depuration was used, and 44% slower impaired swimming speed. Three final experiments looked at enforcing only the default behavior, eliminating flight behavior, and multiplying toxin ingestion by a factor of ten. In all of these except one, growth is increased relative to the control. All are greater relative to the Formation (B) case. Setting initial $G_X$ to zero during monotonically increasing toxicity means that fish will be getting intoxicated without a lower risk area to flee to. Impairment should set in early, and we see that the additional effect of lowering the depuration is negligible. The change to the parameterization results in a slightly increased growth. The greatest relative growth was for slower swimming, and intensified by lowered depuration. This indicates that under some conditions, increased residence time due to intoxication in high profitability areas can increase growth when physiological effects are not considered. Overall, this suggests that the model is sensitive to the parameterization, and to the timing of intoxication. Empirical values will be needed for the approximated parameters, and more encompassing cross-factorial experiments conducted to understand the behavior of the model.

Table B.1 Additional Growth Experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mean Mass</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial $G_X=0$</td>
<td>16.76</td>
<td>100.8%</td>
</tr>
<tr>
<td>Initial $G_X=0$, 50% depuration</td>
<td>16.79</td>
<td>101.0%</td>
</tr>
<tr>
<td>Initial $G_X=0$, 44% slower impaired swimming</td>
<td>17.24</td>
<td>103.7%</td>
</tr>
<tr>
<td>Initial $G_X=0$, 50% depuration, 44% slower impaired swimming</td>
<td>17.27</td>
<td>103.9%</td>
</tr>
<tr>
<td>44% slower impaired swimming</td>
<td>17.82</td>
<td>107.2%</td>
</tr>
<tr>
<td>50% depuration</td>
<td>17.26</td>
<td>103.9%</td>
</tr>
<tr>
<td>50% depuration, 44% slower impaired swimming</td>
<td>18.13</td>
<td>109.1%</td>
</tr>
<tr>
<td>Only default behavior</td>
<td>16.49</td>
<td>99.2%</td>
</tr>
<tr>
<td>No flight behavior</td>
<td>17.49</td>
<td>105.2%</td>
</tr>
<tr>
<td>10x ingestion</td>
<td>17.56</td>
<td>105.7%</td>
</tr>
</tbody>
</table>
APPENDIX C. SOURCE CODE

Source code omitted. Please contact the author at nicholas.keeney@gmail.com to request a copy.
VITA

Nicholas Keeney received his B.A. in Marine Science through Boston University and Sea Education Association, and went on to study electrical engineering before transferring to LSU. He has worked for the New England Aquarium, Pew Charitable Trusts, and as a vessel technician for Louisiana Universities Marine Consortium. His research interests include biological-physical interactions and computational ecology. Nick is a Master's degree candidate for May 2017. He currently manages the Bowdoin College marine laboratory at the Coastal Studies Center in Harpswell, Maine.