UNIVERSITY OF CALIFORNIA,
IRVINE

Human impacts on the Near Coastal Marine Environment

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Earth System Science

by

Jessica Ann Walden

Dissertation Committee:
Professor J. Keith Moore, Chair
Professor François Primeau
Professor Ellen Druffel

2023

DEDICATION

To:

The intelligent women who came before me,

especially my mother and grandmothers.

"Even if you never have the chance to see or touch the ocean,

the ocean touches you with every breath you take,

every drop of water you drink,

every bite you consume.

Everyone, everywhere is inextricably connected to,

and utterly dependent upon,

the existence of the sea."

Dr. Sylvia Earle

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ACKNOWLEDGEMENTS

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Thank you to Professor Claudia Czimczik, for her guidance and assistance during my last year, and to Dr. Peggy Fiedler, whose encouragement and faith made it all feel possible.

Thank you to my family, especially my husband, Chris, for supporting me through all the ups and downs over the last 5 years and being my partner in life, in the lab, and in plastic-free shopping. And finally, to my mom, for giving me perspective, reminding me of my capabilities, and for always being my fiercest advocate.

VITA

Jessica Ann Walden

EDUCATION

2023
Ph. D. Earth System Science
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2017
Master of Science in Oceanography and Marine Environments
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B.S. Marine Biology, Cum Laude, with Honors
University of California, Santa Cruz

AWARDS

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Ecosystem Management and Environmental Change Certificate

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UCI Ridge 2 Reef Fellowship Recipient

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University of California Regents Scholarship

TEACHING

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Docent Trainer, 2012
Seymour Marine Discovery Center
Organized classes and files, set up and broke down classroom, aided with teaching materials and presentations.

RESEARCH

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Kelp Forest Ecology Field Quarter, University of California, Santa Cruz
Designed and implemented an observational study of algal community diversity as a function of wave exposure, depth, and relief within Hopkins Marine Reserve; collaborated on scientific paper with three other individuals.

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Marine Ecology Field Quarter; University of California, Santa Cruz
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2012
Big Sur Wildlands Project; Wildlands Studies
Conducted surveys of sea otter communities, Southern Steelhead communities, and plant communities within the reserve.

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Women in Science Program; USC Wrigley Marine Science Center
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INTERNSHIPS

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5 Gyres, Marcus Eriksen
Separated and classified microplastics from ocean samples taken from the Persian Gulf to add to a global model of plastic circulation in the ocean.

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In preparation.

Walden, Jessica; McGuire, Christopher; Terriquez, Laura; Robertson, George; Taveras, Joana; Mackey Katherine. "Seasonal and spatial variability of oceanographic and biogeochemical parameters in the coastal Southern California Bight."
In preparation.

Jessica Walden, Katherine RM Mackey, Araceli Serrano, Priya Kaur, Christopher McGuire, Erick Partida, Bradley Nussbaum, and Doug Gibson. "Tidal and diel drivers of biogeochemistry and mercury cycling in a Southern California estuary."
In preparation.

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Human influence on the marine environment, oceanography, earth system science.

ABSTRACT OF THE DISSERTATION

Human Impacts on the Near Coastal Marine Environment

by

Jessica Ann Walden

Doctor of Philosophy in Earth System Science

University of California, Irvine, 2023

Professor J. Keith Moore, Chair

Humans, as all members of the animal kingdom, have always interacted with the natural environment. However, as populations grew exponentially due to agricultural and technological advances, their impact on ecosystems has become disproportionate. Anthropogenic influence on coastal marine environments has detrimental effects on ecosystem balance and function. Negative impacts of human activity can be referred to collectively as pollution. Sources of pollution include, but are not limited to, chemical waste, human effluent, plastic, and heavy metals. In this text, I explore the impacts of effluent and microplastic on the marine environment, within the context of annual and diel biogeochemical cycles.

I investigated bottom-up control on phytoplankton growth over two seasonal cycles in the Southern California Bight off the coast of Orange County. I measured nutrient concentrations, in addition to a suite of biogeochemical variables, to assess the dynamics of primary production. I observed moderate Spring nutrient pulses and corresponding high chlorophyll concentrations. Higher nutrient concentrations were attributed to weak coastal

upwelling and a strong Pineapple Express event that contributed a significant pulse of nutrients from shore. Through this I demonstrate that isolated heavy rain events carry the capacity to cause punctuated algal blooms in low-nutrient environments. During Summer, I found strong nutrient drawdown, rendering the upper layer nutrient-deplete. The water column was highly stratified, indicated by both high temperature and salinity signatures. During Fall and Winter, the water column was less thermally stratified, and we observed lower salinity in the upper layer.

I found that wastewater effluent can impact already anthropogenically altered coastal environments, by providing a constant influx of nutrients. Throughout the year, there is a constant influx of wastewater input from the Orange County Sanitation District outflow pipe. Though the pipe has multiple ports, to allow for more efficient diffusion into the seawater, it is difficult to distinguish where the influence of the wastewater effluent ends. A reference sampling site was chosen for comparison with the effluent samples; however, it is unclear whether the reference site is truly outside the influence of the wastewater outflow.

I explored the intricacies of the interactions between microplastic and marine invertebrates and note that microplastic will differentially impact species depending on habitat, feeding strategy, and size. Marine species who inhabit the water column will be most impacted by neutrally and positively buoyant plastic particles, as they are more likely to come into contact. In addition, species who passively filter the water column will ingest more plastic than species who have the capacity to selectively feed and reject microplastic. However, once microplastic enters the marine environment, it is rapidly colonized by microbes. These microbes may make plastic more appealing, fooling marine invertebrates

into thinking they are food particles. Though the particles themselves may rapidly exit an organism, the chemicals embedded within microplastic could adsorb into the digestive tract during passage. In addition, the effect of plastic particles on fecal pellet sinking rates may have significant impacts on carbon export by decreasing sinking rates.

In addition, I show that a marine zooplankton, Artemia salina, does indeed ingest microplastic. As a passive feeder, A. salina will ingest more microplastic as concentration in the environment increases. However, ingestion rate will saturate after 90 minutes of exposure to high concentrations of microplastic. Further, I develop and refine methodologies for studying microplastic ingestion for chitinous zooplankton in situ. The only successful digesting agent was nitric acid (HNO_3) at 50% dilution and 80ºC. Dissolution on plastic or GF/F filters was not possible, because the acid destroyed the vessel. In addition, neutralization of the solution post-digestion was not possible due to the formation of a hydrogel after the addition of NaOH and K_2HPO_4.

Finally, I examine the diel dynamics of biogeochemical variables and mercury in a tidally influenced estuary in Southern California. I found that only a few samples contained detectable mono methylmercury concentrations—all at low tide, indicating the estuary had a high flushing rate. Therefore, the diel dynamics of MMHg could not be analyzed. However, I found that dissolved oxygen and chlorophyll concentrations were highly dependent upon the dominant source of water at the time, with higher DO at high tide and higher chlorophyll at low tide.

CHAPTER 1

Introduction

Marine ecosystems consist of complex interacting biology between and across trophic levels. The food web can be visualized as a semi-hierarchical structure, with higher trophic levels grazing upon lower trophic levels. However, biomass and activity can be controlled via top down (i.e. grazing pressure) or bottom up (i.e. change in resources) dynamics[1]. Top-down predation can have effects that cascade down to lower trophic levels and influence primary producers – macro and micro algae. The classic example of a top-down trophic cascade is within the kelp forest ecosystem, wherein the presence of sea otters allowed for the proliferation of giant kelp due to their steady diet of sea urchins. However, in the absence of sea otters, the sea urchins graze the kelp forest. Similar top-down effects have the capacity to affect phytoplankton biomass, though the effects are not as strong[1]. In contrast, nutrient availability has a clear effect on phytoplankton biomass, but the link between phytoplankton and zooplankton grazers is not as strong – possibly due to interactions among the zooplankton, the quality of algae, or advection of water parcels[1].

Though complex and not entirely understood, the base of the marine food chain is assuredly the phytoplankton. Clear examples of their capacity to impact higher trophic levels can be found by observing the effects of El Nino Southern Oscillation (ENSO) on fisheries productivity. Specifically, during El Nino years, warm water increases stratification along the eastern boundaries of the Pacific Ocean and prevents upwelling of nutrients to the surface ocean. This decreases phytoplankton biomass, negatively impacting the abundance of

zooplankton and planktivorous fishes, which then causes a decrease in biomass of higher trophic level fishes.

Near-coastal biogeochemistry dynamics have significant impacts on phytoplankton abundance, which translates to higher trophic levels. Factors that influence the coastal ecosystem are both natural and anthropogenic and include rain events, riverine input, and nutrient discharge via runoff and wastewater treatment plants. In the Southern California Bight, water has a high retention time due to the influence of the Channel Islands and bathymetry[2,3]. Therefore, nutrients that are added to this system may have a larger impact on the ecosystem than in other coastal environments[4]. Wastewater treatment facilities around the world operate with different mechanisms and treatment levels, however the common goal is to prevent human waste from impacting the coastal zones and causing harm to human and marine life. These areas tend to be point sources of essential nutrients like nitrogen and phosphorous, in addition to human-related pollutants linked to medicines[5]. Recently, wastewater has become a popular method of tracking human pathogens[6]. It is difficult to disentangle the impacts of wastewater on the marine environment in coastal zones that are already under negative anthropogenic influences. This is further discussed in chapter 2.

Phytoplankton occupy the same size class as another environmental pollutant of increasing concern – microplastic. Both in vivo and in situ studies have confirmed that zooplankton ingest microplastic particles. The issues surrounding microplastic ingestion are twofold: (1) the particles themselves can interfere with feeding and motile appendages, alter the sinking rates of fecal pellets, decrease the amount of nutrition received, and be transferred to higher trophic levels[7–11]. (2) The particles themselves can contain thousands

of chemicals; those intentionally added at the time of manufacture to alter properties such as color, malleability, and durability, and those present in the environment[12–14]. Microplastic can adsorb toxins from the environment, and these toxins can be transferred into organisms during ingestion[12]. With their role as a link between primary producers and secondary consumers, accumulation of toxins within zooplankton can have far-reaching impacts on higher trophic levels, including humans. These impacts are discussed in chapters 3 and 4.

Humans have always had a close relationship with the ocean, through needs for nourishment and exploration. Only recently, however, has our relationship become more negative. Through the introduction of invasive species, extinction of native species, climate change, ocean acidification, and chemical and physical pollutants, we have permanently altered the state of marine ecosystems. Of these, plastic debris are the most recent area of concern, having only been introduced into mainstream usage during the 1950s.

Rivers are a major source of plastics to the marine environment[15]. Improper waste disposal and management, wind, and rain transport plastic debris into waterways, and a lack of infrastructure allows waste to enter the coastal ocean directly. Other sources of marine debris are from maritime activities, including ships and fishing, litter from coastal populations, and pulses during catastrophic events. Around 80% of marine debris are from land, while 18% are traced to the fishing industry[16]. In addition, most wastewater treatment facilities are not designed to filter out small synthetic debris, and can therefore be a point source contributor of microplastic to the marine environment[17,18]. Once the plastic enters the ocean, marine organisms, from zooplankton to whales, become entangled in and ingest plastic debris[19]. Researchers identify synthetic microfibers wrapped around zooplankton, while whales wash ashore with 40-90 pounds of plastic in their stomachs[20,21].

Once plastic debris enters the marine environment, they are broken down by physical and chemical processes. Depending on the size, different trophic levels of organisms ingest the plastic. Commonly studied organisms include such animals as albatross, camels, and whales with their stomach contents filled with plastic[20,22–24]. More recently, science has turned toward investigation of microplastic ingestion within the lower trophic organisms, fueled by discoveries about prevalence of synthetic microfiber pollution from textiles[25,26].

Now, increasing numbers of studies in vivo and in situ demonstrate the ingestion and trophic transfer of the smallest plastic debris. However, a lack of uniform methodologies makes comparison across studies difficult. Attempts to establish set protocols are ongoing, including a recent workshop involving researchers and technology companies from around the world at the Southern California Coastal Water Research Project (SCCWRP) in April 2019, followed by drafting standardized methodologies for measuring microplastics in drinking water[27]. However, a set and approved protocol has yet to be established for measuring and tracking microplastic ingestion at the base of the marine foodweb and across marine invertebrates. Various methodologies are tested and evaluated in chapter 3.

The current scientific consensus is that microplastics are indeed ingested by marine invertebrates, however ingestion depends on feeding strategy, location within the water column, size of debris and of animal, and environmental concentration. One of the risks associated with microplastic ingestion by zooplankton is the impact on carbon export, because microplastic can alter the density of fecal pellets[11,28,29], slowing sinking speeds and reducing the flux to depth. Studies have also demonstrated trophic transfer from invertebrates into higher organisms, however the degree of bioaccumulation remains

variable and uncertain. In addition, the accumulation of toxins desorbed from microplastics during ingestion is a topic of debate among scientists; some studies demonstrate concentration of chemicals while others demonstrate that toxins are absorbed by the gut and subsequently desorbed back into the particles before egestion[13,30–33]. This topic is further explored in chapter 4.

Mercury (Hg) is a potent neurotoxin that can enter the aquatic environment via atmospheric deposition. Its biologically available counterpart, monomethylmercury (MMHg), enters the marine foodweb via phytoplankton. Therefore, areas with high productivity and high mercury concentrations tend to be areas of high biomagnification and are most at risk for trophic transfer of MMHg. In addition, the presence of microplastic debris increases the incidence of mercury methylation to MMHg[34]. The MMHg subsequently adsorbs to microplastic particles, decreasing demethylation rates. So aquatic environments with both high Hg and microplastic concentrations tend to favor the more biologically active and biomagnifiable form of Hg, MMHg[34]. Hg is also found in anoxic sediments as a byproduct of microbial respiration. So, aquatic systems that experience anoxia also tend to be areas with higher Hg concentrations; such systems include marshes, lagoons, deltas, and estuaries.

The Hg concentrations tracked over a diel cycle in a Southern California Estuary were primarily driven by relative proportions of the two endmembers (river and ocean) depending on tide, rather than changes in the water chemistry, where the lowest total Hg concentrations corresponded to periods when dissolved oxygen and pH were high (periods of high tide). Phytoplankton's abundance is not the only impact on higher trophic levels. The presence of environmental toxins, such as the potent neurotoxin mercury, can also impact

higher trophic levels via phytoplankton[34–36]. For example, methylmercury accumulates in the cytoplasm of phytoplankton cells and bioamplifies to four times the concentration within zooplankton grazers; planktivorous fish and higher trophic level fishes can experience even greater accumulation[35]. The dynamics of mercury in aquatic systems is discussed in chapter 5.

Marine ecosystems face influence from anthropogenic pollutants via a variety of pathways – wastewater effluent, wind, rivers, runoff, and atmospheric deposition. A range of pollutants are explored in the following four chapters – anthropogenic nutrients, microplastic, and mercury. It is important to establish the natural biogeochemical cycling of nutrients within aquatic ecosystems before assessing the extent that anthropogenic inputs alter them. However, it is difficult to disentangle anthropogenic influence from the marine ecosystem when study sites are under a barrage of human influence from all directions. In addition, just as climate change and ocean acidification are distinct yet related problems, so, too, are wastewater effluent, microplastic debris, and mercury.

CHAPTER 2

Seasonal and spatial variability of oceanographic and biogeochemical parameters in the coastal Southern California Bight

Adapted from:

Jessica Walden, Christopher McGuire, Laura Terriquez, George Robertson, Katherine RM Mackey.

Abstract

The Southern California Bight is a unique region along the coast of California subject to dynamic controls, including varying wind regimes, seafloor topography, and a dominating cyclonic current. Here we report seasonal patterns along nearshore transects off the coast of Newport Beach, an area under the influence of both natural and anthropogenic nutrient inputs. We collected biogeochemical and nutrient data from monthly monitoring cruises and observed overall similar patterns between sites located over a treated wastewater effluent pipe and sites 7km away and found the impacts of anthropogenic nutrients are far-reaching. In addition, we observed a significant nutrient pulse from a heavy rain event that led to a phytoplankton bloom that had sustained impacts on nutrient levels for several months. This study demonstrates the importance of lateral nutrient transfer to a region that does not have the same coastal upwelling impacts observed further north along the coast.

Introduction

The California Current (CC) is an eastern boundary current that flows south from British Columbia to the southern end of the Baja California Peninsula. North of Point Conception, coastal waters are subject to episodic upwelling events triggered by northerly alongshore winds in the summer months[37] that typically last days to weeks and have a strong influence on coastal biogeochemical processes[38–40]. South of Point Conception, the CC diverges 100-300km offshore of the coast and flows equatorward[41]. In this region, winds blow predominantly onshore year-round, and phytoplankton blooms generally occur due to increases in wind-driven mixing and periodic upwelling[42]. In the nearshore regions of the Southern California Bight (SCB), the CC has a stronger effect in the spring, whereas in winter the California Counter Current dominates, flowing north and bringing warm waters from the south[41,43,44]. In addition to the influences of winds, currents within the SCB are influenced by physical features of the region[41]. The bottom topography consists of undersea mountains and submarine canyons that drive complex patterns of circulation[44]. Cyclonic eddies, driven by the interaction between currents, bottom topography, coastal features, and the presence of islands, generates high retention of water within the bight. Together these features give rise to the Southern California Cyclonic Gyre.

The interaction of currents in the SCB has a strong effect on biogeochemical cycles[45]. These have been well-characterized temporally and spatially through the California Cooperative Oceanic Fisheries Investigations (CalCOFI) program that currently maintains a time series of 66 sampling stations dating back to 1984[46]. The stations extend from just north of Point Conception at 35.5ºN to just south of San Diego at 29.5ºN, from the coastline to about 125ºW, roughly 700km offshore. Measurements taken at these stations and

moorings include temperature, salinity, oxygen, nutrients, phytoplankton, zooplankton, bacteria, pH, and pCO_2[46].

Based on CalCOFI data, Hayward and Venrick identified three regimes in which surface chlorophyll concentrations were driven by different physical processes: offshore, northern nearshore, and southern nearshore[47]. The offshore and inshore regimes are separated by a steep salinity gradient at the edge of the CC. The inshore region is further separated into northern and southern regimes based on the degree of isopycnal shoaling, which drives higher chlorophyll levels in the north compared to the south. In the southern inshore regime, wind driven mixing is an important process for introducing nutrients to surface waters because the pycnocline rarely shoals to the surface.

The SCB generally experiences spring phytoplankton blooms, with episodic blooms occurring year-round[43,48–52]. The spring bloom season occurs during the months of March, April, and part of May as the water column begins to stratify[53]. However, high chlorophyll concentrations have been observed beginning in mid-February and extending through early-autumn in the SCB, indicating earlier "spring" blooms and late-season blooms do occur[42,49]. In the SCB, phytoplankton growth is generally limited by the availability of nitrogen, particularly in the summer when vertical mixing is minimal. The degree of both upwelling and seasonal mixing strongly influences chlorophyll dynamics in the Bight[54,55].

Near-coastal biogeochemistry within the Bight is additionally influenced by land-based processes[48]. A nuanced understanding of the physical and biogeochemical processes that affect water quality in the near-coastal SCB is essential in order to characterize the effects of natural and anthropogenic drivers of coastal water quality and inform coastal management practices. Over 22 million people live along the Southern California coast, and

tourism to Southern California yields over $14 billion in revenue per year; hence, monitoring and maintaining the health of coastal waters is of great importance to state and local communities.

Newport Beach, California, is an interesting location to study near-coast processes in the SCB because it is subject to both natural and anthropogenic influences on coastal biogeochemistry. Surface water biogeochemical parameters measured at Newport Beach show strong seasonal patterns characterized by higher nutrient levels in the winter and spring due to greater mixing, followed by limited nutrient availability in the summer and fall[48,56]. This pattern gives rise to a seasonal succession of phytoplankton groups, which results in variable elemental stoichiometry in the particulate phase throughout the year[56,57].

Hydrological processes, such as rainfall, affect near-coastal biogeochemistry by introducing natural and anthropogenic constituents to the ocean. Winter rains transport nutrients and other pollutants from land into the ocean via river discharge and overland flow, concentrating urban runoff in the coastal zone. Additionally, rainwater itself is an important source of nutrients like nitrogen (N) and phosphorus (P)[58]. Rainfall input varies considerably from year to year in Southern California and is influenced by the El Niño Southern Oscillation (ENSO). Therefore, the effect of storms on coastal water quality is episodic and variable but may nevertheless influence water quality during winter months.

In addition to natural inputs, anthropogenic processes also affect near-coastal dynamics. One major anthropogenic influence in the vicinity of Newport Beach is the release of treated wastewater from the Orange County Sanitation District (OCSD) water treatment facility. The OCSD is the third largest wastewater treatment facility west of the Mississippi River, servicing 2.6 million people in central and northwest Orange County and processing

179 million gallons ($6.8x10^8$L) of wastewater per day. Treated wastewater is discharged to the ocean via an effluent pipe that extends 5 km offshore of Newport Beach (Fig. 2.1).

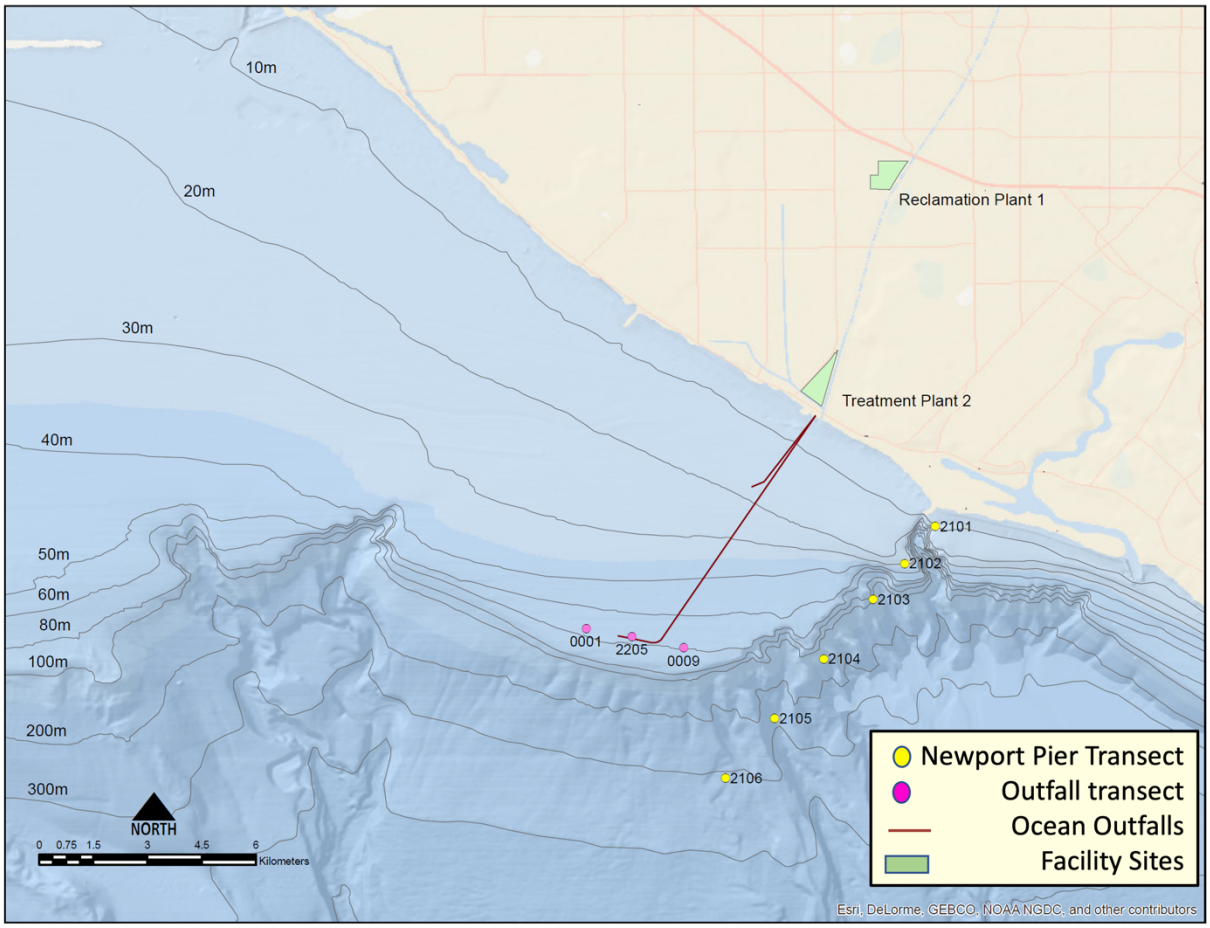


Figure 2.1: Map of station locations in the study area (points) and location of effluent pipe (red lines).

In this study, we examined the seasonal and spatial variability in physical and biogeochemical characteristics of near-coastal waters off Newport Beach, CA over two annual cycles. We characterized potential natural and anthropogenic impacts on water quality and found that natural seasonal and episodic weather events have a strong impact on coastal biogeochemistry, even in a region already under the influence of anthropogenic nutrients.

Methods

Samples were collected on 22 cruises between February 14, 2017 and January 23, 2019 (Table 2.1) aboard the OCSD-operated research vessel M/V Nerissa. Monthly monitoring cruises are conducted by the OCSD to ensure that certain water quality parameters in the vicinity of the plume comply with regulatory limits. Water samples were collected from six stations (2101, 2102, 2103, 2104, 2105, 2106) along a coastal offshore transect from the surface down to 60 m (or less for shallower stations) at 10m intervals (Table 2.2, Fig. 2.1). Additionally, samples were collected at a minimum of one of three stations in an alongshore transect over the treated wastewater effluent pipe (0001, 2205, 0009; Fig. 2.1). The effluent station was selected based on the direction of the plume flow as determined using real-time CTD measurements of colored dissolved organic matter (CDOM), which is a tracer of the wastewater plume (reference). To look for effluent influence outside the area directly over the discharge pipe, we examined station 2104 in more detail. All materials used in sample collection were acid cleaned prior to use.

Table 2.1: Sampling dates for the offshore transect and outflow stations.

2017	2018	2019
2-14-17	1-16-18	1-23-19
2-28-17	2-5-18	
3-28-17	3-12-18	
4-18-17	4-18-18	
5-8-17	5-7-18	
6-19-17	6-13-18	
8-3-17	7-10-18	
9-12-17	8-6-18	
10-26-17	9-20-18	
	10-16-18	
	11-6-18	
	12-12-18	

Table 2.2: Depths of sampling in the study region.

STATION	DEPTHS MEASURED
2101	0, 10
2102	0, 10, 20, 30
2103	0, 10, 20, 30, 40, 50, 60
2104	0, 10, 20, 30, 40, 50, 60
2105	0, 10, 20, 30, 40, 50, 60
2106	0, 10, 20, 30, 40, 50, 60
0001	0, 10, 20, 30, 40, 50, 60
2205	0, 10, 20, 30, 40, 50, 60
0009	0, 10, 20, 30, 40, 50, 60

CTD Ocean Sampling and Water Collection Procedure

Each cruise utilized a CTD (Sea-Bird Electronics SBE9/SBE 11 Deck Unit) with a carousel water sampler (Sea-Bird Electronics SBE32/SBE33) equipped with 12 Niskin bottles. At each station, conductivity (for salinity), temperature, pressure (for depth), dissolved oxygen (DO), pH, chlorophyll-a fluorescence, and colored dissolved organic matter (CDOM) were measured. SEASOFT software was used for real time data display, acquisition, and sensor calibration. Potential outliers were removed from downcast data, as well as possible instrumental errors, electronic noise, or physical interruptions from the sensors[59].

Chlorophyll-a Analysis

Calibration of chlorophyll values from the CTD were verified in the first five cruises by directly measuring chlorophyll levels as described previously. Briefly, 200ml of seawater was filtered through a 25mm glass fiber filter (GF/F, Whatman), extracted for 24 hours in 90% acetone at -20^{o}C, and the fluorescence measured on a Turner Trilogy Fluorometer (model 7200-000). The values measured by the CTD were found to accurately report the values measured via the extraction method. This is consistent with prior observations for

the SCB that show a strong correlation between extracted chlorophyll and CTD fluorescence-determined concentrations[48]. Therefore, CTD derived chlorophyll values were used in these analyses due to the greater spatial resolution.

Chlorophyll data were plotted with Ocean Data View (Mac edition 5.2.1) and displayed using Data Interpolated Variational Analysis (DIVA) gridding. This method has been shown to better account for coastlines, eliminating false mixing, and advection by using a finite-element method, accounting for observational constraints, smoothness constraints, and dynamical constraints[60].

Nutrient Analysis

Nutrient analyses were performed on seawater samples filtered using GF/F filters (Whatman, nominal pore size 0.7 μm) and stored frozen in 50 mL conical centrifuge tubes until analysis. Samples were analyzed for nitrate (including trace amounts of nitrite) and soluble reactive phosphorus (hereafter phosphate) using a Lachat's QuikChem® 8500 Series 2 Flow Injection Analysis System according to manufacturer's specifications. The detection limit for nitrate was 0.014 μM and the detection limit for phosphate was 0.054 μM.

Results

Temperature and salinity

A consistent seasonal pattern was observed for temperature along the offshore transect (Fig. 2.9A, Fig. 2.2). A weak thermocline was observed in the fall and winter months. The surface waters warmed and the thermocline intensified in the spring and summer. Winter thermocline depths were ~20 m, whereas the depth of the thermocline deepened in

the spring and summer, occasionally reaching depths of 30-40 m. The warmest temperatures occurred in July and August, reaching up to 20 - 24^{o}C in the surface (Fig. 2.9A, Fig. 2.2). Water column temperature did not vary appreciably with proximity to the coast (Fig. 2.2).

Salinity values varied between 33-34 PSU for all depths and locations (Fig. 2.3). During several months, intrusion of deeper water was observed from higher salinity values measured toward the base of the depth profiles (Fig. 2.3; April, May, and June 2017 and 2018). This is consistent with shoaling of the pycnocline as deeper, more saline waters flowed up from the submarine canyon at the offshore end of the transect (Fig. 2.1). In June, July, and August of 2018, higher salinity values were observed in surface waters (Fig. 2.3) that coincided with warm surface temperatures (Fig. 2.2) and very low nutrient levels (Fig 2.5), likely the result of entrainment of offshore Pacific gyre waters into the SCB. Dissolved oxygen (DO) and pH levels (Figure 2.4) showed similar depth profile characteristics as temperature. The range of pH values was 7.4-8.3, and the range of DO values was 3.1-9.5 mg/L (Fig. 2.4), with the highest values occurring in surface waters during the summer stratified period for both 2017 and 2018.

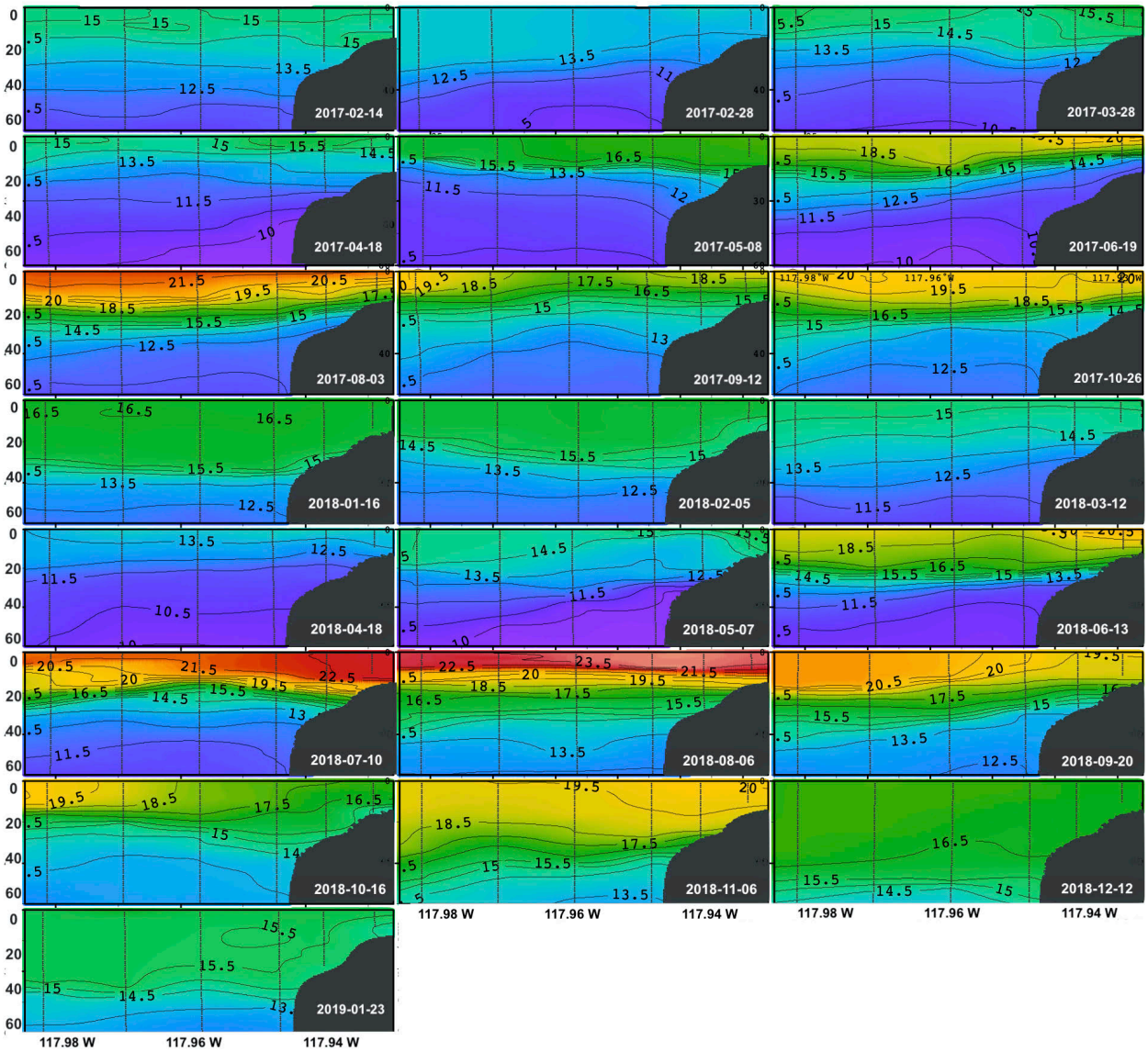


Figure 2.2: Transect plots of temperature data along the offshore transect.

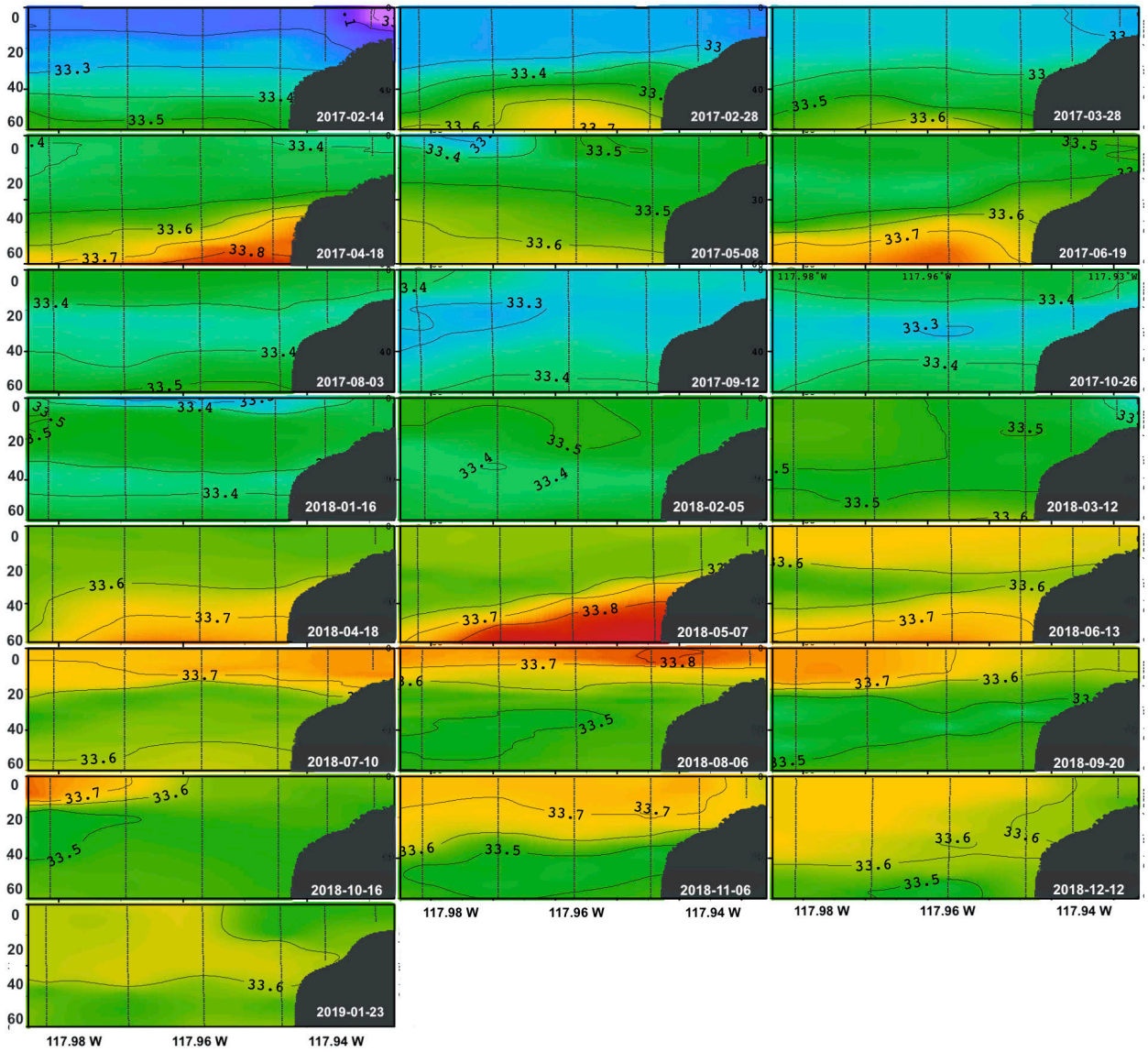


Figure 2.3: Transect plots of salinity data along the offshore transect.

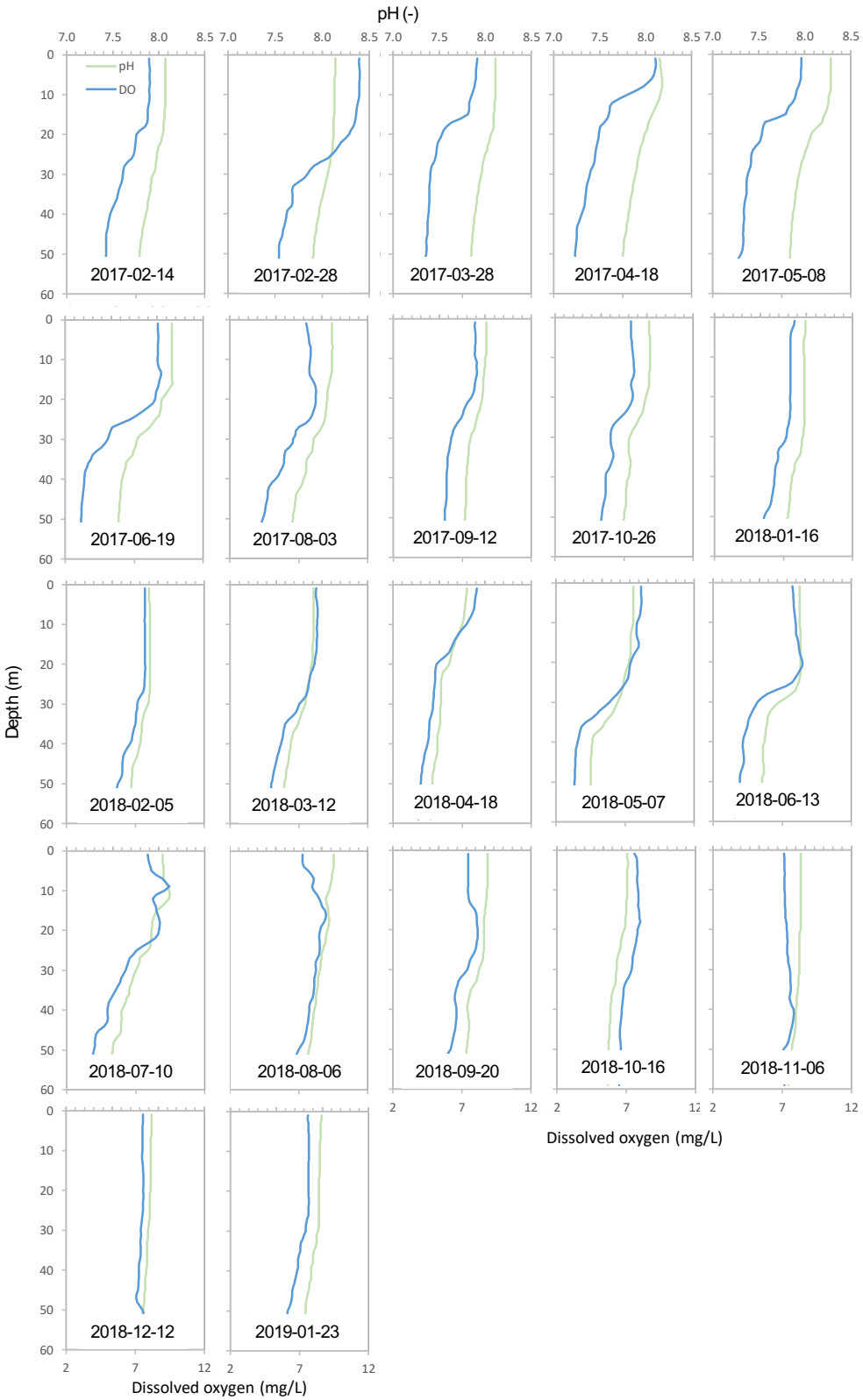


Figure 2.4: DO and pH depth profiles at station 2104 during the study period.

Biogeochemistry

Nitrate and phosphate concentrations shared similar spatial and temporal patterns (Fig. 2.5, Fig. 2.6). Throughout the year, both nutrients were depleted in surface waters at station 2104, and increased with depth (Fig. 2.7), consistent with the thermocline depth (Fig. 2.8). Nitrate ranged from below detection in surface waters to ~5-10 μM at depth depending on the month, whereas phosphate concentrations were generally 1 μM or lower in the surface (Fig. 2.5). Phosphate concentrations exceeded nitrate concentrations in all but two months (April 2018 and June 2018 at some depths, Fig. 2.7), leading to nutrient N:P ratios that were generally well below the Redfield value of 16:1 in surface waters (Fig. 2.8). N:P ratios in the deeper water below the thermocline exceeded Redfield, reaching values of up to 72:1 (January 2018, Fig. 2.8). The transition from low to high N:P ratios with depth corresponded to the depth of the thermocline. Following extreme rains in March 2018, the N:P ratio was elevated throughout the water column, reaching a value of 88:1 in surface waters at station 2104 (Fig. 2.8).

Variability in nutrient levels was apparent between the two years. While nitrate levels were generally similar in 2017 and 2018, phosphate levels declined by more than half in the summer of 2018 compared to 2017, although they rebounded to similar levels beginning in September 2018 (Fig. 2.6; Fig. 2.9B). Second, nitrate levels deeper in the water column were occasionally influenced by pulses of nitrogen rich deep water (e.g., August 2017 and February and June 2018). Nitrate and (to a lesser extent) phosphate were both highly enriched in the nearshore stations in April 2018 following heavy rains (Fig. 2.5, Fig. 2.6).

To determine the extent to which treated wastewater effluent was source of nutrient enrichment to the area, concentrations from the effluent depth profiles were plotted against

concentrations from corresponding depths at site 2104. There was a significant linear relationship between the two data sets, with an R^2 value of 0.43 ($p<0.05$, Fig. 2.10), suggesting nutrients from the outflow site were influencing the offshore transect region.

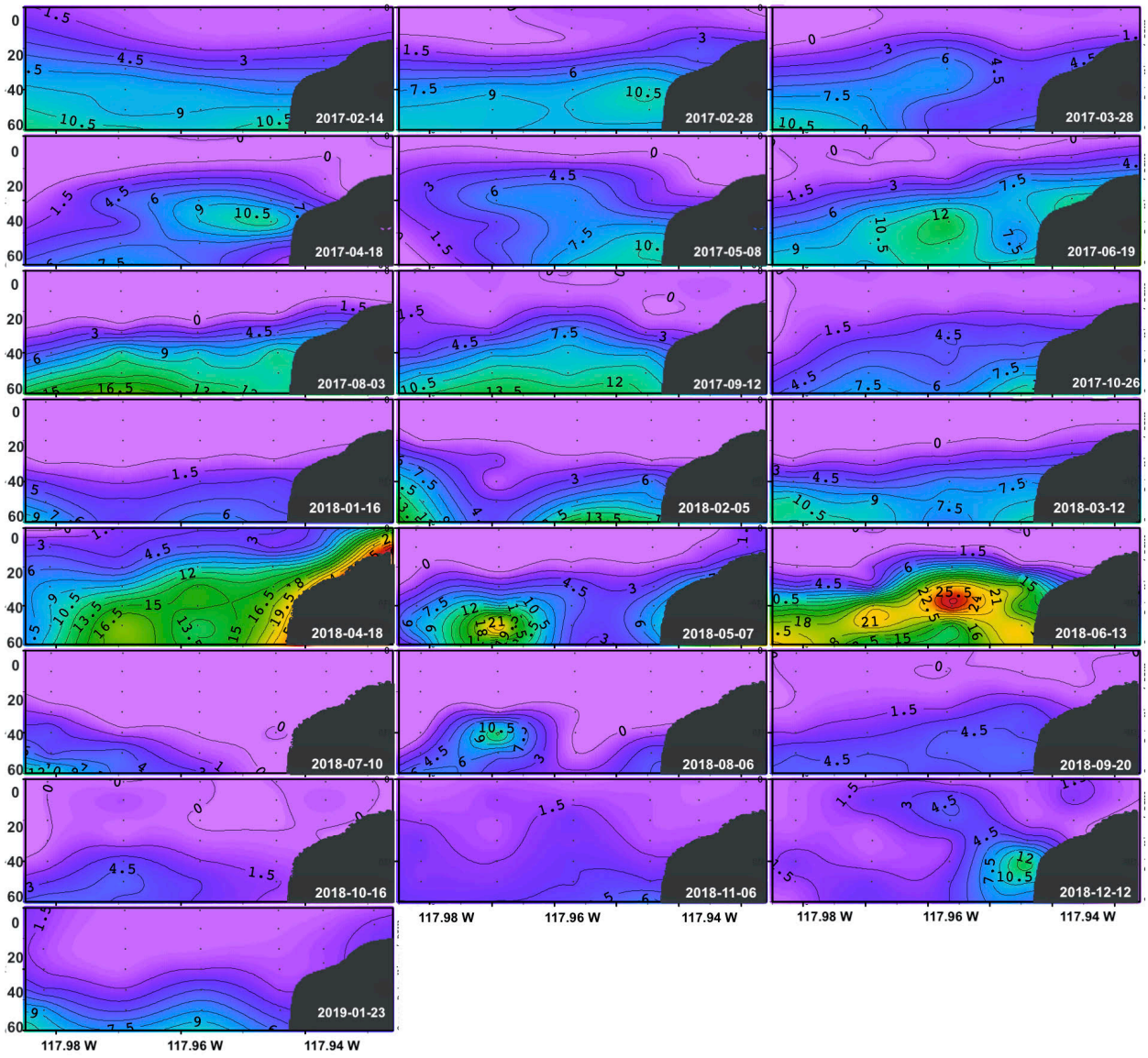


Figure 2.5: Transect plots of nitrate along the offshore transect.

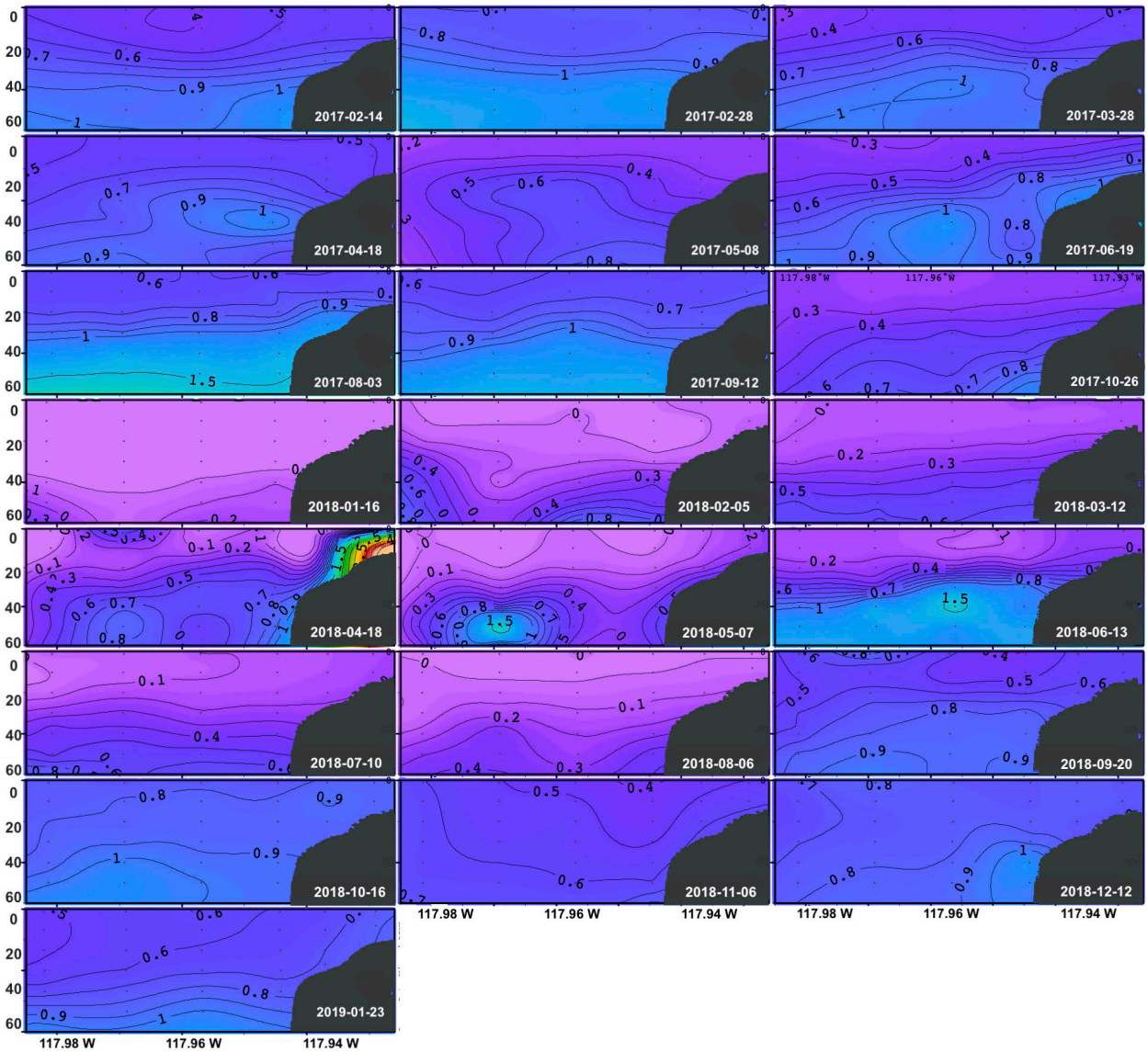


Figure 2.6: Transect plots of phosphate along the offshore transect.

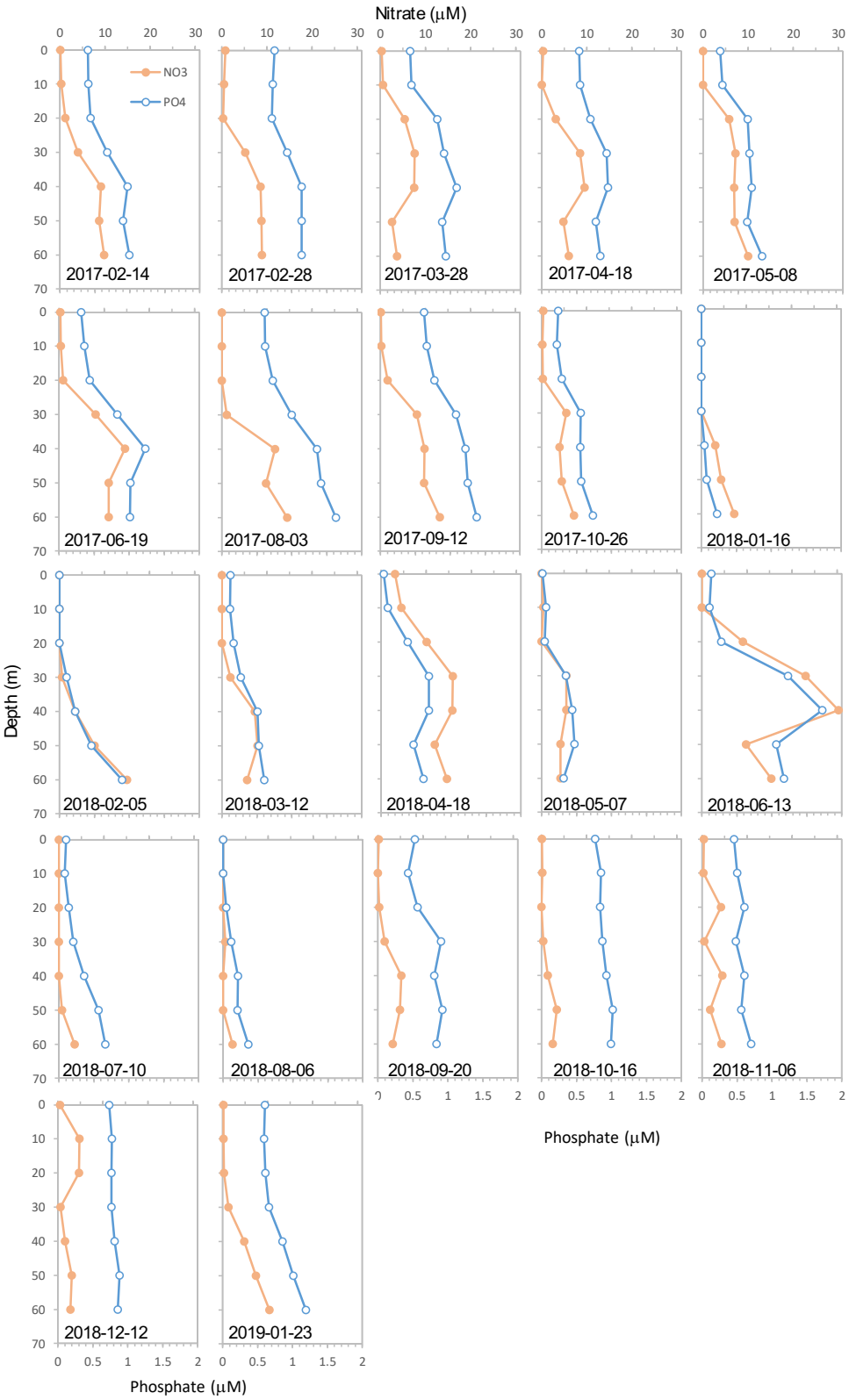


Figure 2.7: Nitrate and phosphate concentration depth profiles at station 2104.

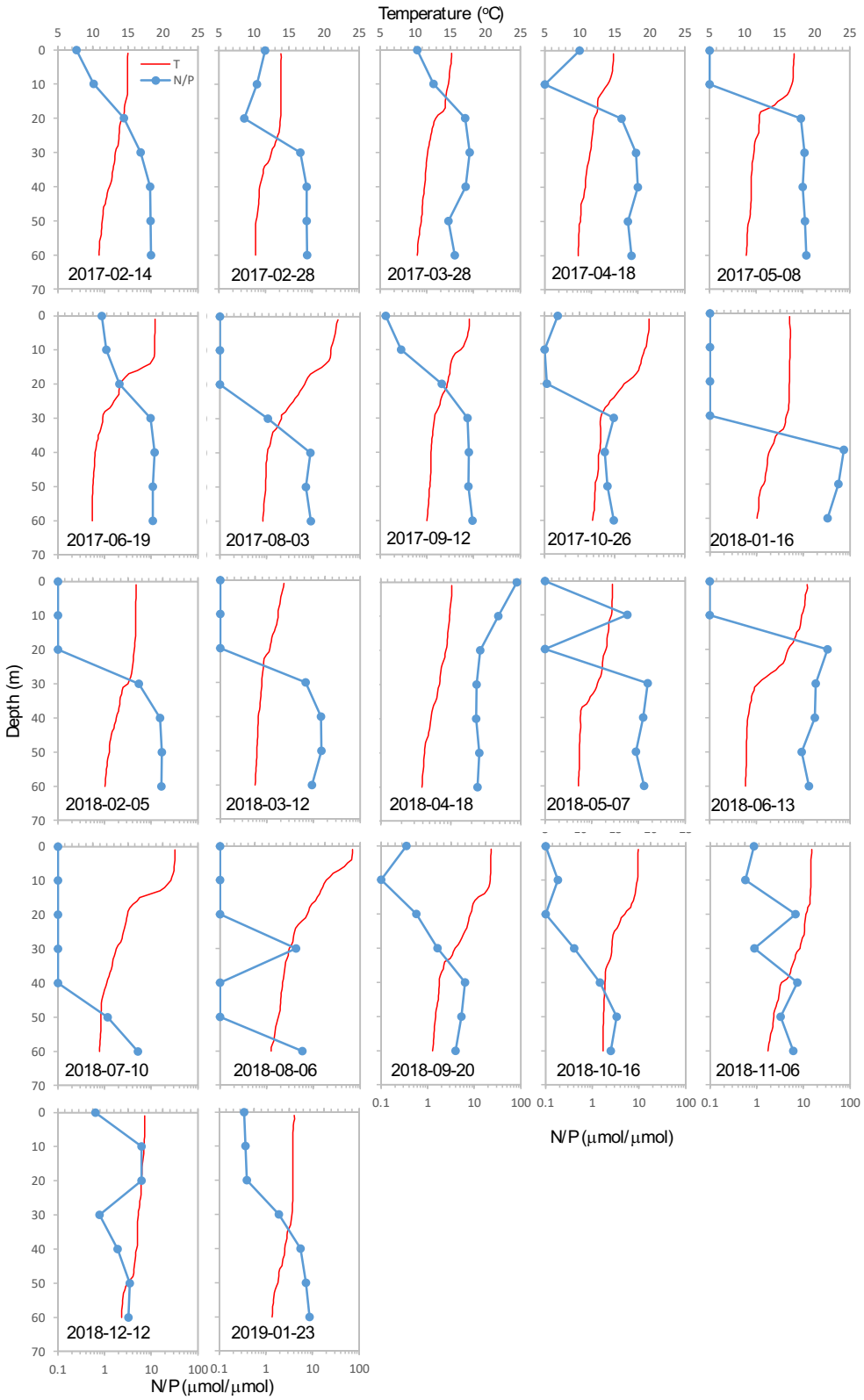


Figure 2.8: Temperature and Nitrate : Phosphate depth profiles at station 2104.

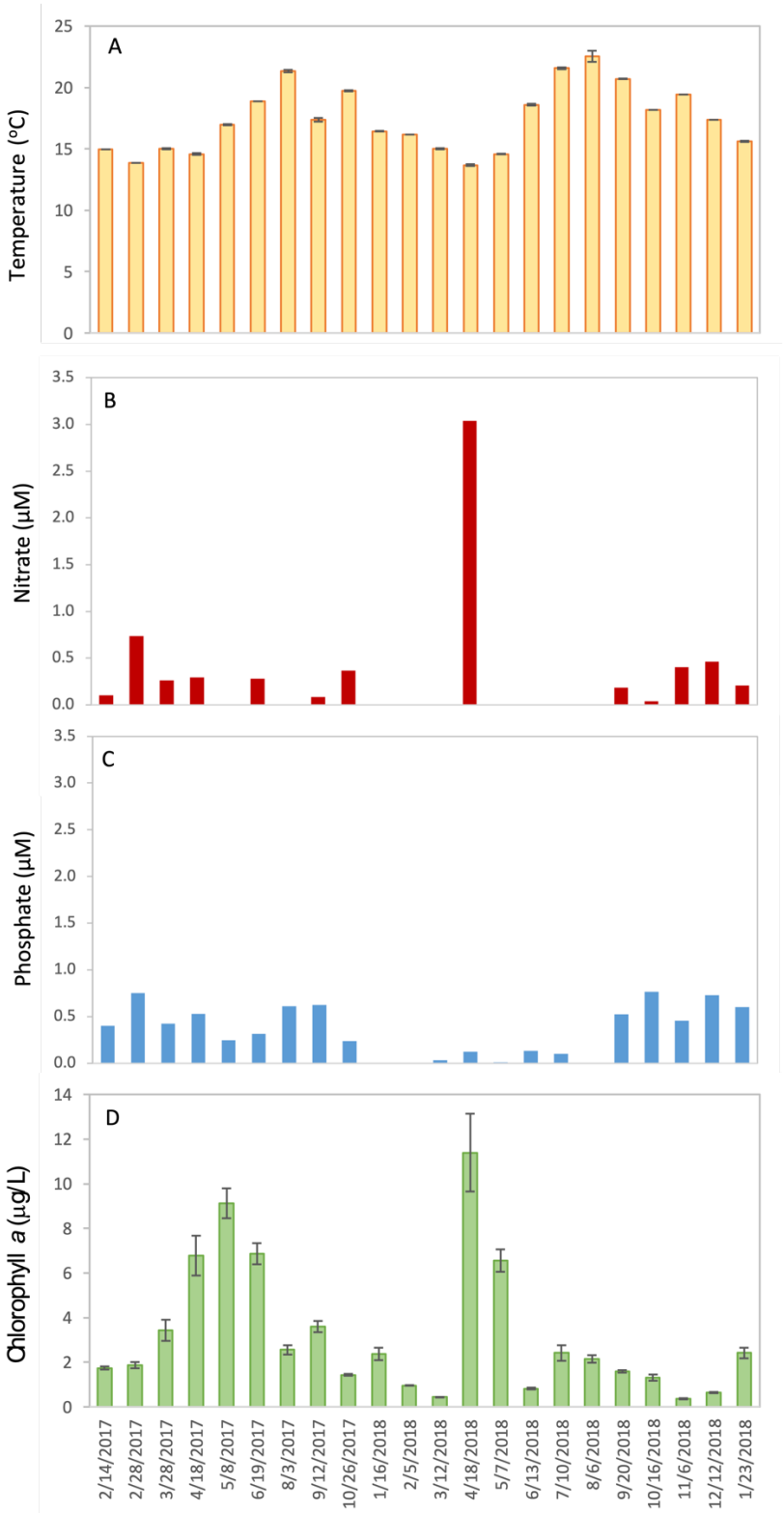


Figure 2.9: (A) Temperature, (B) nitrate, (C) phosphate, and (D) chlorophyll-a concentrations in surface water (upper 10 m) during the time series at station 2104. No bar indicates a concentration of zero.

Chlorophyll-a

Chlorophyll-a concentrations varied considerably with season, distance from shore, and depth. At station 2104, surface chlorophyll-a levels in the upper 10 m showed a relatively consistent seasonal pattern, where the lowest levels occurred in the fall and winter, and the highest occurred in spring and summer (Fig. 2.9D). Winter chlorophyll levels were typically below 5 mg/m3 throughout the water column, whereas concentrations in the deep chlorophyll maximum (DCM) layer reached high values of 15-20 mg/m3 in the summer months (Fig. 2.10). A rapid increase in chlorophyll concentration occurred in April and persisted into June and July and was more pronounced closer to shore (Fig 2.11).

The highest chlorophyll levels (>15μM) were observed close to shore during summer months when the water column was stratified (Fig. 2.11). The maximum chlorophyll levels moved further offshore and deeper in the water column throughout the summer as stratification progressed and surface nutrients were depleted (Fig. 2.11). The pulse of nutrients observed in April 2018 (Fig. 2.5, Fig. 2.6) and associated increase in N:P ratio (Fig. 2.8) induced by strong rains coincided with increased chlorophyll in surface waters compared to the prior month (Fig. 2.11).

To determine if the effects of the treated wastewater effluent could be observed at 7km away from the effluent pipe, chlorophyll concentration depth profiles were compared between station 2104 (reference station) and the effluent stations (Fig. 2.10). The DCM depths and concentrations were generally similar between the stations.

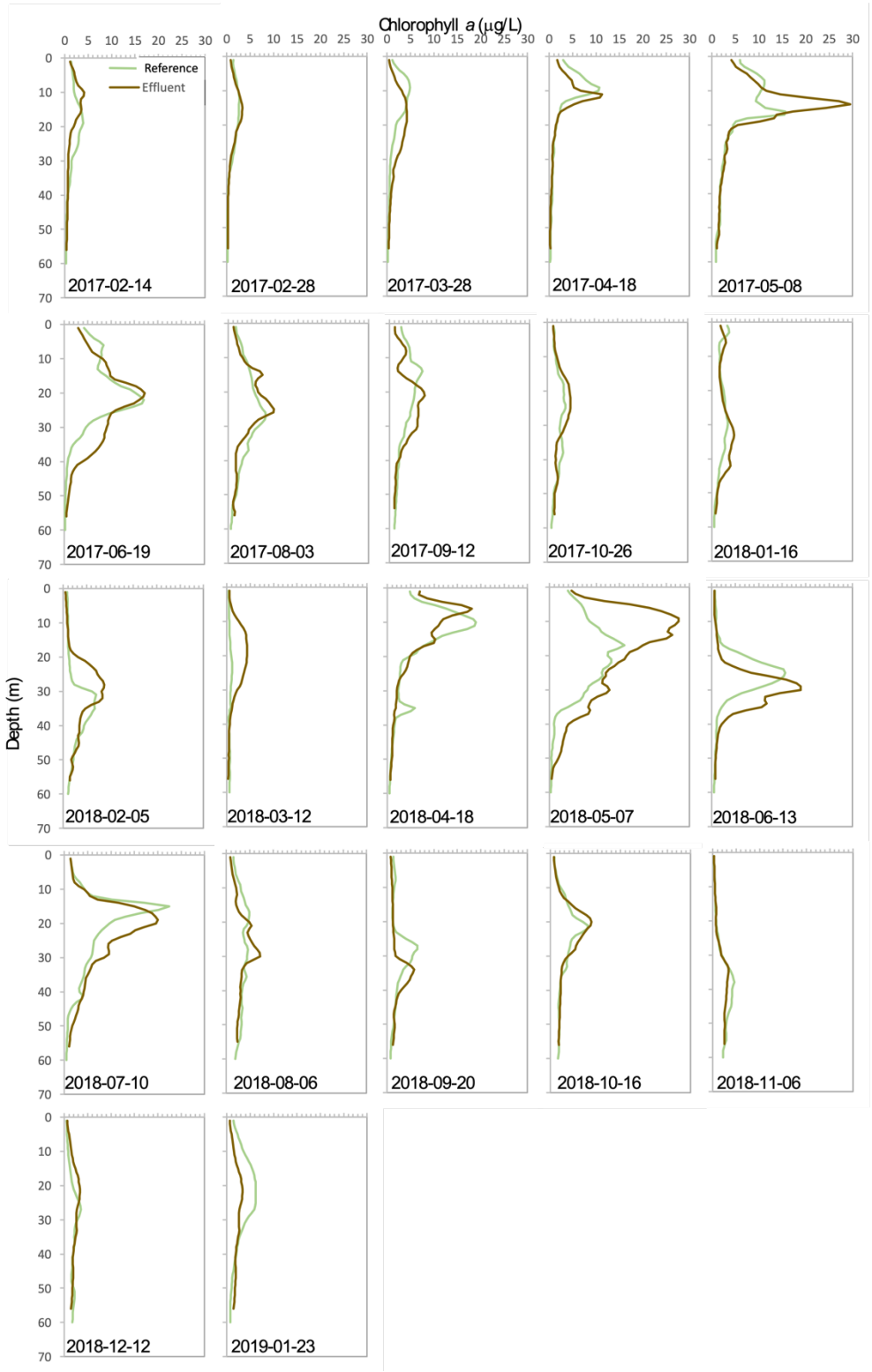


Figure 2.10: Chlorophyll concentration depth profiles at the effluent and station 2104.

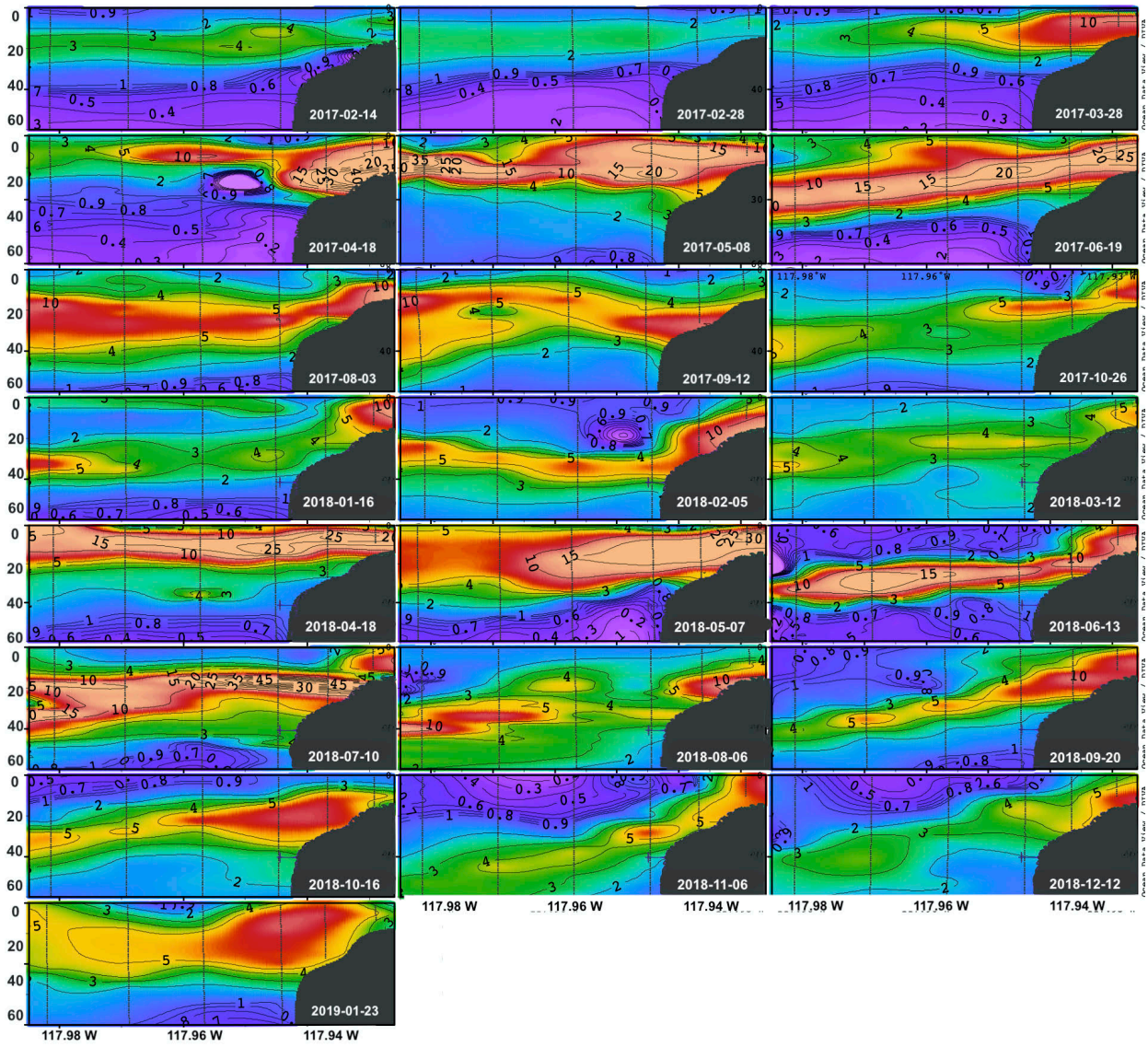


Figure 2.11: Transect plots of chlorophyll along the offshore transect.

Discussion

Near-coastal waters, which are used to describe waters within ~5 km of the shore, are subject to natural and anthropogenic factors that have the potential to affect coastal biogeochemistry. Understanding how these factors interact in space and time is important for coastal management. Newport Beach is a near-coastal environment representative of many Southern California beaches.

The near-coastal waters off Newport Beach have similarities and differences with waters elsewhere in the SCB. The seasonal patterns observed along the Newport transect agree well with observations of winter mixing and summer stratification observed further offshore that drive patterns of nutrient availability and chlorophyll abundance. We observed lower chlorophyll concentrations in the winter, which is likely the result of light limitation induced from water column mixing. A phytoplankton bloom occurred each May at the onset of stratification, as has been observed previously[43,56]. Chlorophyll concentrations within the DCM were elevated throughout the summer months during stratification, but declined upon onset of mixing in the fall and winter. DO and pH levels closely mirrored water column stratification and mixing. In the stratified summer months, phytoplankton blooms in surface waters caused oxygen levels and pH to increase compared to deeper water, which is influenced by the California Undercurrent[61] (Fig. 2.4).

Newport Beach is located at the boundary between the northern and southern inshore regimes of the Bight identified by Hayward and Venrick[47]. In the north, frequent outcropping of the pycnocline supports higher chlorophyll levels, whereas in the south outcropping is less common and wind driven mixing plays an important role in delivering nutrients to the surface. The two years of observations in this study suggest that near-coastal Newport Beach is more similar to the southern inshore regime, as although shoaling of the pycnocline did occur, it did not outcrop.

However, near-coastal physical dynamics and biogeochemistry in the SCB are strongly influenced by ENSO[62], with El Niño conditions suppressing upwelling and La Niña conditions favoring enhanced upwelling. Although 2017 and 2018 were weak La Niña years and significant upwelling was not observed in this region, it is possible that during stronger

La Niña events the Newport Beach transect could more strongly resemble the northern inshore regime.

Also consistent with offshore waters in the SCB, nitrate is the limiting nutrient for phytoplankton along the Newport Beach transect, where nutrient N:P ratios were consistently low compared to Redfield value of 16 in surface waters. Based on the extensive CalCOFI dataset, Mantyla and coworkers identified a consistent relationship between depth of the nitricline (taken as the depth where nitrate concentration is 1 μM), and the depth of the DCM layer within the Bight[54]. Specifically, they observed that as the water column stratifies and phytoplankton begin to bloom, and nitrate levels become depleted in surface waters. Consumption of nitrate by phytoplankton causes the depth of the nitricline to deepen. This, in turn, causes the depth of the DCM layer to deepen, as phytoplankton move deeper in the water column to access nitrate. Below a certain depth, light limitation prevents phytoplankton growth, generating an offset between the depths of the nitricline and the DCM. They found that overall, in the SCB the DCM is generally 12 m shallower than the nitricline when the nitricline is near the surface of the water column, and 20 m shallower than the nitricline when it is deeper in the water column.

In our near-coastal dataset, we did not observe a consistent relationship between the depths of the nitricline and the DCM. The difference in depths varied considerably between stations, suggesting that spatial variability plays an important role in determining phytoplankton-nutrient interactions in this near-coastal environment. This is likely due to the difference in bottom depths between the stations, particularly for the shallow stations closest to the coast. Looking only at the deeper stations along the Newport pier transect (2104, 2105, and 2106), the DCM is at times shallower, deeper, or at the same depth as the

nitricline depending on season (Fig. 2.7, Fig. 2.10, Figure 2.12). However, in the summer months when the waters are stratified, the DCM does follow the pattern of being ~10-20 m shallower than the nitricline, as observed for waters further offshore in the Bight[54] (Fig 2.12).

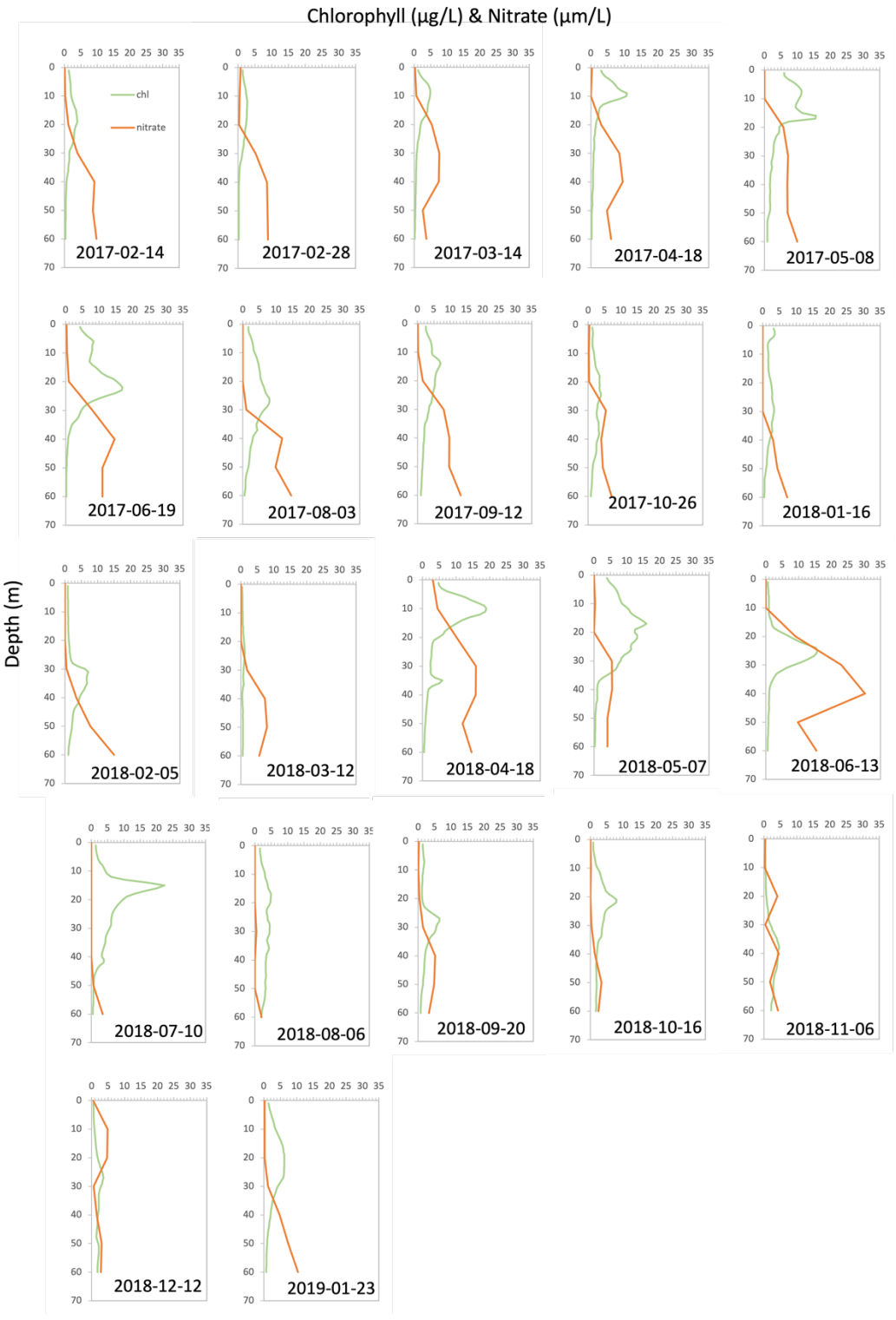


Figure 2.12. Nitrate and chlorophyll concentrations at station 2014.

Near-coastal waters have the potential to be affected by natural and anthropogenic land-sea interactions. Extremely high rainfall occurred along the California coast in the end of March 2018 that was associated with a "Pineapple Express" atmospheric river. The rainfall was concentrated between Santa Barbara and San Francisco, whereas further south, rains were less intense. A pulse of nutrients was present in the nearshore stations of the Newport pier transect in April 2018, and was also observed 28 days following the rains. To determine if the rains could be a source of the nutrients, we calculated the transit time for water originating in Santa Barbara and San Francisco to arrive at Newport Beach. Santa Barbara and San Francisco are located ~250 km and ~800 km, respectively, from Newport Beach. Assuming the average velocity of the CC is 25 cm/s, we calculate that direct coastal transport would take ~11 days to arrive from Santa Barbara and ~37 days to arrive from San Francisco, in agreement with the 28-day delay between the timing of the rains and the April sampling date. Although we did not observe a signal in salinity, this is likely due to the fact that the nutrient-rich water parcel traveled south and was not derived from local stormwater runoff. Thus, there was substantial time to dilute the salinity signal from the rain event up the coast.

The distribution of nutrients in April along the transect and with depth suggests that the nutrients were being consumed as the water was transported, resulting in lower concentrations in surface waters compared to deeper in the water column. Although there does appear to be a slight shoaling of deep water in April 2018 (Fig. 2.3), the pulse of nutrients is not likely driven by deep water input because in months with stronger shoaling, nutrient levels did not reach concentrations as high as those in April 2018 (Fig. 2.x). Additionally, the extremely high ratio of N:P associated with this water parcel is consistent

with high N:P ratios in rain water[63,64]. This event led to very high N:P throughout the water column (e.g., 88:1 in surface waters at station 2104, Fig. 2.8), and was the only sampling event in which nitrate concentrations exceeded phosphate concentrations (Fig. 2.7).

The elevated N:P ratio in April 2018 along with the more frequent shoaling of the pycnocline in summer 2018 had a sustained effect on nutrient inventories that lasted throughout the summer. Specifically, there was a notable decline in phosphate levels throughout the water column in summer 2018 compared to 2017 (Fig. 2.6, Fig. 2.9B). The greater number of pulses of deep water with elevated nitrate concentrations would serve to relieve nitrogen limitation and allow greater phosphate drawdown. Taken together with the large pulse of nitrogen delivered with the atmospheric river rains in late March that caused nutrient enrichment in April (Fig. 2.5), 2018 appears to be less nitrogen limited compared to 2017. This likely gave rise to the low phosphate concentrations that persisted until September 2018, when stratification began to weaken, and phosphate levels were restored by winter mixing.

In addition to natural nutrient sources like deep water and rain, near-coastal sites like Newport Beach have the potential to be affected by anthropogenic nutrient sources. Our initial study was motivated by the presence of the Orange County Sanitation District's effluent outfall pipe. Orange County Sanitation District releases on average 22.9mg/L of nitrite (4.6), nitrate (13.4), and organic nitrogen (4.9) each month[59]. Given that there are 62.01 g/mol of nitrate, OCSD releases about 7.2 μM nitrate per day (using an average of 30 days per month to calculate). Assuming rapid conversion of all organic nitrogen to nitrate via bacteria upon entry into the marine environment, there could be up to 12 μM nitrate per day contributed by the effluent.

To determine how the treated wastewater effluent impacts water quality elsewhere in the bight, we compared nitrate and chlorophyll values from depth profiles at the effluent site and Newport pier transect stations. For nitrate, agreement between the stations was generally closer for lower concentrations (Fig. 2.13). Several higher concentrations did not follow as closely, but there was no consistent trend in the source of this variation. The outliers included both surface and deep samples and appear to be related to shoaling of the nitricline (when station 2104 had higher concentrations, due to its closer proximity to the submarine canyon) or occasional high pulses of nitrate from the effluent (when the effluent site had higher concentrations).

Interestingly, on the occasions where we observed higher concentrations at the effluent, the difference correlates quite nicely with the 12μM contribution estimated from OCSD's effluent nutrients (Fig 2.13; August 2017, April-May 2018). It is difficult to directly compare, because our samples were a snapshot of one day of a month, whereas the daily average calculated above is an estimate based on the monthly average. In order to better capture the contribution of nutrients, daily samples of both effluent outflow and the water column near the pipe would need to be done.

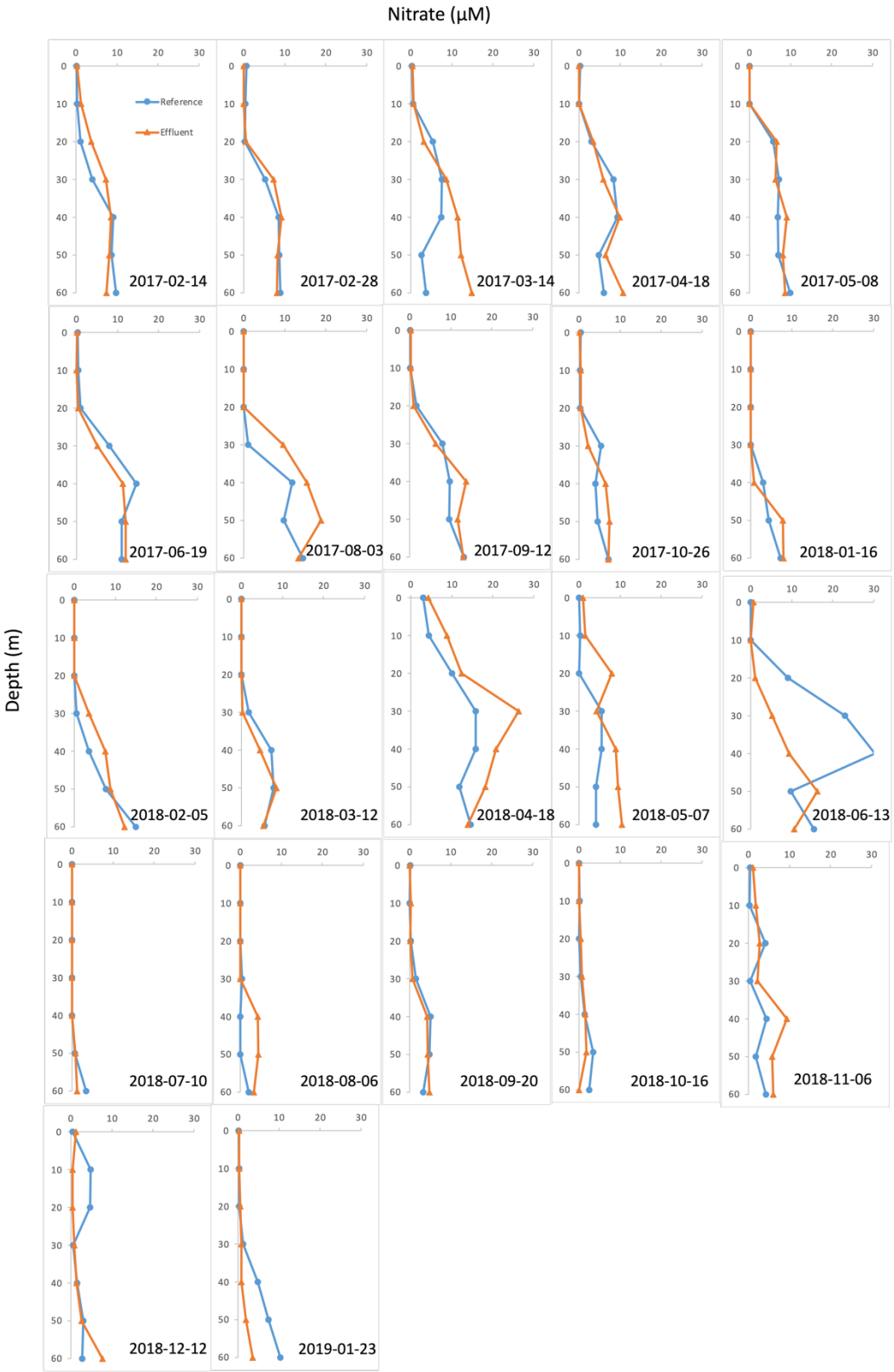


Figure 2.13: Nitrate concentration depth profiles at station 2104 and effluent sites.

Chlorophyll concentrations between the two stations were remarkably similar in terms of the size and depth of the DCM, suggesting that nutrient inputs from the effluent may be widespread throughout the region; this is also supported by the similarity in nutrient inventories between the two stations (Fig. 2.10). For a better comparison, sampling several reference sites further from the effluent site, away from the entrained nutrients that may still be found at station 2104 would be beneficial. There were two months (May 2017 and 2018) in which the effluent station chlorophyll levels exceeded those at station 2104 that coincided with the onset of spring stratification. However, this is most likely due to faster progression of stratification on the shelf, where the effluent station is located, compared to waters closer to the edge of the submarine canyon where station 2104 is located. Earlier stratification on the shelf would provide more time for the bloom to develop at the effluent station, leading to slightly higher chlorophyll concentrations compared to those at site 2104 during those months.

To determine the extent that the wastewater effluent impacts this near coastal region, a reference site further away and outside of the cyclonic entrainment that traps waters in the region would be necessary. Further research is needed to be able to distinguish nutrient pulses and subsequent increases in chlorophyll concentrations from natural sources and treated wastewater. Clearly, outside nutrient sources can have large effects on areas that are generally nutrient poor, as demonstrated by the impact of the Pineapple Express event. It is unknown whether the combination of a constant influx of treated wastewater and the cyclonic circulation that keeps water parcels trapped within this region supports higher than natural nutrient levels.

The two years of data collected along a transect in near-coastal Newport Beach show seasonal patterns in which water column physical dynamics appear to drive the biogeochemical characteristics at the site. Like waters further offshore in the Bight, winter mixing introduces nutrients that support phytoplankton blooms in the spring as the water column stratifies. Newport Beach shares characteristics with the southern inshore regime of the SCB[47] in that wind-driven mixing has a stronger influence on nutrient availability and chlorophyll levels compared to upwelling. Runoff from intense rain events also has the potential to cause large scale changes in near-coastal biogeochemistry that can influence water column nutrient characteristics for the duration of the stratified season by increasing nitrate availability and allowing greater drawdown of phosphate.

Acknowledgements

This work was funded by a generous gift from Greg and Donna Jenkins and the Jenkins Family (to C.I.C.). We thank The Orange County Sanitation District for the use of their vessel and access to the CTD biogeochemical data.

CHAPTER 3

Microplastic Ingestion by Artemia salina as an Entry Point into the Marine Food Web

Abstract

The issue of plastic pollution touches every ecosystem on earth, and its effects on organisms are just recently being studied. This paper explores varying methodologies for studying how microplastics impact the marine foodweb. Previous studies employ a wide range of methodologies and organisms to demonstrate that microplastic is ingested by zooplankton, however there is no methodological consensus. Here we demonstrate that Artemia salina ingest microplastic after 90 minutes of exposure, and that ambient concentrations of microplastics impact ingested concentration. In addition, we show that 1) many commonly used digestion methods do not work on chitinous organisms and 2) the type of plastic used in a study can impact data analysis. These findings indicate that methods need to be tailored to the study organism, and that uniform methodologies across all zooplankton may not be possible.

Introduction

The "age of plastics," so-called due to the rampant rise in use of synthetic polymers since the 1950s, is characterized by widespread marine and terrestrial plastic pollution[65]. Research on ocean plastic pollution began in earnest in the early 1970s, after Carpenter and Smith reported plastics in the North Atlantic Ocean for the first time [66]. Publications tapered during the 1990s, but resurged after 2001 when Charles Moore documented the "Great Pacific Garbage Patch", which he described as floating plastic within the Pacific subtropical gyre[67,68]. Soon thereafter, plastic accumulations were discovered in all five subtropical gyres, whose compositions are now characterized more akin to a "plastic smog" of micro- and nano-particles swirling from the sea surface to depth[68–72].

Studies on the feeding strategies and natural diets of zooplankton demonstrate great variety. Off the coast of Southern California, researchers observed differences across species with regard to time of feeding, gut contents, and environmental conditions[73].

In order to feed, zooplankton filter water equal to 10^6 times the volume of their body[74]. There are four main types of feeding strategies observed in zooplankton: passive ambush feeders, active ambush feeders, feeding-current feeders, and cruise feeders[74]. Passive and active ambush feeders will either encounter and intercept prey or perceive and attack prey, respectively. Feeding-current zooplankton obtain prey by generating a feeding current that draws particles toward their mouths, while cruise-feeders swim through the water to capture prey. Zooplankton have also been shown to feed on large marine snow aggregates that contain microplastic[28,74,75]. The brine shrimp, Artemia salina is a passive,

filter feeding branchiopod – it is non-selective and will ingest whatever it encounters in the water column[76].

Previous incubation studies of zooplankton with microplastic demonstrate a range of methods utilized and impacts observed. Most studies employ copepods, exposing them to both virgin and biofouled microplastic beads and particles[21,29,77–80,80,81]. These studies all demonstrate that zooplankton ingest microplastics, however the amount ingested is highly dependent upon the feeding strategy of the organism, the concentration of the particles, and whether particles are virgin or biofouled. For example, Xu et al., 2022 found that the copepod Temora longicornis rejected 80% of microplastics to which it was exposed. Cole et al., 2013 found that ten out of thirteen experimental copepod species ingested a significant amount of microplastic spheres, including T. longicornis[77,81]. Further, an incubation study of Artemia parthenogenetica with polystyrene beads resulted in observed abnormalities to the intestinal epithelial cells[82]. Some authors argue that, although microplastics are ingested, the amount transferred to higher trophic levels decreases with each level up, while others demonstrate results to the contrary[32,83]. However, the issue of microplastic ingestion is not solely the transfer of the particles themselves, but also of the chemicals contained within the synthetic polymers. For instance, benzo[a]pyrene, a polycyclic organic hydrocarbon and a persistent organic pollutant, has been reported to transfer from Artemia nauplii into zebrafish via ingestion of inoculated microplastic particles in the lab[12].

A. salina is an ideal experimental organism and has been used for decades in laboratory studies due to its ease of culture and ability to thrive in a range of temperatures and salinities[76]. The purpose of my microplastic incubation experiments is not to determine

whether organisms are harmed by microplastics, but to observe the ways that microplastics enter the food web. Therefore, utilizing a highly sensitive species may impede the experiment from progressing, generating inaccurate results. Further, the Artemia genus is found worldwide in brackish waters, with A. salina native to areas around the Mediterranean and closely related to the species A. franciscana found in the Americas.

Recently, microplastics (<0.5mm) have been detected both in tap water and in human waste—the ubiquity of plastics in the environment is thus mirrored in the bodies of organisms[84,85]. Plastic does not decompose into smaller organic and inorganic molecules – they are instead broken apart by physical, chemical, and biological weathering processes, including mechanical disintegration, photodegradation, oxidation, hydrolytic degradation, and microbial colonization[84,86,87,88].

Microplastics have two origins: primary microplastics are small by design, while secondary microplastics form when larger plastics are broken into smaller pieces by weathering. Secondary microplastics are typically separated into five categories: fibers, sheets, films, fragments, and foam (Fig. 3.1). Rivers are a major source of plastics to the marine environment[15]. Improper waste disposal and management, wind, and rain transport plastic debris into waterways, and a lack of infrastructure allows waste to enter the coastal ocean directly. Other sources of marine debris are from maritime activities, including ships and fishing, litter from coastal populations, and pulses during catastrophic events, such as extreme flooding or tidal waves[16,89].

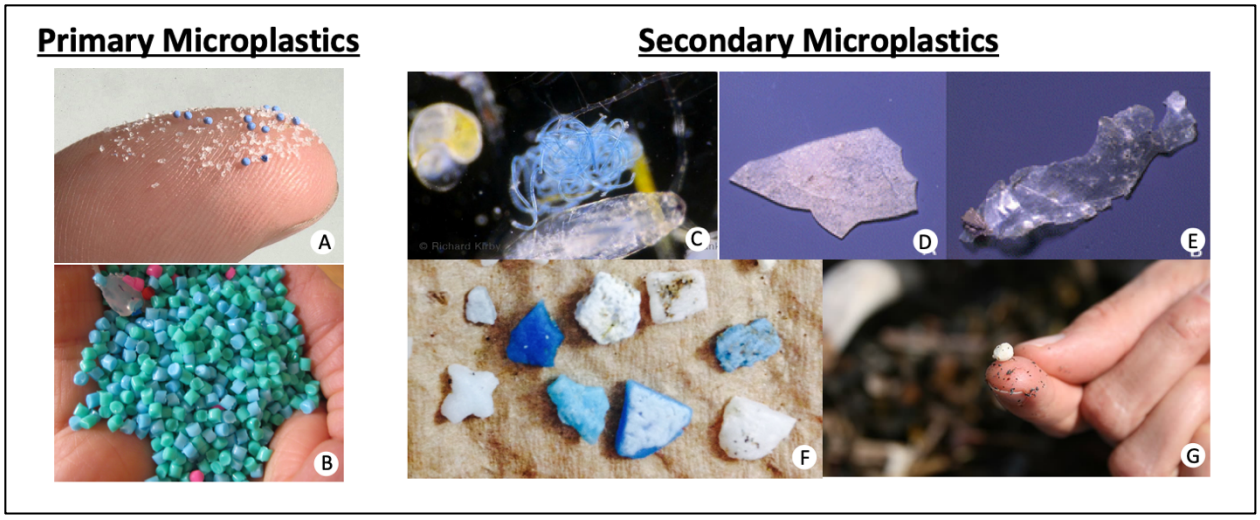


Figure 3.1: Two categories of microplastics – primary and secondary – and further classifications within each category: (a) microbeads, (b) nurdles, (c) fibers, (d) sheet, (e) film, (f) fragments, and (g) foam.

Microplastics carry with them environmental pollutants in addition to the chemical cocktail of additives already contained from manufacture[88]. Along their paths down storm drains, sewers, and rivers, plastics encounter chemicals and pollutants of varying origin, including fertilizers, pesticides, aerosols, and "contaminants of emerging concern" or CECs. Because of the hydrophobic nature of plastics, environmental pollutants adsorb to its surface[16]. Therefore, in addition to additives introduced during processing, plastics serve as a vector for additional contaminants to enter the food web. This provides one reason as to why plastics found inside of organisms are a threat to health—though the particles themselves are eventually excreted, the toxins they contain are transferred into humans and higher trophic level organisms during digestion. Upon leaching, acute toxicity and endocrine disruption may occur[90]. The extent of these impacts is still being researched, though studies have demonstrated harmful effects of chemicals including bisphenol A, phthalates, and other endocrine disruptors[90–92].

Depending on the chemical composition of the particular plastic, it will either float on the surface, have neutral buoyancy, or sink. Polyethylene (PE), polypropylene (PP), and polystyrene (PS) tend to float, while polyamides are more neutrally buoyant. Polyester, polyvinyl chloride (PVC), and polyethylene terephthalate (PETE) sink. PE, PP, and PS dominate plastic production, so the majority of macroplastic in the environment floats[16,93-94]. However, as macroplastics undergo weathering, and as organisms aggregate on their surfaces, their buoyancy decreases[72,95]. Recent enumerations of floating marine debris are less than estimated based on loading; therefore, biofouling may prove a mechanism by which plastics are removed from the surface waters[79,95].

Biofouling has further impact beyond altering the density of plastic particles. Microbial organisms that constitute biofilms may produce chemicals that alter the attractiveness as food and may contribute to increased predation by actively feeding zooplankton[79,96]. Most incubation experiments to date use sterile, pristine microplastics to study zooplankton ingestion. It is important to include microplastics coated with natural microbes in incubation studies to expose another factor that can contribute to microplastic ingestion in the environment[79]. Polystyrene beads, both pristine and inoculated with biofilms, were incubated with two copepod species; both species ingested more of the artificially aged plastic spheres than the pristine spheres[96]. However, as beads are not found in the ocean in concentrations as high as fragments and fibers, studies need to incorporate these other types of plastic to further expand knowledge on how biofilms influence microplastic ingestion among various marine primary consumers.

Though the production of positively buoyant plastics is high, synthetic fibers in textiles are the greatest source of plastic microfibers to the environment[7,97–99]. Every time

an article of clothing is washed, small fibers are released through the machine's drain and enter the environment. This category of microplastic has become of great concern, because microscopic organisms are often found associated with fibers[7,80,97]. Understanding the impact of plastic microfibers in natural samples is complicated by the fact that distinguishing natural fibers, such as cotton and silk, from synthetic fibers during microscopic analyses is difficult[100]. Using Ramen and Fourier Transform Infrared Spectroscopy (FTIR) techniques to identify chemical species within samples helps elucidate between natural and synthetic materials[100,101].

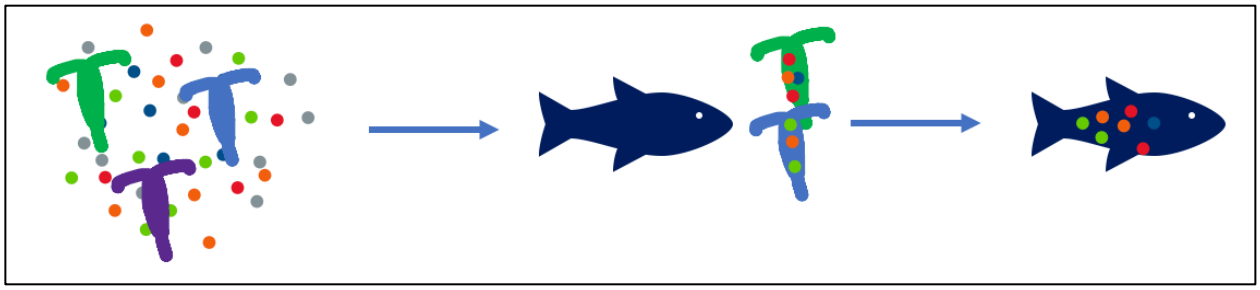


Figure 3.2. Zooplankton ingest microplastics (colored dots) and are then eaten by fish. The fish then contain the plastics and associated chemicals. Microplastic toxins may become increasingly concentrated as they move up the food chain to longer-lived species, called biomagnification.

The potential sink within higher trophic levels merits additional research, because mechanisms of transfer and the categories of plastics transferred are poorly characterized at present. To discover which plastics are accumulating in large organisms, we must understand potential selection and ingestion by the smallest marine animals—zooplankton. Phytoplankton, zooplankton, and microplastics occupy overlapping size classes in marine ecosystems. Because zooplankton are primary consumers, they graze on phytoplankton and

similarly sized particles, and can therefore ingest microplastic in the process[10,21]. In this way, zooplankton serve as an entry point for microplastics into the marine food web (Fig. 3.2).

The interactions of zooplankton with microplastics influence the types of plastics that bioaccumulate into higher trophic level organisms. Secondary consumers will ingest zooplankton laden with microplastics, potentially leading to a bioaccumulation effect of both the plastics themselves and of associated chemicals and bacterial assemblages[32,102,103]. However, the mechanisms of this transfer are unknown, including how long particles remain in zooplankton before excretion, and how that will determine the amount of plastics transferred through trophic levels[11,32,83,104]. In addition, several primary consumers are capable of particle distinction, which might influence the concentration of microplastics available to secondary consumers[105,106]. Finally, the size, shape, and chemical composition of microplastics might influence consumption and trophic transfer[7,105,107,108]. Current experts in the field stress the need of identifying local entry points and fates of microplastics and associated chemicals to determine relevant ecological impacts (need refs here, perhaps restate).

Hypotheses

To address the issues surrounding the fate of microplastics, incubation experiments were conducted with zooplankton, phytoplankton, and microplastics. These incubation experiments determined microbead (primary) ingestion across different concentrations and time exposures. In addition to the qualitative incubation experiments, different methodologies were tested and evaluated for use in future incubation studies attempting to quantify microplastic ingestion rates[109–115].

Several research questions and associated hypotheses were addressed through this research and methods development. First, we knew that brine shrimp would ingest microplastic, given previous research demonstrating ingestion by other marine zooplankton species and our pre-trials. Then, we formulated two main hypotheses: (1) ingestion of microplastic by bring shrimp will increase as concentration of microplastic increases and (2) ingestion rate will plateau over time exposed to plastic.. We found that both of our hypotheses were correct: ingestion of microplastic is concentration-dependent and the rate of ingestion plateaus over time.

Methods

Organisms

Seawater was obtained from Newport Beach, sterilized, and inoculated with F/2 medium[116] to provide essential nutrients and vitamins to the study organisms. The phytoplankton species Isochrysis galbana and the zooplankton species Artemia salina were obtained from the aquarium supply store Algae Research Supply. I. galbana is a hearty, easily cultured single-celled haptophyte (brown algae) within the size range of food normally ingested by the chosen zooplankton species, A. salina (8-11μm). A. salina is a passive-feeding brine shrimp and can survive in a wide range of salinities, temperatures, and oxygen concentrations[76]. Using these robust lab species is beneficial for incubation experiments, because it eliminates confounding factors that might interfere with variables to be measured. Active selective feeding strategies, sensitive reproductive cycles, and strict light,

temperature, and feeding requirements could all impact the success of the incubations and the confidence in results.

To optimize the health of I. galbana, cultures were observed over the course of several months (Fig. 3.3), taking daily fluorescence and Fv/Fm measures. Fv/Fm measures the variable fluorescence versus the maximum fluorescence, which represents the maximum potential efficiency of photosynthesis. This measure can give insight into the health of the organism, using photosynthetic performance as a proxy[117]. I. galbana was maintained at a temperature of 22°C in a controlled-environment incubator under continuous white light (Darwin Chambers Co.), and cultures were diluted with fresh media once every 10-12 days. The cultures were maintained in sterilized 200ml Pyrex flasks with polystyrene foam stoppers wrapped in cotton cheesecloth.

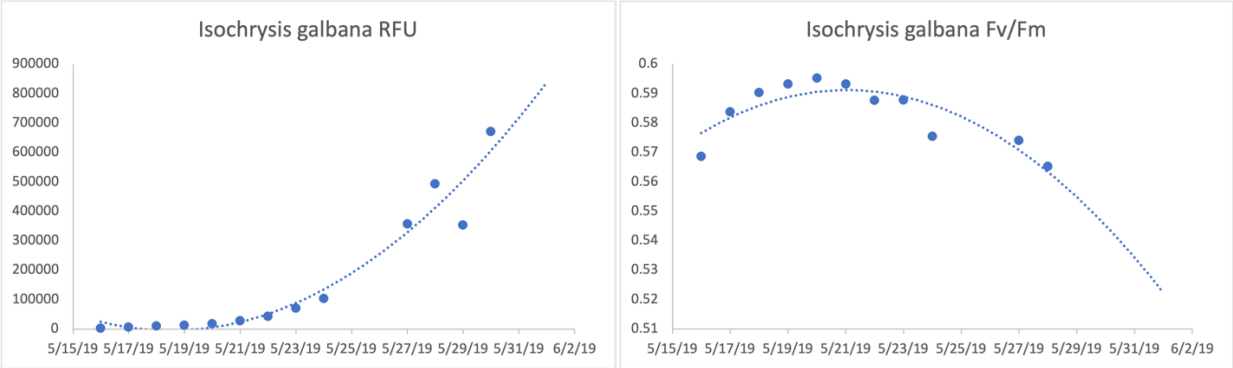


Figure 3.3. I. galbana observed RFU (raw fluorescence units) on the left and Fv/Fm values on the right.

A. salina are relatively large, ranging from 8-15mm, making them easy to visually isolate and move from culture to experiment. In addition, they are easily sexed, with males having larger secondary antennae. Egg sacs on females are easy to identify, and these

organisms were avoided for the experiments to allow for maintenance of culture and to avoid alteration of behavior due to attribution of energy towards reproduction. A. salina were fed with a highly concentrated culture of I. galbana every 10-12 days. Each time the phytoplankton culture was diluted with fresh media, the "discard" was used as food. They were kept in sterilized 1000ml Pyrex flasks and the water was refreshed roughly monthly to dispose of fecal matter and dead organisms and replenish the food supply.

Microplastic

The microplastic beads were of a similar size class to the selected phytoplankton and within range of the usual prey of the selected zooplankton. Color should be uniform for initial assessment, and then could be varied for future experiments to test whether the color of plastic influences ingestion rates. Fluorescent particles are easier to count via flow cytometry or under fluorescent microscopy. Several beads were used during the method development: 6μm polystyrene beads from Spherotech impregnated with Nile Red dye, 10 μm melamine beads impregnated with Fluorescein-5-isothiocyanate (FITC) from Sigma Aldrich, and 10-20 μm polyethylene beads also impregnated with FITC from Cospheric.

Microscopy and Fluorescence

A Nikon Eclipse Ti2 microscope with an Excite Series 120Q fluorescent bulb was used under FITC excitement (548nm) was used to qualitatively assess microplastic ingestion by visualizing microplastic within the guts of A. salina.

In an effort to streamline measurements of phytoplankton and microplastic count post-dissolution, fluorescence values were compared with particle counts under flow cytometry (Fig. 3.4). The Chl-a filter exhibited high raw fluorescence unit (RFU) values for the microbeads, nearing the detection limit for the Turner fluorometer. Thus, the Chl-a filter was determined insufficient for estimating bead count. Either the orange or green filter demonstrated more reliable detection for the microbeads.

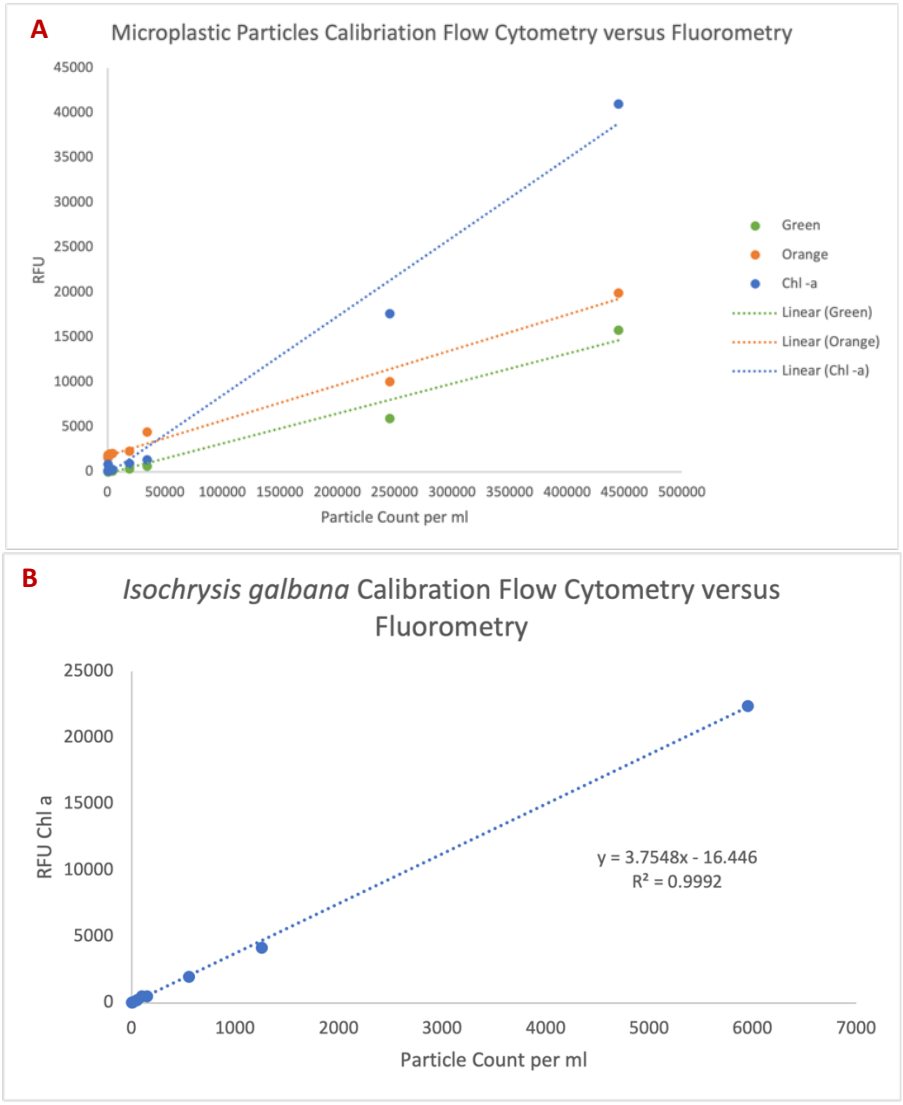


Figure 3.4: Panel A, FITC-labelled melamine resin beads under fluorometry versus flow cytometry; Panel B, I. galbana cultures under flow cytometry and fluorometry.

Incubation – Ingestion Rate

In order to verify whether a 24-hour incubation was optimal, short-term incubations were evaluated to visually observe ingestion rates. A simple incubation was set up with 7 40-ml test tubes filled with 30ml sterilized seawater and inoculated with a bead concentration of 10^4 beads/ml. Each test tube was also inoculated with the same concentration of phytoplankton at 10^4 cells/ml. Two individuals of A. salina were added to each test tube and the time exposed was as follows: T1 (5 minutes), T2 (20 minutes), T3 (40 minutes), T4 (60 minutes), T5 (90 minutes), T6 (120 minutes), and T7 (150 minutes).

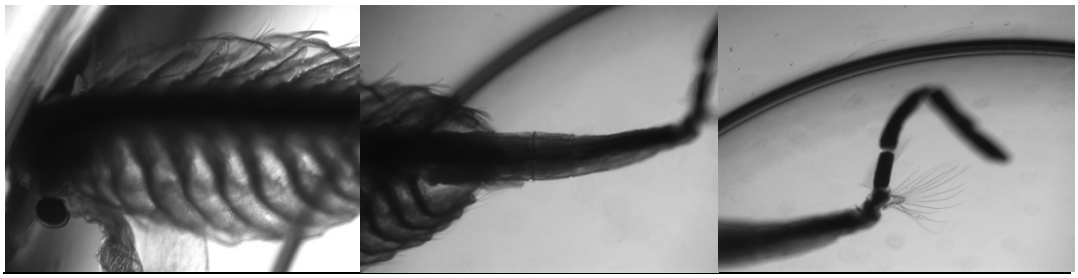


Figure 3.5: Organism T1 under DIC at 4x

Organisms were fixed with formaldehyde and photos were taken after each time step was completed under Differential Interference Contrast Microscopy (DIC) and FITC fluorescence for each organism at 4x. These photos were used to qualitatively assess the ingestion of microplastic after each prescribed time. This incubation was repeated to verify confidence in results.

Incubation – Concentration-Dependent Ingestion

To determine whether the concentration of microplastic particles in solution impacts the overall quantity of beads ingested, a 4-hour incubation was set up under the following parameters. Five 40ml test tubes were filled with 30ml sterilized seawater and 4 individual A. salina per tube. The control tube had no microplastic particles, while the remaining 4 tubes had concentrations of 10^2, 10^3, 10^4, 10^5 beads/ ml, respectively. Bead concentrations from 10^2-10^5 beads/ ml are within an environmentally relevant range, the higher being found in heavily polluted areas[99,109,118–120]. Adult males and females were selected; juveniles and females with egg sacs were not included in the incubation to avoid confounding energy needs. Results were qualitatively assessed via fluorescent microscopy.

Digesting Zooplankton

Current literature provides a myriad of methods for digesting organisms to count the microplastics within. However, there is not a clear consensus as to which method is most effective. Several methods can successfully digest soft tissue organisms but cannot process chitinous organisms. In addition, the methods that can digest chitin are either cost prohibitive or cause measurable damage to the microplastic particles. The use of heat is controversial, because it speeds up the reaction time and can exacerbate damage to plastic particles. Finally, the ideal method will be different depending on the origin of plastic particles. If the polymeric composition of the microplastic is known to be resistant to damage by nitric acid digestion, this would be a suitable method. However, when analyzing organisms collected from the natural environment, using nitric acid would be ill-advised as

the composition of plastic particles is unknown and therefore some particles could be susceptible to damage, yielding false results.

Fenton's Reagent

Fenton's Reagent has been demonstrated as an effective method for digesting organic material for the extraction and measurement of microplastic[113,114]. The reagent involves utilization of 30% hydrogen peroxide (H_2O_2) as the digestive solution, catalyzed by ferric sulfate ($FeSO_4$) at ambient temperature. The following procedure was implemented to test whether Fenton's reagent would effectively digest A. salina in order to recover the ingested microplastic beads.

One brine shrimp was added to a 50ml plastic Falcon Tube with 5ml $FeSO_4$ and 10ml 30% H_2O_2. An additional 2.5ml H_2O_2 was added every minute for 10 minutes, resulting in a total addition of 35ml H_2O_2. At this point, the solution was adjusted to a pH of 3-4 with 2ml H_2SO_4 to dissolve the iron precipitate. Unfortunately, after several attempts, the chitinous exoskeleton remained visible, indicating that this reagent could not be used to digest this organism.

Alkaline Digestion

Previous studies have successfully utilized and verified alkaline digestion of zooplankton with KOH and NaOH, with no significant break down of microplastic particles after digestion[109,110,121]. One A. salina individual was placed in a glass test tube with 500µl of

1.8M KOH at 40C for 48 hours and another placed into a test tube with 500µl of 10 M NaOH at 60ºC for 24 hours. Neither solution was able to break down the exoskeletons after the prescribed time. Each was left an additional 24 hours with no discernable difference. Finally, A. salina was exposed to saturated KOH (11.7M) and NaOH (19.1M) solutions at 60ºC for 24 hours and a soapy liquid formed, with the exoskeleton having remained undigested.

Acidic Digestion

Nitric acid (HNO_3) has proven effective in digesting copepods that contain chitin in their exoskeleton[80,110]. However, HNO_3 has also demonstrated significant destruction of microplastic particles during digestion; one study found that polystyrene beads, polyethylene terephthalate, and high density polyethylene particles were degraded and nylon fibers completely disappeared[110,121]. It is also highly caustic and must be handled in a fume hood. Because a known concentration of beads was being added in these incubations, slight degradation in this study would be acceptable if beads were still countable post-digestion. To first test whether nitric acid could digest A. salina, one individual was placed into a test tube with 500 µl HNO3 and immersed in a water bath at 80 ºC for 30 minutes. After 30 minutes, the individual was completely digested.

Interaction with Formaldehyde

Two A. salina were incubated with 10^5 beads/ml in a test tube with 30ml F/2 media for 24 hours[116]. A. salina were placed in well plates and inspected under the microscope at

FITC fluorescence to confirm ingestion of microplastic. In order to better photograph the individuals, 4% formaldehyde solution was added to quickly kill them. Once photographed, individuals were placed in tubes to digest via HNO_3 digestion. Within 5 minutes, a brown vapor (potentially NO_2) began escaping from the tubes and the liquid was an opaque brown, presumably, due to interactions with the formaldehyde and nitric acid. Therefore, it is advised to avoid fixing individuals with formaldehyde before digesting with HNO_3.

Neutralization

To simplify counting the beads ingested by A. salina, flow cytometry is ideal. It eliminates counters bias and human error and delivers accurate results when uniform beads like the ones in these incubations are used. However, due to the extreme acidity of the nitric acid solution, neutralization is a necessary step before introduction into the Novocyte capillaries.

A test tube filled with 500 µl 15.7 M HNO_3 was placed on ice. 15.7 M NaOH was added drop by drop until the solution turned an amber color and a pH strip read 14. The solution could not achieve neutrality without a buffer, as each solution is a strong acid or base. To test a buffered solution, 500µl HNO_3 was added to 500 µl of H2O on ice. Then, 320 µl of NaOH was added, drop by drop, until the liquid changed color and a pH strip verified pH 14. To this mixture, 1.075 g of KH_2PO_4 powder was added and allowed to react. A pH strip confirmed the solution was finally near neutral, at pH = 8. This method was then utilized on a nitric acid solution post-digestion of A. salina, and the liquid turned into a gel upon the addition of KH_2PO_4. This was repeated three more times, yielding the same results. Something about the

chemistry involving the dissolved components of the chitinous organism modified the viscosity. So, flow cytometry as an option for counting particles was ruled out.

Melamine Beads

The melamine beads lost their fluorescence under acidic conditions, but the fluorescence returns under neutral or basic conditions. Immediately after digestion, NaOH was added until the pH flipped to 14. If too much time passed where the beads remained in the acidic conditions, they would never fully recover their fluorescence.

Following dissolution and alkalization, the solution was poured through a vacuum filtration system onto 3µm polycarbonate filters. Upon inspection of filters after dissolution and alkalization, it was determined that most of the beads remained inactivated by the HNO_3. Further, under DIC, the beads were difficult to distinguish from the pores on the filter. Finally, an attempt was made to elute the microplastic from the polycarbonate filter. This would produce a neutral solution that could be run in the flow cytometer. However, a small and inconsistent percentage of microplastic were able to be recovered from the filter.

I abandoned counting with fluorometry, and used dissolution in well plates that could fit onto the microscope stage . This way, there would be no particle loss due to transfer from test tube to well plate and there would be no need for fluorescence because particles would not be confused with pores on a filter. However, the well plates became damaged and warped from the acid and the heat. In addition, upon inspection under the microscope, it appeared that the melamine beads were dissolving under the strong chemical conditions. So, it appears

that HNO_3 is an inappropriate tool for incubations that involve melamine beads. Similar to nylon, the strong acid dissolves this resin.

Polyethylene Beads

New fluorescent polyethylene beads were purchased from Cospheric (Fluorescent Yellow Polyethylene (PE) Microspheres 1.00g/cc 10-20µm - 0.1g). These beads came in powder form and were hydrophobic. To suspend the hydrophobic PE microbeads in water, a surfactant was necessary to coat the outside. Tween 20 is a biocompatible surfactant (polyoxyethylene sorbitol esteris) solution provided by Cospheric to suspend their particles in aqueous solution. A 0.1% solution of Tween 20 was made by adding 0.1g Tween 20 per 100ml of boiled Milli-Q water. The mixture was subjected to an immersion blender for 30 seconds, until very foamy. Once cooled, the foam settled, and the solution was ready to use. The proper ratio to suspend particles is 5:1 Tween solution to microplastic by volume. Ten µl microplastic was added to 50 µl Tween in a microcentrifuge tube and vortexed. The particles were then suspended in seawater.

I repeated incubations and digestions with the PE beads and observed minor degradation of beads, however they remained mildly fluorescent and visible. Therefore, it is recommended to use PE beads when digesting with HNO_3, but to alkalize the solution as soon as possible in order to preserve the beads for counting.

Well Plates

Dissolution on well plates would circumvent the need for counting via flow cytometry, because the plates could be transferred directly from the water bath onto the microscope and beads could be counted in the well. This would ensure that no beads were left behind if shrimps were digested in a glass test tube, stuck to the walls or inside of a pipette. An adapter was made to fit a glass-bottom, well plate with polycarbonate siding onto the microscope stage. Unfortunately, well plates could not be found that were 100% glass, which would be ideal for dealing with acidic conditions and high heat. So, the plates would get slightly warped and could not be used reused after heated digestion in a water bath.

After incubation, organisms were transferred into individual wells on the plate. Each individual was covered with 50µl concentrated HNO_3 and observed under the microscope. The nitric acid kills shrimp instantly and begins to dissolve fecal pellets and soft tissue within 10 minutes at room temperature. However, the exoskeleton remained unless the plates were subjected to heat, and the beads clumped together so it was not possible to visually count beads close to the remaining exoskeleton.

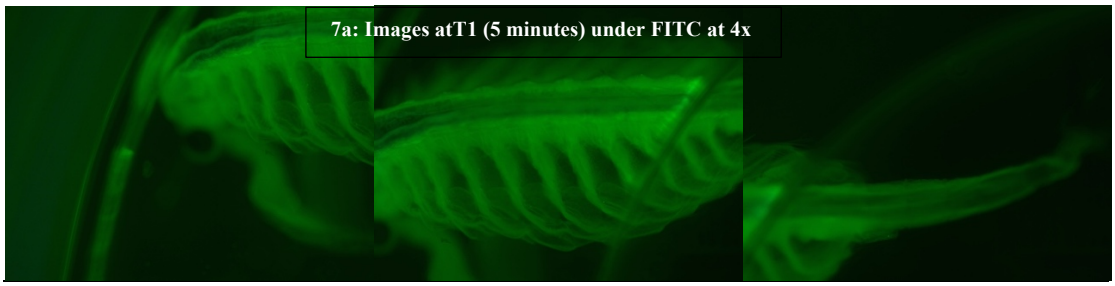
Results

First, I explore rate of ingestion of microplastic beads by A. salina through an incubation with 7 time points. Then, I examine whether ingestion is concentration-dependent by varying the concentration of beads in solution over a 4-hour incubation.

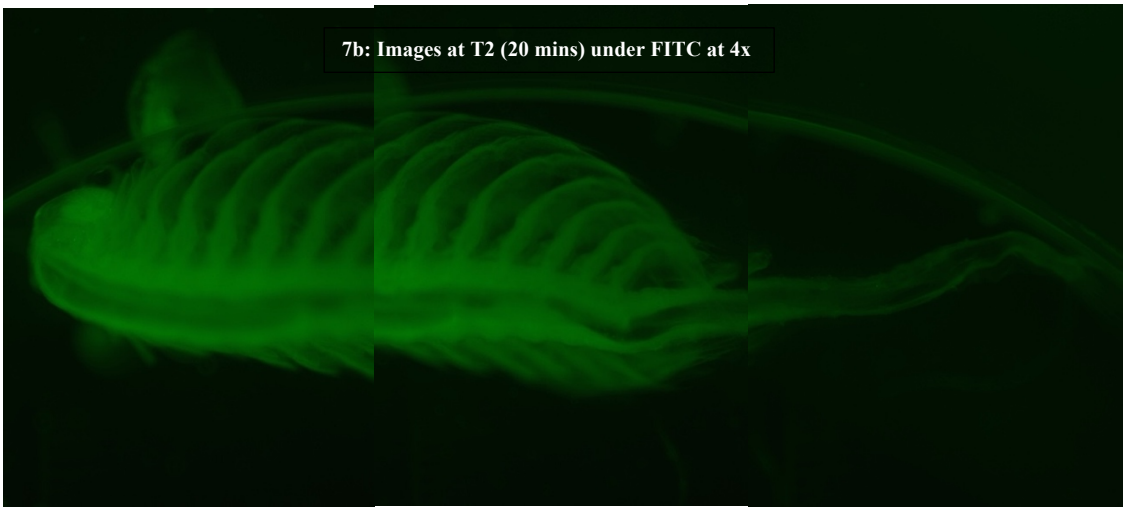
Ingestion Rate

Through this short incubation, it was determined that, under high microplastic concentrations, A. salina reached peak ingestion rate at 90 minutes after introduction to inoculated media (Fig. 3.6). Therefore, 24-hour incubations could be an inaccurate representation of ingestion, as organisms ingest and subsequently egest microplastics, shifting the proportion of microplastic floating in solution to fecal pellets at the bottom of the vessel.

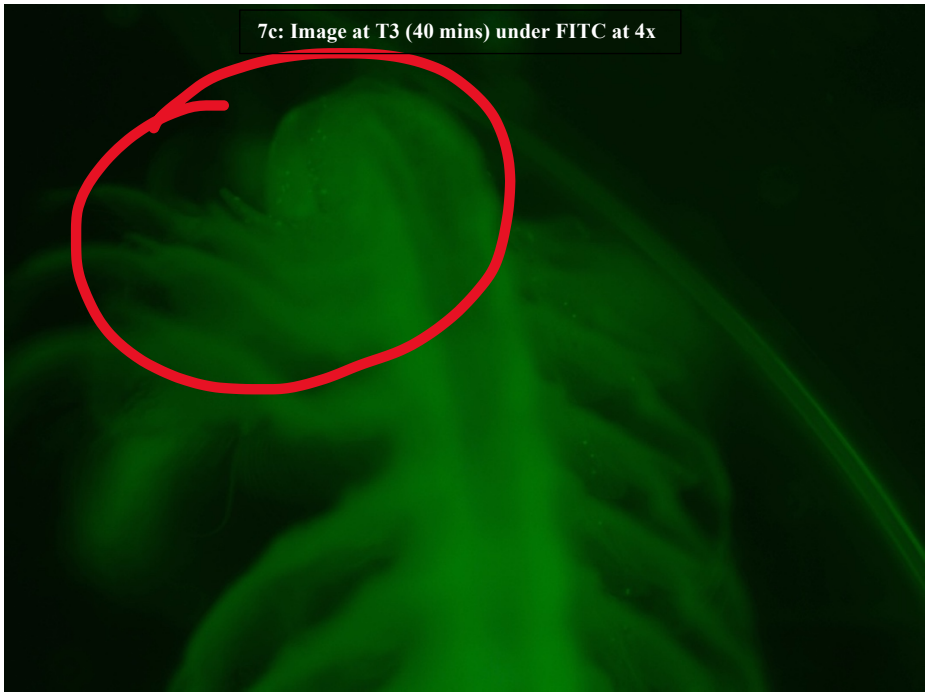
After 5 minutes (T1), no visible microplastic were ingested or could be seen near feeding appendages. At T2 (20 minutes), microplastic could be seen near the mouth, but had not yet been ingested. At 40 minutes (T3), the concentration of microplastic increased around the mouth and appeared to have begun to enter the mouth. After 60 minutes (T4), both feeding and swimming appendages are covered in microplastics and beads are visible around the mouth. At 90 minutes (T5), the microplastics have made their way into the gut and several fecal pellets are visible dotted with glowing spheres. As time continues, at both T6 (120 minutes) and T7 (150 minutes), microplastic concentrations continue to increase in the gut and the number of fecal pellets dotted with spheres continues to grow, with the highest number of fecal pellets found at T7.



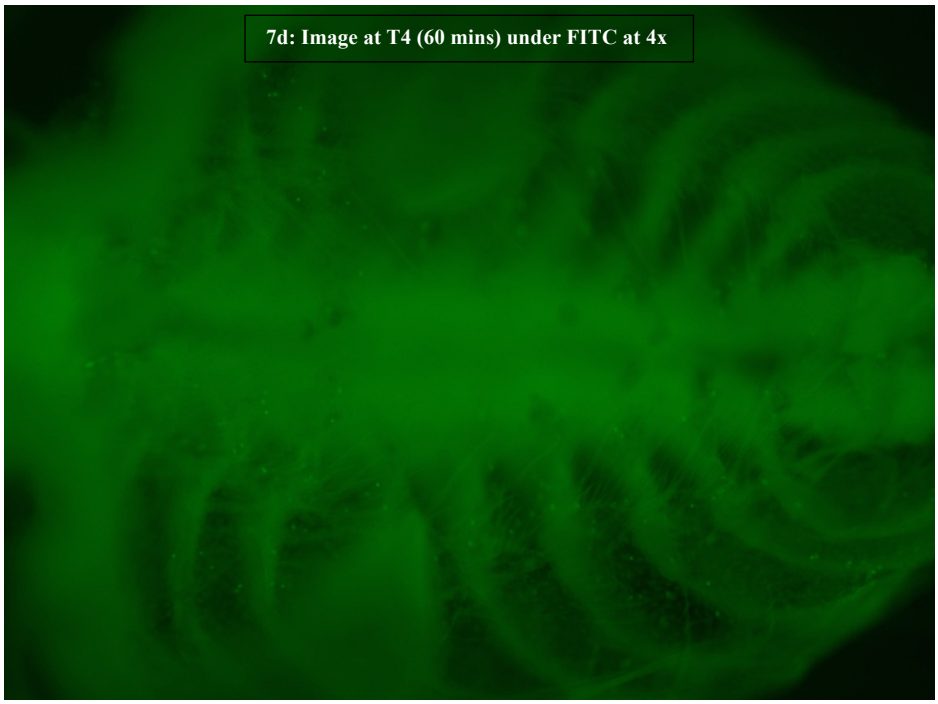
7a: Images atT1 (5 minutes) under FITC at 4x



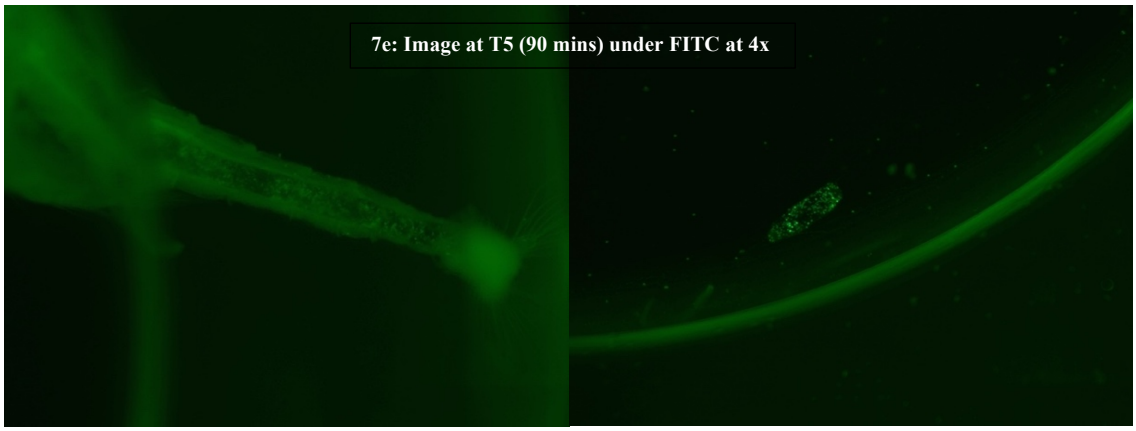
7b: Images at T2 (20 mins) under FITC at 4x



7c: Image at T3 (40 mins) under FITC at 4x



7d: Image at T4 (60 mins) under FITC at 4x



7e: Image at T5 (90 mins) under FITC at 4x

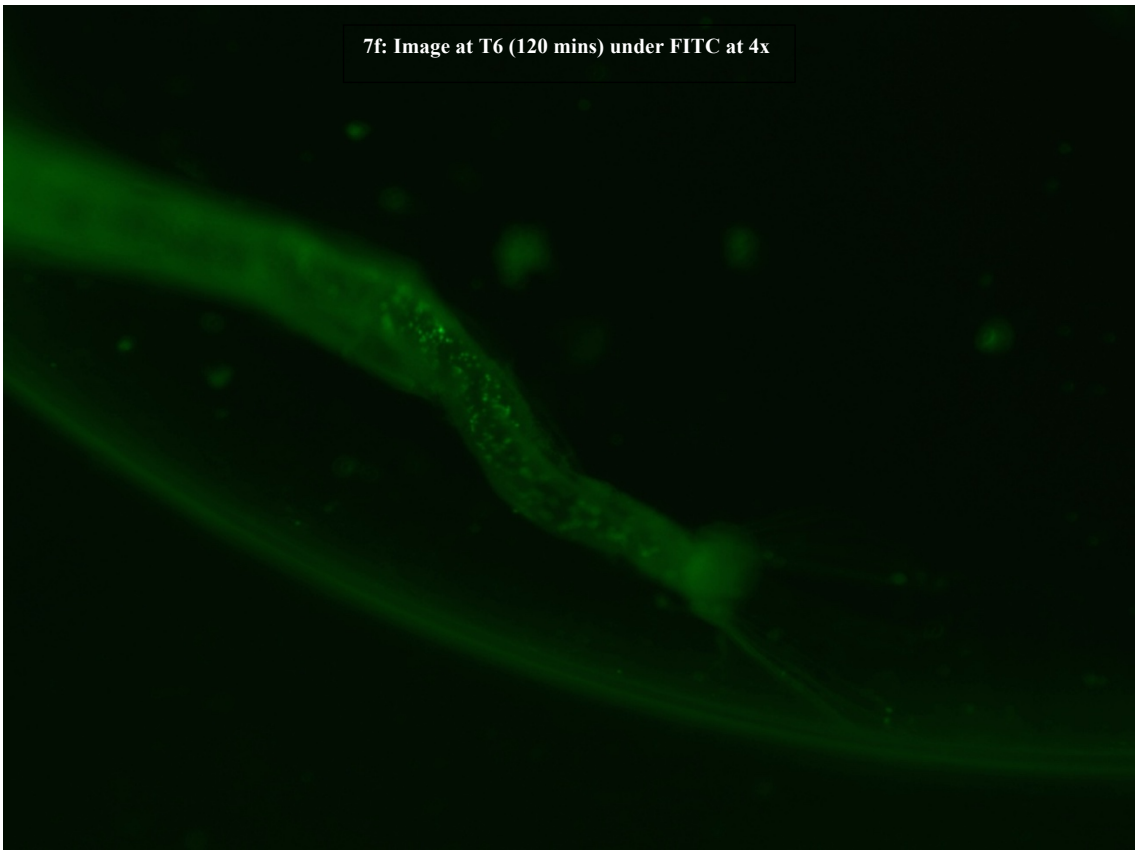


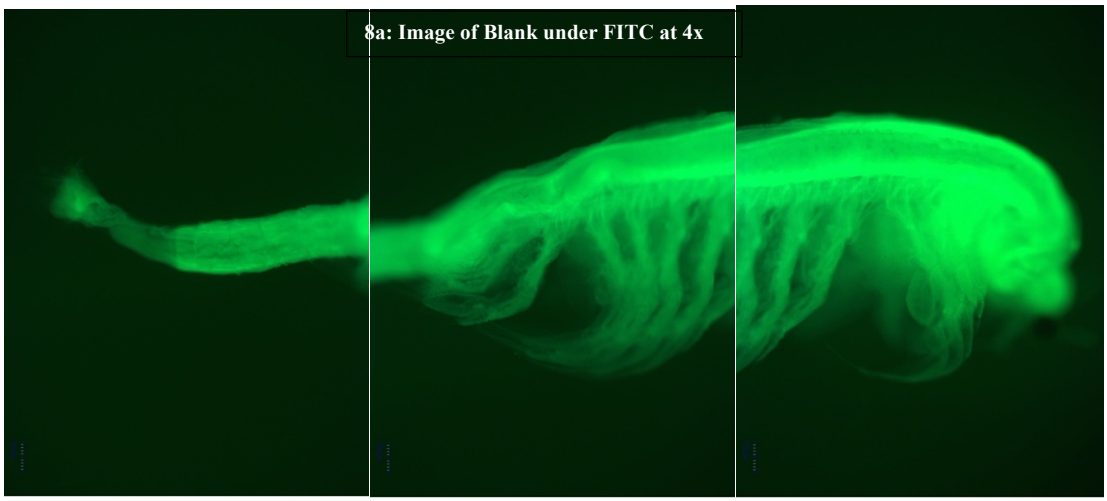
Figure 3.6: Images of A. salina under FITC fluorescence for the various time points.

Concentration-Dependent Ingestion

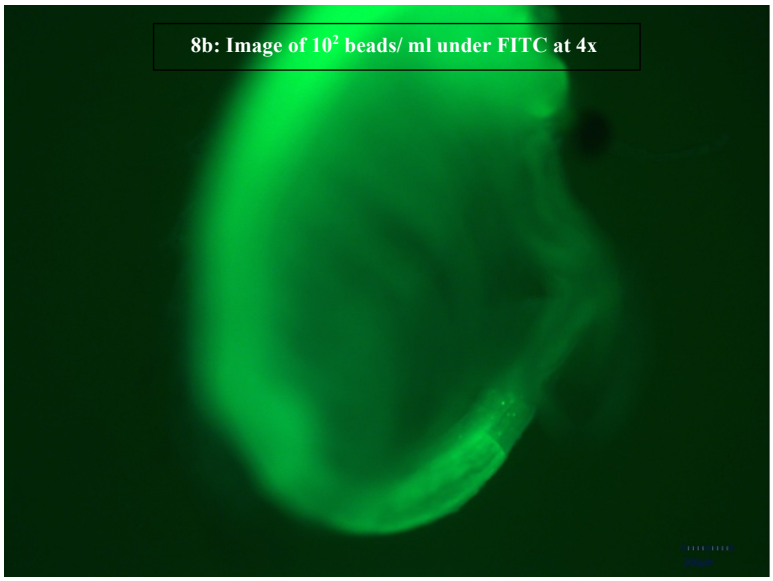
Concentration-dependent ingestion was determined qualitatively through analysis of overall FITC fluorescence in organisms after exposure to the variable treatments for 4 hours.

Results demonstrate that concentration of ingested beads increases as the concentration in the environment increases (Fig. 3.7).

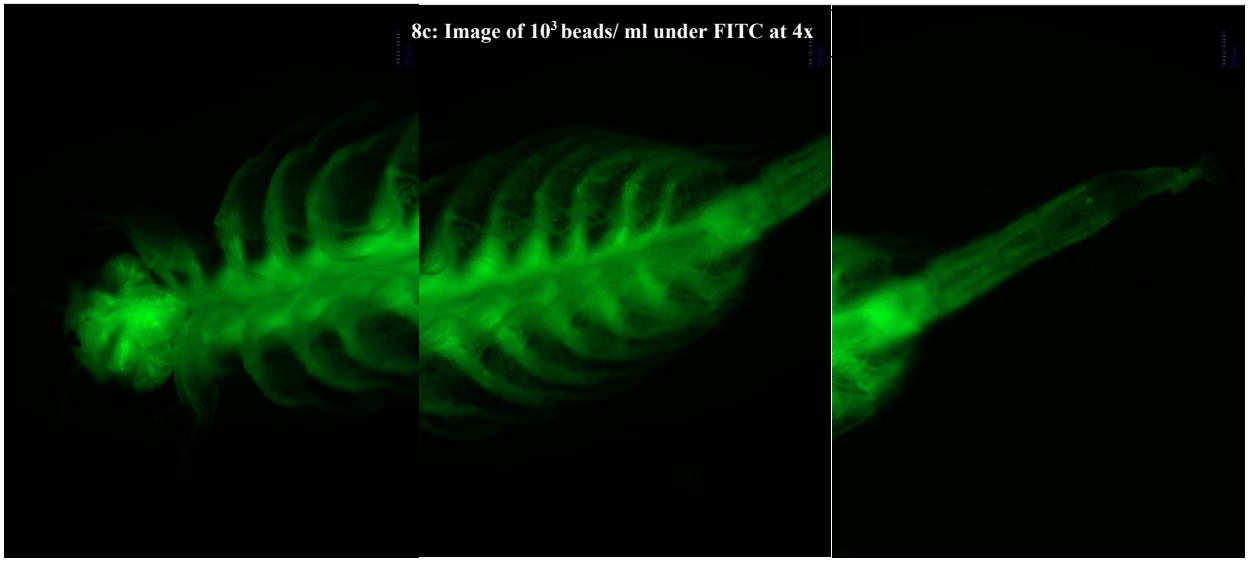
No microplastics were visible in the blank treatment (Fig 3.7a). At 10^2 and 10^3 beads/ml (3.7b,c), low concentrations of microplastic are visible in lower gut tract and near the mouth. Under the higher concentration conditions 10^4 and 10^5 beads/ml, high concentrations of microplastics appear in the lower gut, reflected in the increased fluorescent brightness in the images (Fig 3.7c, d).



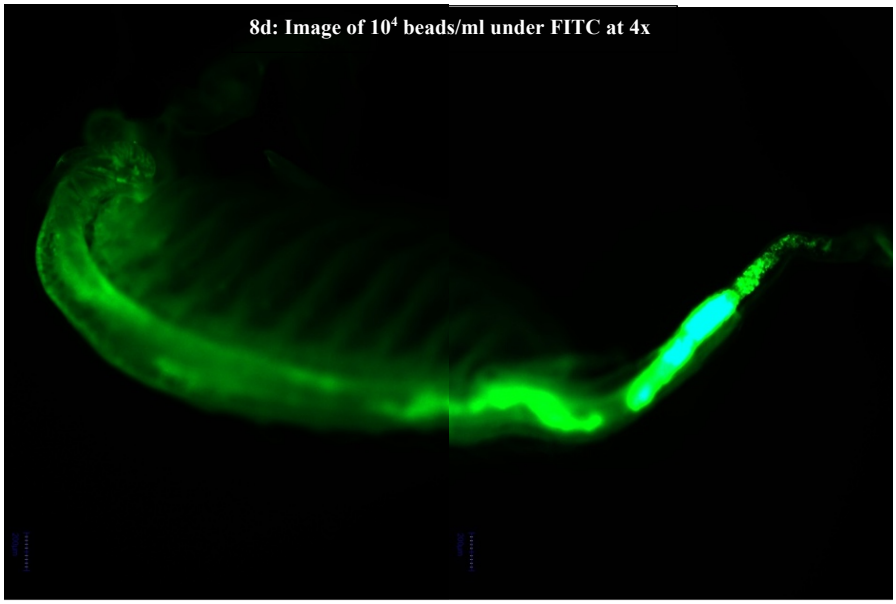
8a: Image of Blank under FITC at 4x



8b: Image of 10^2 beads/ ml under FITC at 4x



8c: Image of 10^3 beads/ ml under FITC at 4x



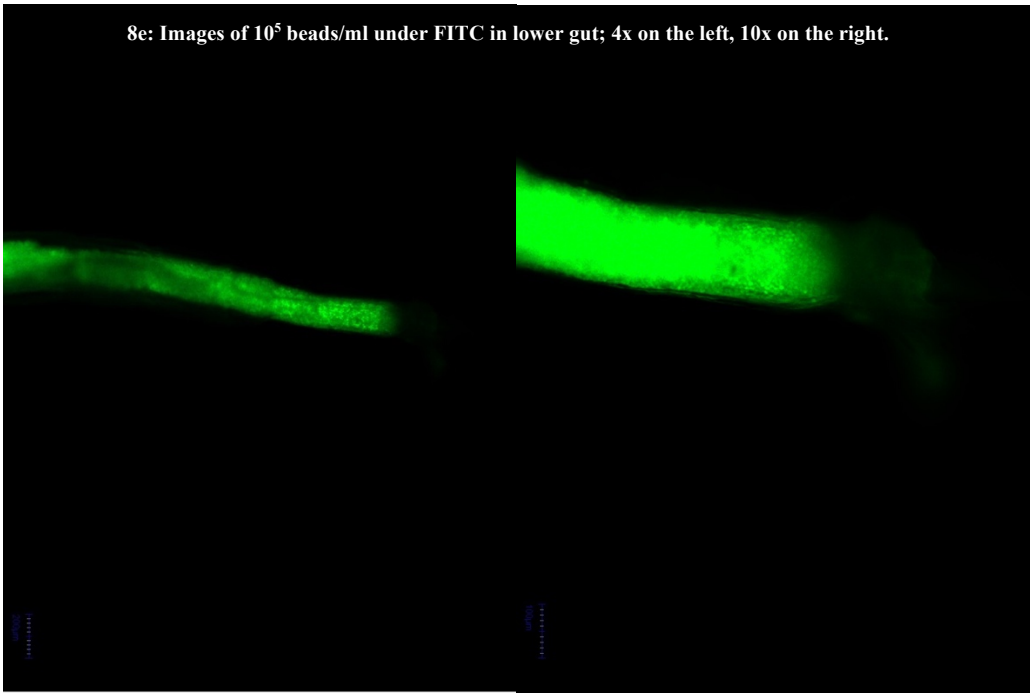


Figure 3.7: Images of A. salina under fluorescence for the various concentration treatments.

Discussion

Most microplastic incubation studies to date look at ingestion after a 24-hour period. However, here we found that rates of ingestion saturated between 90-120 minutes after exposure to beads. This rate may be unique to the study species, therefore future studies should determine the unique ingestions rates of their study species before implementing a 24-hour incubation. In addition, it would be helpful to compare the rates of ingestion across a diversity of zooplankton, as this could give clues as to which species may do better in microplastic-saturated environments. Because A. salina is a passive filter feeder, they ingest all particles of the proper size class that they come across. So, ingestion rates and selectivity of microplastic will differ in species that are active or selective feeders and incubation times should vary accordingly.

There does appear to be a concentration-dependent ingestion of microplastics, where ingestion of particles increases with their concentration in the water column. This agrees with A. salina's classification as a passive filter feeder, whereby the organism will filter all particles and ingest those that will fit in their mouth. If there are fewer particles present, less particles will be filtered and ingested. This may not be true for organisms who are selective or active feeders. Such organisms could be affected by size, shape, color, smell, and texture of microplastic particles, regardless of overall concentration in the water column; although, a higher concentration increases the likelihood of encounter.

Because Fenton's reagent is often employed in digesting sludge in wastewater treatment plants, it seemed a promising method of dissolution. It is also very inexpensive, easy to implement, and has been utilized successfully in the dissolution of invertebrates for assessment of microplastic[99,110,113,122–124]. However, it was ineffectual at dissolving the chitin

in the exoskeletons. Strong bases, such as KOH and NaOH, have been used to dissolve marine organisms, with varying concentrations, temperature, and time[115,125,126]. However, neither were effective at dissolving completely the A. salina in this study, even at the extreme end – saturated solutions of each base at high temperature.

Once it was determined that concentrated nitric acid could completely dissolve the exoskeletons, the next step was counting the microplastics ingested. In order to count the microplastics in the flow cytometer, the solution had to be neutralized. The addition of 15.7M NaOH drop by drop would remain pH 1 until suddenly changing to pH 14. When a buffering solution was implemented, pH of 8 was able to be achieved. However, this method did not work due to the creation of a hydrogel.

The melamine beads (Sigma Aldrich) dissolved completely in the nitric acid, and the polyethylene beads (Cospheric) lost their fluorescence under acidic conditions. If the NaOH was added immediately after dissolution and allowed to sit for 24 hours, some of the beads would reactivate. However, we found that not all beads reactivated and therefore visual inspection for counting could not be implemented accurately.

It is possible that, in the process of dissolving and neutralizing the solution with the chitinous components of the A. salina exoskeleton, a hydrogel similar to chitosan was produced. A hydrogel is a network of cross-linked polymers that have a great proclivity for water absorption[127]. To convert chitin into chitosan, deacetylation is performed via hydrolysis of acetamide groups with a strong base, such as NaOH or KOH at 40-50% in water at high temperature[127–129]. My dissolution steps included HNO_3 for 30-60 minutes at 80ºC, followed by the addition of 15.7M NaOH (100% saturated solution) for 24 hours. Then, buffering the solution to achieve neutral pH of 8 with KH_2PO_4. These steps may have

provided the necessary components for inducing this hydrogel. As soon as the buffer was added to the solution, the liquid congealed, and the tube could be flipped upside down and poked with a forceps. Therefore, another method of dissolution might provide a more efficient and successful way of counting the microplastic within the zooplankton, such as employing an enzyme targeted at the exoskeleton, like chitinase.

HNO_3 is the cheapest option to completely dissolve the chitin from A. salina. However, it must be done under high temperatures and beads must not be damaged. This is not an ideal solution for beads of unknown composition, as studies done on plastic collected from the environment demonstrate differential reaction to this strong acid[110,121].

Fenton's reagent is a cheap and easy digestion method, however only viable for organisms who do not have chitin, such as the soft tissues of invertebrates and fish[113,114,126]. With more funding, the chitinase enzyme is ideal for digesting zooplankton like shrimp and copepods post-incubation[109,121]. This eliminates the problems associated with strong acid, such as dissolution of plastic and inability to count via flow cytometry.

The insight obtained from following microplastics into primary consumers increases our understanding of the rates and mechanisms by which microplastics enter the marine food web and informs further research efforts. As it stands, researchers are still developing uniform methodologies for studying microplastics in the environment and within organisms. The method developed by Cole et al. (2016) for creating microfibers is an attempt to unify research so that cross-study analyses can be performed and minimize experimental differences. In order to establish uniform methodologies, they must be tested and refined. Therefore, by including microfibers in addition to microbeads in an incubation experiment,

future studies can replicate the procedure with different sizes and materials of plastic fibers, different species of phytoplankton and grazers, and at different concentrations.

It would be beneficial to compare ingestion of polystyrene and polyethylene microbeads to polypropylene, polyesters, polyamides, or acrylics, all of which are also found in high concentrations in marine debris[94]. It is difficult to cross-compare studies that utilize PS beads and PE beads, for example, because it is not known whether organisms interact with the different polymers in a similar fashion.

Further, studies can vary the ratio of microplastics to phytoplankton to represent different environmental concentrations and could also use multiple species to quantify the variance in ingestion rates amongst the vast population of zooplankton. Previous incubation studies with plastic microbeads demonstrate species-specific capabilities of selective or indiscriminate feeding behaviors, which can affect how many plastic beads are ingested[11,77,80,81,83,106]. Nonetheless, even when species can avoid ingestion of microbeads, their presence always lowers overall filtration and ingestion rates. As a result, grazers are eating less than they would in lower ratios of plastic to phytoplankton[105].

The surface of plastics are soon colonized by microorganisms upon entry into the ocean, and research demonstrates the presence of this biofilm promotes microplastic ingestion by zooplankton[79,96]. Following the incubation of both pristine and inoculated microbeads and microfibers, would provide evidence as to whether the presence of biofilm promotes greater ingestion of microfibers across study species, or has different effects based on active or passive feeding styles. Because biofilm presence produces significantly different results[96], future studies should to incorporate biofilms into their incubations. This also

provides further impetus for analysis and characterization of the bacterial colonizers that constitute plastic biofilms in different oceanic regions.

Parallel microbead and microfiber incubation experiments would explore any differences in uptake for the various categories of microplastic. In addition, further research is needed into the topic of shape-dependent ingestion because the dominant microplastic found in the environment are secondary microplastics, not perfectly spherical beads. And, within the subsect of secondary microplastics, microfibers dominate. Therefore, studies on ingestion of beads may not accurately depict the way organisms interact with plastic in the marine environment. Because synthetic fibers are freely released into the environment, this form of microplastic is of primary importance to understand and track through ecosystems and into organisms.

Any and all of these proposed studies would yield great insights into the reality of microorganisms and microplastics in the marine environment. Further research that contributes to information regarding the mechanisms with which microplastics infiltrate the food web is greatly needed. This information will continue to help identify the most vulnerable organisms in terms of particle ingestion and further studies which will evaluate where and how microplastics and associated toxins are accumulating in larger organisms.

CHAPTER 4

Interactions and effects of plastic pollution on invertebrates and marine ecosystems

Adapted from:

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Abstract

Marine invertebrates are a diverse group of organisms comprising most of the macroscopic animal life in the ocean and a significant portion of the ocean's microscopic life. Invertebrates are often the primary consumers in marine systems, and as such they form an important link by which microplastics can enter marine food webs and be transferred to higher trophic levels. The group includes animals with a variety of benthic and pelagic habitats that can adopt either active or passive grazing strategies. This diversity of life strategies makes the issue of microplastic consumption by invertebrates a challenging yet important topic to understand because it affords numerous ways by which microplastics can enter the food web and affect the health and functional activities of these organisms. Accordingly, it is important to understand the factors that affect plastic ingestion rates by invertebrates and their role in introducing plastic to the food web (including the potential to bioaccumulate plastics, transfer toxins and affect nutritional quality to higher trophic levels). Additionally, characterizing the ability of invertebrates to raft and their susceptibility to plastic-borne diseases is likewise important because it carries implications for the carbon

cycle and the sinking rates of particulate carbon. This review summarizes the sources and types of plastics in the marine environment and synthesizes the current literature on how plastic bioaccumulates, transfers toxins and pathogens, and impacts organismal health – both its own and that of its consumers – across a range of invertebrate taxa.

Introduction

Marine Invertebrates

Marine invertebrates are both microscopic and macroscopic, and they occupy both the pelagic and benthic environments. Invertebrates are often the primary consumers in marine ecosystems, interacting with the ocean's smallest organisms. There are six phyla of invertebrates commonly found in the ocean: Porifera (sponges), Cnidaria (corals, jellies, anemones), Annelida (segmented worms), Molluska (octopus, squids, snails, clams, mussels, and scallops), Arthropoda (euphausids, copepods, barnacles, shrimp, and crabs), and Echinodermata (sea stars, urchins, and cucumbers). Benthic invertebrates live on the seafloor and within the sediments; some are stuck on the seafloor (like sea cucumbers) while others can alternate between the benthic and near-benthic water column (like shrimp and octopi). Zooplankton are pelagic invertebrates and include larval stages of all six phyla.

Because microplastic, phytoplankton, and zooplankton occupy the same size class, the capacity for microplastic to disrupt normal predator-prey interactions is significant. In addition, the benthic-dwelling invertebrates filter-feed through water and sediment, making ingestion of microplastic suspended in the water and buried within sediments likely. Here, we discuss studies that attempt to assess the effects that microplastic has on various aspects of invertebrates in the marine environment. Invertebrates directly graze upon microplastic;

plastic can transfer chemical additives into the gut tissues, alter the buoyancy of the organisms themselves or their fecal pellets, and have impacts on higher trophic levels. In addition, microplastic can be a vector for both pathogens and invasive species.

Marine invertebrate interactions with plastics have been observed in every major ocean basin across a variety of phyla and subphyla or class. However, crustaceans and bivalves currently dominate the literature[26,130–132]. Furthermore, within these class/subphyla blue mussels (Mytilus edulis) was the most common species. Because of the large number of studies on M. edulis plastic ingestion, they have been proposed as an indicator species of coastal microplastic pollution[133]. While an indicator species would be useful in comparing microplastic concentrations in tissues harvested from varying geographic locations, the emphasis of plastic field studies on such a small number of invertebrates makes laboratory studies on a variety of marine invertebrates seem out of context.

Sources and transport of plastics

Plastics enter the marine environment via a variety of pathways. Plastic can enter the ocean as either primary or secondary microplastics. Primary microplastics are small by design, while secondary microplastics form when larger plastics are broken into smaller pieces by weathering[14,16,134].

Litter from land can be blown or otherwise deposited in the ocean from waterways. A scaling analysis of litter entering the marine environment from mismanaged land-based waste estimates 4.8 to 12.7 million metric tons of debris enters the ocean annually, with China and other Southeast Asian countries as the largest sources[135]. However, these

estimates may have changed as China implemented governmental management techniques in 2018, and African countries are improving plastic waste mismanagement[136,137]. Plastic also enters the ocean via rivers; it is estimated that 10 rivers transport 88-95% of the global plastic load into the sea, carrying 0.41 to 4 million tons per year[138]. Cargo lost at sea from container ships, lost fishing gear, and discharges from ships can be a source of both manufactured plastic products and plastic raw materials such as pre-production pellets[69,139].

Sewage outfalls contribute small plastics to the coastal ocean because current water treatment technologies designed to clean sewage before it is released into the environment are not designed to capture plastics that enter the sewage system as microbead scrubbers in personal care products or microfibers released from synthetic clothing during washing[134,140]. Though, some facilities have been found to inadvertently retain upwards of 83% of microplastics, depending on filtration methods, over tens of thousands of particles to millions of particles are still released each day, depending on treatment plant size and location[122,123,141].

Once plastics enter the ocean, they are subject to a variety of weathering and transport mechanisms. Through biodegradation, photo-oxidation via ultraviolet (UV) light, and thermal degradation followed by exposure to frictional forces (e.g. abrasion, wave action), plastics can fragment into microplastics (particles < 5 mm in diameter)[14,16,142]. UV-induced weathering is accelerated for buoyant plastics floating on the ocean surface while darker colored plastic experience enhanced thermal weathering[16]. A recent study found evidence to suggest UV light can weather plastic into dissolved organic carbon (DOC) that can then be consumed by phytoplankton, which may alter the structure and activity of lower trophic levels[143]. Even if photodegradation does not decrease the size of plastic, it can alter

the color by bleaching brighter colored plastic. Plastic color may impact ingestion preferences in invertebrates, because studies demonstrate dominant ingestion of less vibrant hues, blues, white, and transparent microplastic[120].

Plastic that is denser than water can sink when it first enters the ocean, whereas buoyant plastic can increase in density when colonized by fouling organisms, such as barnacles, polychaete worms, hydroids, mollusks, and bryozoans, eventually sinking as a result[69]. Animal ingestion of plastic serves as another sink for plastic from the ocean[144,145]. Ingestion of sinking plastic has been observed in benthic organisms, including anemone, sea cucumber, zoanthid, sea pen (cnidaria), hermit crab, and squat lobster, before it is buried[146]. The most comprehensive estimates of benthic plastic waste suggest that 11.1 billion plastic items are entangled on coral reefs across the Asia-Pacific regions and forecast this to increase 40% in the next 5 years[147].

Buoyant plastic that floats in the ocean is transported by currents to convergence zones, like the 5 subtropical gyres in the Atlantic, Pacific, and Indian Oceans, where concentrations of floating plastic are higher than in the rest of the ocean[69,86,148,149]. Surface concentrations of plastic in convergence zones can exceed 200,000 pieces per km^2, and therein may be especially impactful areas for marine invertebrate interactions[69,150]. Deposition of plastic along coastlines from the ocean is common, sometimes in areas where currents that have travelled across long distances of open ocean.

As water travels, the accumulated plastic may deposit on the first land it encounters[151]. An estimated 170 trillion plastic particles, or 2 million tons, are afloat in the ocean[152]. However, estimates of 19 to 23 million tons of plastic entered aquatic systems in 2016, suggesting that a significant proportion of plastic remains unaccounted for[153]. This

implies that the majority of plastic have either sunk, been eaten, been deposited on shorelines, or been otherwise removed from the floating debris portion. Clearly, more research and accounting are needed into the various fates of microplastic once it enters the ocean.

There are several limitations on sampling and estimating microplastic abundance in the ocean. In general, there is an absence of uniform collecting, separating, counting, and identification methods. A significant problem arises from using a mesh size for sampling nets that inhibit plastics less than 0.3 mm from being captured and thus enumerated[108,142]. In addition, the microplastics that are most prevalent in the marine environment are not often the plastics used in lab-based experiements[7]. In order to effectively understand how plastic and marine organisms interact, future studies should utilize environmentally-relevant particles.

Classification of plastics

Marine plastics can be classified via a variety of different methodologies. One key method is by their chemical composition (Fig 4.1). Plastic products are made from a variety of resins that can be augmented and combined to have ideal properties for a specific purpose, e.g. flexibility for plastic grocery bags or rigidity for food containers. The chemical composition of plastics depends on their constituent mixture of polymers, additives, copolymers, composites, and surface coatings[142]. Some of the most common plastic resins used in products include polyethylene terephthalate (PET), high-density polyethylene (HPDE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), and polystyrene

(PS)[14]. As these six plastic resins comprise 90% of plastic production globally, they are expected to be the most observed plastics in the ocean[144].

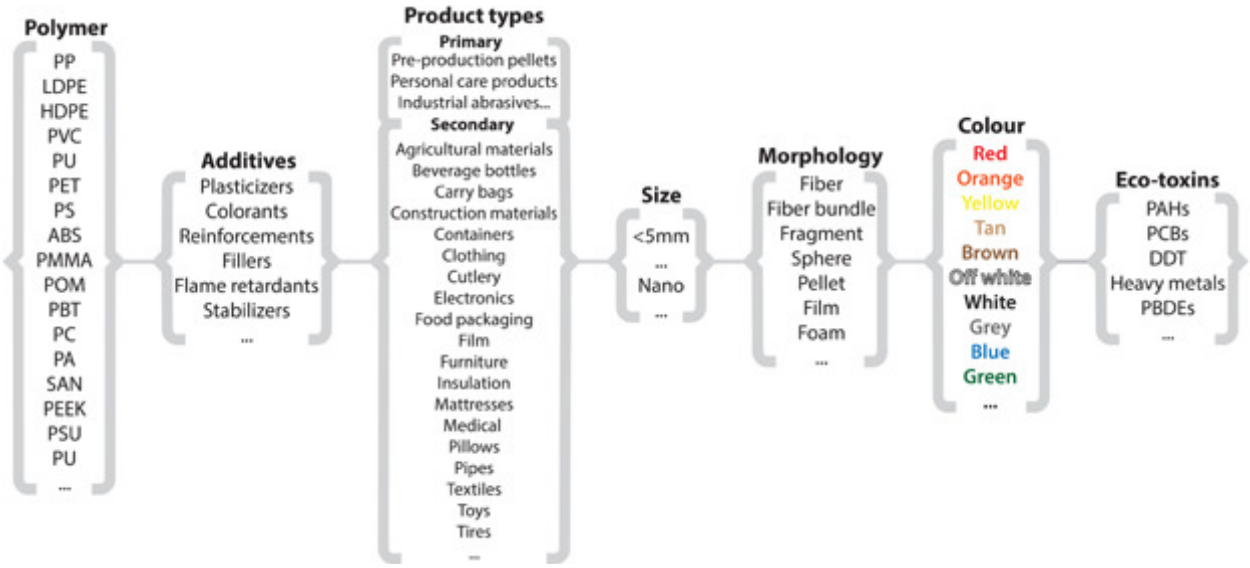

Figure 4.1: Ways of describing microplastic[14]

In particular, the PS and LDPE are low density plastics that float, and most marine plastic studies occur in the upper regions of the ocean and in shallow oceanic systems[154]. However, plastics made from polyacrylonitrile, polyester, and polyamide have also been observed in the guts of benthic marine animals in the deep sea, including the phyla cnidaria, Echinodermata, and Arthropoda, indicating other plastic resins are also present in the ocean[146,155]. A summary of plastic densities is shown in table 4.1. A density of less than 1.02 g/cm^3 (density of seawater) is required for plastic to float in seawater.

Table 4.1: Densities of most common plastic resins observed in the marine environment.

Plastic Resin	Density (g/cm^3)
Polyethylene Terephthalate (PET)	1.38
High-Density Polyethylene (HDPE)	0.93 to 0.97
Polyvinyl Chloride (PVC)	1.30 to 1.45

Low-Density Polyethylene (LDPE)	0.92 to 0.93
Polystyrene (PS)	0.96 to 1.04
Acrylic	1.18
Polyester	1.38
Nylon	1.15

Another key method of categorization for microplastics is according to their origin and morphology (Fig. 3.1). Primary microplastics are plastic microspheres and resin pellets, while secondary microplastics are broken into five categories: fragments, film, foam, fibers, and fiber bundles[7,14]. Microspheres or microbeads are perfectly spherical microplastics used in cosmetic products. These products have been banned from production in many countries, including the United States, Canada, and China. Plastic resin pellets, often called virgin plastic or nurdles, are the raw bulk material from which plastic products are created. Resin pellets are cylindrical and have been observed in high concentrations in some areas of the Pacific where loss of plastic resin pellets from cargo ships occurred[69,156].

Microplastic fragments are the worn and weathered remaining (often jagged) pieces of hard plastic. Fragments are one of the most common plastic morphologies observed and can be comprised of any type of plastic[157,158]. Plastic films include thin flexible pieces of plastic broken down from various types of plastic bags. Plastic film is most often composed of HPDE or LPDE, as the flexible nature of these resins works well for manufacturing plastic bags[157,158]. The foamed plastic category includes any plastic with a foam-like structure of light density. This category includes the trademarked brand Styrofoam, which is derived from PS, but it can include any extruded foamed plastic[157,158]. The last categories are plastic fibers and fiber bundles. One of the most common plastics found anywhere, fibers are

derived from synthetic plastics such as acrylic, polyester, or nylon used in textiles, fishing gear, and cigarettes; fibers resemble strands of fabric, while fiber bundles are tangles of 20 or more fibers[14,159].

Chemical additives

The additives present within plastic before it enters aquatic systems also have the potential to harm invertebrates because they leach into the water and into organisms that ingest plastic[160,161]. In addition to additives added to plastic during production, persistent organic pollutants (POPs) in the environment accumulate on the surface of plastic particles; legacy pollution from terrestrial environments, such as the pesticide DDT, can also sorb onto plastic and be carried to the ocean[159,162]. The potent neurotoxin, mercury, has also been shown to sorb onto plastic particles in the marine environment[30]. Upon ingestion these contaminants have the potential to leach into the tissues of organisms[160,163]. Interestingly, plastic can also act as a sink for chemical contaminants in tissues, by absorbing toxic chemicals, depending on the concentration of said contaminant within the tissues and the plastic, gut retention time, and food present within the gut[103]. A list of POPs as defined by the Stockholm Convention can be found in Table 4.2.

Table 4.2: Persistent Organic Pollutants defined by the Stockholm Convention[164].

Chemical	Date	Use	Decision
Aldrin	1995	Soil insecticide	Eliminate
Chlordane	1995	Crop insecticide	Eliminate
Chlordecane	2001	Agricultural pesticide	Eliminate

Decabromodiphenyl ether (commercial mixture, c-decaBDE)	2001	Flame retardant, plastic additive, textiles, adhesives, sealants, inks	Eliminate
Dicofol	2001	Crop pesticide	Eliminate
Dieldrin	1995	Soil pesticide	Eliminate
Endrin	1995	Crop insecticide	Eliminate
Heptachlor	1995	Soil pesticide	Eliminate
Hexabromobiphenyl	2001	Flame retardant	
Hexachlorobenzene (HCB)	1995	Crop fungicide	Eliminate
Mirex	1995	Insecticide; Plasticizer	Eliminate
Toxaphene	1995	Crop & livestock insecticide	Eliminate
Polychlorinated biphenyls (PCBs)	1995	Electricity technologies; paint, plastic, carbon paper additive	Eliminate
Dichlorodiphenyl-trichloroethane (DDT)	1995	Agricultural nsecticide	Restrict
Dioxin	1995	By-products of high temperature processes (pesticide production, incomplete combustion)	Reduce
Polychlorinated dibenzofurans	1995	By-products of high temperature processes (pesticide production, incomplete combustion)	Reduce
Chlordecone	2001	Agricultural pesticide	Eliminate
α-Hexachlorocyclohexane (α-HCH) and β-Hexachlorocyclohexane (β-HCH)	2001	Insecticide; by-product of lindane production	Eliminate
Hexabromodiphenyl ether (hexaBDE) and heptabromodiphenyl ether (heptaBDE)	2001	Main components of commercial octabromodiphenyl ether (octaBDE).	Eliminate

Lindane (γ-hexachlorocyclohexane),	2001	Seed, soil, leaf, tree, wood pesticide	Eliminate
Pentachlorobenzene (PeCB)	2001	Pesticide; unintentional by-product	Eliminate
Tetrabromodiphenyl ether (tetraBDE) and pentabromodiphenyl ether (pentaBDE)	2001	Industrial chemicals and the main components of commercial pentabromodiphenyl ether (pentaBDE).	Eliminate
Perfluorooctanesulfonic acid (PFOA)	2001	Production of fluoropolymers	Eliminate
Endosulfans	2001	Crop & livestock pesticide; wood preservative	Eliminate
Polychlorinated naphthalenes	2001	Insulating coatings for electrical wires. Wood preservatives, rubber and plastic additives, capacitor dielectrics and in lubricants.	Eliminate and Reduce
Hexabromocyclododecane (HBCD)	2001	Flame retardant	Eliminate
Short-chain chlorinated paraffins (SCCPs)	2001	Additives in transmission belts, rubber conveyor belts, leather, lubricant additives, tubes for outdoor decoration bulbs, paints, adhesives, metal processing, plasticizers	Eliminate; exemptions exist

Accordingly, an emerging role of plastics in ecosystems is the transfer of toxins to biota. PET and PS are among the most common synthetic polymers both produced and found in aquatic environments and have the ability to act as vectors for POPs and other environmental contaminants such as polycyclic aromatic hydrocarbons (PAH)[159,163,165]. Hydrophobic POPs have been observed in sea water, even though many POPs have been banned from production and use[166–168]. POPs have been found to sorb onto plastic particles,

which then carry the POPs to the ocean[162,169,170]. Smaller particles with higher surface area to volume ratios are particularly important in this process, as the surface area available for contaminant sorption increases as plastic breaks into smaller pieces.

Some POPs that reach the ocean are added at the time of manufacture; for example, bisphenol A (BPA) is integrated into plastic to increase sturdiness while maintaining clarity. BPA is an endocrine disruptor like many POPs that impair reproduction across many invertebrates including amphipods, crustaceans, and marine polychaete worms[91]. In addition, phthalates are added to plastic to make it a more rubbery and malleable material; sometimes phthalates can make up more than 50% of PVC's total weight[14].

Males of the amphid Corophium volutator developed less pronounced secondary antenna when exposed to the surfactant 4-nonylphenol, a known endocrine disruptor. As the secondary antennae are a sex-based characteristic, shorter antennae may lead to less success in recruiting females[171]. There is evidence that exposure to endocrine-disrupting POPs negatively impact the ability of barnacle larvae to settle by inhibiting the production of a protein required for settlement. A laboratory study observed up to a 50% reduction in barnacle settlement rates in treatment groups exposed to an estrogenic POP compared to the control[172]. Another plastic additive is pyrene, a PAH. Marine mussels, Mytilus galloprovinvialis, were exposed to PET and PS particles laced with pyrene, and an accumulation of pyrene in the digestive glands of the mussels was observed. Conversely, another study found that earthworms who ingested Zn-sorbed microplastics did not exhibit accumulations of Zn in their guts[173]. Further, some researchers purport that plastic is a negligible pathway for nonylphenol and BPA exposure in a marine lugworm[174]. These chemical responses, which were observed in a relatively short amount of time and at

concentrations higher than found in the environment, indicate that toxic effects could occur following chronic exposure to contaminated microplastics.

Methods

Researchers employ a variety of methods to study the interaction between marine invertebrates and microplastic. Techniques vary with lab versus field experiments, and the types of marine invertebrates under study.

Lab-based experiments involve incubating chosen organisms with various concentrations of microplastic[81,160,175]. Some studies utilize virgin microplastic beads or fibers, while other studies use microplastic collected from the environment. In addition, studies inoculate virgin microspheres with seawater in order to encourage formation of biofilms to mimic environmental microplastics in a controlled setting[96,163,176].

Field experimental techniques vary based on the study goal. Research on the presence of microplastic in the guts of various species involve collection of species and digestion of organic tissues to reveal ingested particles[107,146,155,177]. Tissues are digested with a variety of protocols, including acidic, alkaline, enzymatic, and oxidative methodologies[109]. Trophic transfer studies involve collection of organisms from a range of trophic levels in order to analyze and compare the concentration of microplastic particles in gut contents. Several lab experiments have also been conducted to mimic a trophic transfer in a more controlled setting[31,33].

Ingestion Rates

The likelihood of ingesting plastic depends on the physical characteristics of the particles like size, polymer composition, age (biofouling and aggregation), and shape[96,178]. However, the habitat and feeding strategies of a particular invertebrate, along with the concentration of microplastic within the water column, both affect the amount of microplastics ingested[9,179]. The four main feeding strategies employed by zooplankton are: (1) Passive ambush feeding, (2) active ambush feeding, (3) feeding current feeding, and (4) cruise feeding. Passive and active ambush feeders are nonmotile predators that encounter mobile prey; they differ in that the passive feeder waits for prey to collide while the active feeder will attack in response to prey detection[180]. Zooplankton that employ a feeding current will either intercept or filter feed on organisms that become trapped in their feeding current, whereas cruise feeders actively swim in order to find prey[74].

Many benthic invertebrates are suspension feeders; some benthic organisms ingest particles that are large enough to be detected, while others passively filter large volumes of water that contain small particles and allow the concentration to build up[181]. Still other benthic invertebrates filter through the sediment to find food, part of the phenomenon of bioturbation on the seafloor; this strategy also serves to resuspend settled particles into the water column[182].

In a laboratory setting, 7 invertebrate species were studied: bivalves, mysid shrimp, amphipods, and polychaetes; the bivalves ingested significantly more polystyrene beads than any other species tested, while free-swimming crustaceans contained more beads than the benthic organisms. Organisms that graze on the surface of sediment for food, such as polychaetes and some amphipods, have limited access to buoyant particles for ingestion. In

contrast, the behavior of some swimming amphipods and mysids stirs sediment into the water column, resuspending sunken particles and making them more available for ingestion. However, the highest plastic ingestion rates were observed in bivalves, which passively filter water for food. The passive ingestion of all suspended particles makes these invertebrates particularly sensitive to heightened concentrations of microplastics. In general, for all species studied, the number of beads found within an organism increased as the concentration of beads in the water column increased. At the highest concentration of microplastics (250 beads /mL), all individual bivalves, mysids, and Gammarus sp. contained plastic, while less than 50% of the deposit feeders contained beads[160].

Many laboratory incubation experiments utilize microbeads; however, microfibers are much more environmentally relevant in terms of concentration in the ocean and in terms of observed ingestion. For example in 2020, fibers accounted for over 90% of microplastic ingested by 39 zooplankton species obtained from the Bohai Sea[108]. Moreover, fibers represented 95% of microplastic ingested by two deep sea benthic invertebrates collected between 1976 and 2015[155].

Sea anemone were exposed to various polymers of microplastic in the presence and absence of brine shrimp. In the absence of brine shrimp, anemones ingested more nylon than any other polymer. However, when brine shrimp were added, over 80% of the anemones ingested all polymers[178]. This implies that likelihood of ingestion is not only dependent upon the polymer type, but the cues from the surrounding environment and biota.

Organisms that actively select food particles tend to choose food that is more nutritionally appealing. Grazers that feed on marine snow aggregates are more likely to ingest microplastics due to their associations with marine snow[75,79,130]. In addition, biofilms

tend to disguise microplastics as food particles[87,176,183]. Upon entry into the marine environment, a film of organic and inorganic compounds sticks to the surface of plastic[79]. This thin layer serves as a "conditioning layer" upon which microorganisms can colonize. Biofilms are collections of microbes which include algae, fungi, bacteria, viruses and protozoans embedded in an extracellular polymeric substance (EPS)[79]. These assemblages are desirable for microbes as the particle provides stability and the assemblage allows for horizontal gene transfer, nutrient accumulation, and protection.

The term 'plastisphere' has been assigned to describe the communities of microorganisms that colonize microplastics versus other materials and differ from seawater[184,185]. This microbial community is taxonomically distinct from, and less diverse than, communities that assemble on other materials[184]. The specific organisms that are able to form a biofilm depend on the conditioning layer formed, which is determined by the chemical makeup of the particle itself [79]. After the first layer of organisms establish, a second layer can form due to the way the primary colonizers modified the chemical composition of the biofilm[79]. Recently, researchers have found that the microbial communities within plastispheres may contain species who transform mercury into mono-methylmercury, which is the bioavailable form of this potent neurotoxin[34]. This implies that microplastic can influence the biogeochemical cycling of mercury in the marine environment, leading to increases in concentration of bioavailable mercury[34].

Ingestion of plastics by invertebrates has been shown to cause several harmful effects to the organisms themselves, as well as larger organisms that consume them[12,13,31,33,149,160,177,186]. Particle size influences likelihood of ingestion, as the copepod Centropages typicus exhibited a decline in feeding when exposed to 7.3 □m beads, which

were observed lodged in the feeding apparatus, swimming legs, furca, and antennae. However, the organism did not exhibit a lower grazing rate when exposed to 20.6 □m beads that were too large to become entrapped[81]. The decreased rate of algal ingestion by C. typicus in the lab setting observed when exposed to smaller beads followed a strong logarithmic relationship between microplastic concentration and total algal ingestion. Even when microplastics are not ingested, they indirectly affect grazing rates. Microplastics can induce mechanical problems for grazers by clogging the digestive tract and adhering to external appendages, thus interfering with mobility[160]. In addition, the ascidian Ciona intestinalis could not distinguish between phytoplankton and microbeads, and juvenile development was hindered, most likely due to microplastics taking the place of some food[9].

However, not all observed ingestions of microplastic and nanoplastic in the lab result in negative impacts. When exposed to polystyrene nanoplastics, oyster larvae Crassotrea giggs did not produce measurable impact on its development or feeding capabilities[175]. In addition, though sea urchin larvae ingested microplastics at environmentally relevant levels, they egested particles within a few hours and there were no effects observed on development and survival[179]. Some species of invertebrates can retain microplastics for longer periods of time; however, the majority quickly egest the particles within their fecal pellets[11]. For example, the copepod Calanus helgolandicus retained polystyrene beads for up to 7 days, while the shore crab Carcinus maenas retained microspheres up to 14 days after ingestion and up to 21 days after particles crossed the gills[81,187]. The variation in retention time makes assessing trophic transfer effects more difficult, as rates will depend not only on likelihood of ingestion by primary and subsequent secondary consumer, but the amount of plastic that remains within the primary consumer's gut.

Buoyancy of Plastic and Marine Invertebrates

Another potential outcome of plastic consumption is the effect of the plastic on the buoyancy of invertebrates. Ingestion of high-density plastics, such as PVC, could increase the density of free-swimming invertebrates to the point that these organisms must increase their energy expenditure to stay afloat. Conversely, animals that consume low-density plastics, such as LDPE, may have difficulty migrating vertically in the water column, as has been proposed for pelagic planktivorous fish[188]. The extent of this effect would depend on the size of the organism, the type and amount of plastic they ingest, and whether the plastic remains within the organism or is excreted in fecal pellets[29]. Ingestion of only positively buoyant or only negatively buoyant plastics would likely increase the effects, while rapid gut clearance rates would decrease the effects. However, the degree to which consumption of plastic alters an invertebrate's density is largely unknown and should be a consideration in future research studies.

Biofilm formation can also alter plastic buoyancy, as it increases the density of plastic, causing otherwise buoyant particles to sink out of the water column[79,95]. The buoyancy of a particle is highly dependent on the volume; however, the susceptibility to fouling depends on the surface area to volume ratio. As microplastics are smaller than larger marine debris, they are typically removed much more quickly from the water column[95]. In addition, the stickiness of a microplastic particle will increase after the biofilm forms, contributing to the formation of "heteroaggregates," an accumulation of other microplastics, detritus, and microbes; these further decrease buoyancy[130]. As biofouled and aggregated microplastics reach the seafloor, they are now available for filtration and bioturbation by benthic fauna, who will either ingest or resuspend the particles into the water above the sediment.

Copepods energetically link primary producers and higher trophic organisms through their production of fecal pellets. These pelagic invertebrates cycle marine nutrients by consuming primary producers and repackaging them in this form of much larger particulate organic matter, fecal pellets. This allows for consumption by larger organisms, promoting trophic transfer. Fecal pellets are nutrient-rich and dense, which means they sink quickly out of the water column and provide food for deep pelagic and benthic organisms – an important component of the biological pump[11]. Fecal pellet density and sinking rates are highly dependent upon the foods consumed; neutrally buoyant pelagic microplastics are typically low density and cause fecal pellets to sink much more slowly when incorporated. There was a 2.5-fold reduction in fecal pellet sinking rates when Calanus helgolandicus ingested microplastics[11]. These pellets were also more vulnerable to fragmentation, which can further slow the sinking rate. In addition, they demonstrated that fecal pellets are also a food source for many organisms, as C. helgolandicus ingested microplastic-laden pellets from C. typicus. This finding demonstrates that the slower sinking rate of fecal pellets not only contributes to a slower biological pump, but also promotes increased ingestion of microplastics by pelagic organisms[11,28,29].

Pathogen Vectors

Plastic waste can host pathogens that are frequently implicated in disease outbreaks of marine invertebrates, with high densities of surface bacteria increasing the likelihood of disease transmission[147,189]. Disease risk in reef-building (scleractinian) corals increased 20-fold when in contact with plastic debris in surveys from over 120,000 corals in Asia-

Pacific[147]. Although the mechanisms are not yet clear, the influence of plastic debris on disease development may have several routes.

Plastic debris promote pathogenic invasion and drain resources for immune system function during wound-healing processes via physical damage and scraping of coral tissues[147,190–192]. Additionally, microplastic can create low-light microenvironments, which can lead to anoxic conditions favoring anaerobic pathogens[147,193]. Plastic debris may directly introduce local and alien pathogens, thereby indirectly influencing beneficial microbial symbionts[147,194].

Microbial communities colonizing polypropylene were found to be dominated by the genus Vibrio, a group of opportunistic pathogenic bacteria implicated in disease outbreaks affecting a wide range of phyla including arthropods, echinoderms, and cnidarians[185,195–197]. Moreover, using laboratory trials, temperate coral polyps (Astrangia poculata) were fed microbeads with biofilms composed of green fluorescent protein (GFP)-labelled Escherichia coli[177]. After two weeks of ingestion, there was an increased GFP signal within the polyps that ingested the microbead followed by localized mortality, providing the first experimental evidence for the transfer of a bacterial pathogen from microplastic to an animal host. This demonstrates the ability of microplastics to transfer pathogens to animals via ingestion, providing a novel pathway for disease. This could increase incidence of outbreaks in ecosystems with high concentrations of microplastic debris. In addition, it could have devastating effects in aquaculture, as pathogens could rapidly spread to all organisms within the farm and to adjacent ocean environments[196].

Trophic transfer and bioaccumulation

Both plastic particles and the chemicals they contain have the potential to bioaccumulate within organisms[32,83]. This means that associated chemicals build up in tissues of grazing invertebrates and are then transferred to higher trophic consumers, where toxins will reach greater concentrations. However, retention time within organisms may significantly alter the concentration of chemicals that will bioaccumulate to higher trophic levels. Primary consumers, like invertebrate grazers, are a crucial link between primary producers and higher trophic consumers. Thus, the plastic ingested at this base level may have far reaching impacts.

Laboratory experiments in which zooplankton with ingested microbeads were fed to mysiid shrimp and found that the shrimps contained the zooplankton prey and their microspheres after a three-hour incubation[83]. Further, an experiment was conducted to replicate the trophic transfer between blue mussels and shore crabs (Carcinus maenas) and notable concentrations of microplastics were measured throughout the crab body, including the stomach, gills, hepatopancreas, and ovaries[104]. In another study, the excretory phase time of C. maenas after ingestion of microspheres was studied; researchers found that the time increased by 6-fold compared to that of non-plastic food particles. Specifically, the time from ingestion through excretion of the microspheres by the shore crabs was 3 weeks, which indicates that during this amount of time, a trophic transfer could occur[187].

Marine species that are exposed to microplastic in a short period of time may have brief side effects, but more long-term experiments are needed to gain a better understanding of the effects of chronic exposure[163]. In a partial review of studies on trophic transfer of microplastics found that, although all trophic levels studied contained microplastic, the

concentrations in lower trophic organisms were proportionally much higher than concentrations in higher trophic organisms, implying that the physical transfer of microplastics may not bio magnify[32]. However, further studies must be conducted to address the issue of bioaccumulation of chemicals associated with these plastic particles.

Transfer of plastics and their associated chemicals from invertebrates to higher trophic levels is an important emerging field, although many challenges remain. Gut content analysis of wild animals provides information on plastic ingestion, but differences in location and foraging behavior can introduce a high degree of variability between individuals[198]. Grey seal (Halichoerus grypus) scat samples and the gastro-intestinal tract of their known prey, Atlantic mackerel (Scomber Scombrus) was examined for plastic content[198]. In that study, 48% of the seal waste showed at least 1-4 microplastic particles, while 32% of the Atlantic mackerel had at least 4 microplastic particles. These results demonstrate the potential of high trophic level contamination; however, proving the exact origin of microplastics found in tissue of these animals can prove to be difficult due to the complexities of the marine food web.

Several laboratory studies were able to show that sea cucumbers can ingest PVC, nylon, and other microplastic fragments and fibers[199,200]. Surprisingly, the sea cucumbers analyzed contained a higher ratio of plastic to sediment than the ratio of plastic to sediment in the substrate, indicating that the organisms selectively ingested plastic particles over sediment[199]. In addition, sea cucumbers collected from the field demonstrate presence of microplastic within the gut contents, the majority ranging in size from 0.51μm to 2μm[201]. While field experiments prove difficult for exact comparisons to lab experiments due to

confounding factors that exist in the natural environment, the presence of plastic in the gut contents of in situ invertebrates underscores the importance of further lab studies.

Fish larvae were exposed to ciliates that contained DDT-laden microspheres and ciliates that did not. They found that the fish larvae not only ingested more DDT-laden than DDT-free ciliates, but also had a lower wet weight; this demonstrates that trophic transfer of chemicals can have negative effects on higher trophic levels[31]. More targeted studies to determine the role of invertebrates at lower trophic levels in transferring plastics to their predators are needed to understand the effects on nutritional quality and the bioaccumulation of plastics and their associated chemicals.

Rafting transport

The studies discussed thus far have focused on the physiological effects of plastics on marine invertebrates and their consumers. However, plastics can also affect invertebrate distributions and ecology by providing surfaces on which these organisms are able to attach and be transported via rafting[202–204]. Plastics tend to be colonized by sessile encrusting and fouling epibionts, but barnacles, tube worms, foraminifera, coralline algae, hydroids and bivalve mollusks are also commonly encountered[19]. Many studies have noted that marine invertebrates are transported by rafting on natural substrates such as driftwood and Sargassum; however, important differences have been observed in the characteristics of communities that colonize buoyant plastics[202,205,206].

First, the communities colonizing plastics appear to be less diverse compared to natural rafting communities, suggesting that ecological shifts in community composition and interspecies competition can result from plastic pollution[207]. Plastic debris collected along

the coast of Florida that originated in the Caribbean supported encrusted communities of invertebrates that were dominated by the bryozoans Electra tenella, in contrast to the more diverse communities typically observed on Sargassum at this site[208]. One possible mechanism that gives rise to the decline in diversity could be that competitive interactions among species are altered on plastic substrates. For example, researchers observed that rafting barnacles could either serve as a foundation species or as strong competitors depending on environmental conditions that affect organismal interactions[209]. Community composition could also be influenced by the ability of certain organisms to bore into plastics, creating pits and grooves that allow them to adhere more strongly to the material[210].

Observations of plastics collected on land and at sea have shown that plastics may also be affecting the types of organisms that raft compared to natural materials, and this has the potential to change the distribution patterns and behaviors of organisms. A study on a rafting community on plastic recovered from Antarctica showed the rafting community was dominated by invertebrates that are endemic to the Southern Ocean, and included five cheilostomatid bryozoans, two demosponges, two polychaetes, a hydroid and a gastropod[207]. Although the species were all endemic to the region, the authors note that none of these species are known to raft on natural substances, and only one species is normally found in the intertidal zone where the sample was recovered.

Similarly, a study in the Mediterranean Sea found that the native Columbus crab (Planes minutes) and Arch-fronted swimming crab (Liocarcinus navigator) were both observed rafting on plastic debris[211]. L. navigator had not been observed to raft in any prior studies, showing a change in behavioral patterns in the presence of buoyant plastic debris. These studies suggest the potential for habitat shifts to occur among naturally occurring

species in a region due to rafting. Rafting also has the potential to increase the range of invasive species, especially when trans-oceanic rafting occurs. In a study along an Atlantic Ocean transect from 68°S–78°N, the exotic barnacle species Elminius modestus was found in northern latitudes, but found fewer exotic species on plastics in the southern hemisphere, suggesting a possible geographical difference in the likelihood of the spread of invasive species[206]. The brooding reef coral (Favia fragum) was also observed on plastic in waters of the North Sea, having rafted from the Southeast USA on the Gulf Stream[212]. The authors note that the organisms were mature enough to self-fertilize, which could allow a seed population to colonize new areas but that environmental variables like temperature would ultimately modulate the spread of this species via rafting.

In addition to the effects of free-floating plastics, the expansion of invasive species is strongly affected by the plastics associated with maritime activities like aquaculture and shipping. The invasive bivalve Pinctada imbricata was found attached to a floating rope on the Uruguayan coast; it represented a possible stage I invasion and was likely transported to the region via shipping[213]. Aquaculture facilities similarly increase the likelihood of invasive species expansion, due to both the use of plastics for growing the organisms, as well as the potential for the cultivated species to escape confinement and colonize surrounding waters[203]. For example, a study of plastic rafting communities in the Mediterranean Sea near aquaculture sites identified eight aquaculture-related non-native, invasive species (Amphibalanus amphitrite, Austrominius modestus, Balanus trigonus, Hesperibalanus fallax, Hydroides elegans, Hydroides sanctaecrucis, and Magallana angulata) growing on plastics that originated from both maritime and terrestrial origin[203].

As debris float through facilities, species within aquaculture attach themselves as the litter continues its journey back out of the site. Perhaps monitoring debris that enter and leave mariculture operations might reduce the amount of invasive species transfer, even if it is limited to macroplastic debris. For sites that utilize plastic for growth within their farms, modification of material or methods might ensure the plastic doesn't escape to the surrounding marine environment with rafters in tow. Both the introduction of invasive species from aquaculture sites and the disruption of native community composition are evidence that plastic debris has the effect to alter marine invertebrate communities.

Conclusion

The growing body of literature on invertebrate interactions with plastics points to the ecological significance of this topic. It is an important factor in the transfer of plastics and their associated toxins to higher trophic levels, the spread of species and diseases via rafting and biofilm formation that can affect the carbon cycle by altering the buoyancy of organisms and their fecal pellets.

Invertebrate rafting on floating plastic debris demonstrates the potential for plastic to promote the spread of invasive species, which can have negative and lasting impacts on local populations. In order to remediate this, better waste management and plastic recuperation practices must be implemented to prevent further damage to ecosystems. Furthermore, most field studies of plastic-invertebrate interactions to date aim to study ingestion rather than rafting and displacement of invertebrates by plastic, with rafting studies limited to only a few locations. Given the impact of rafting on species transport and biodiversity, there is a need for rafting studies to fill in the geographical blanks.

The relationship between contaminated microplastics and their precise effect on an organism is difficult to understand because ocean biogeochemistry models often do not accurately represent many physiological (pH and temperature effects) and biological processes[165]. In addition, experimental evidence is difficult to precisely compare, as a uniform methodology has not yet been developed. Some lab-based studies purport that there is a negligible effect from plastic-associated chemicals on marine invertebrates[13,103,174]. Conversely, others find there is significant evidence that ingestion of chemically laden microplastic can inhibit certain bodily functions[7,163].

A variety of laboratory and field studies have been used to characterize plastic interactions with a variety of invertebrates; however, most field studies have focused on coastal areas, and certain geographical regions are understudied. An important step moving forward is to determine how representative laboratory studies are of the natural environment, and what the geographical differences are in invertebrate-plastic interactions. In general, invertebrate-plastic interaction studies are much more common in coastal areas. However, certain areas, such as the waters surrounding Antarctica, Africa, and Russia are relatively understudied. Filling in these geographical gaps should be a priority for researchers in the invertebrate-plastic interaction realm.

Clearly, more field studies of invertebrates must be carried out to provide ground-truthing for the laboratory experiments. The disparity between laboratory and field studies is even more pronounced when considering laboratory studies often expose invertebrates to concentrations of plastics well in exceedance of environmental levels and with plastic morphologies more homogeneous than is found in the environment[214,215].

Prevention of plastics from entering the marine environment is one of the most powerful tools we have to regulate the growing crisis. Using a membrane bioreactor treatment in wastewater facilities can prevent 99% of microplastics from entering the marine ecosystem, therefore technological improvements exist and should be implemented to decrease microplastic input[141]. However, recent findings purport that waste management alone is not enough to reach ambitious reduction goals, even with innovations in technology for removal from aquatic environments[152]. Reduction in plastic production is key for reduction in pollution. Further studies on the impacts of microplastics on the marine environment, marine species, and toxin accumulation can influence future environmental policy and laws, therefore continued research is urgent and necessary.

CHAPTER 5

Tidal and diel drivers of biogeochemistry and mercury cycling in a Southern California estuary

Adapted from: Jessica Walden, Katherine RM Mackey, Araceli Serrano, Priya Kaur, Christopher McGuire, Erick Partida, Bradley Nussbaum, and Doug Gibson

Abstract

Mercury (Hg) is a neurotoxin that bioamplifies in aquatic food webs, yet little is known about Hg cycling in estuaries. In this study, we sampled at San Elijo Lagoon over a diel cycle in summer to (1) characterize how biological and chemical characteristics of the estuary changed over tidal and diel cycles, and (2) determine how these factors influenced Hg cycling. Principal component analysis showed that total Hg concentrations (Hg_T) were primarily driven by the tidal cycle, with the highest concentrations occurring at low tide. Tidal mixing likewise drove the fraction of Hg_T in the dissolved phase, although the diel cycle provided a secondary influence, with a higher dissolved fraction during daylight hours. Monomethylmercury (MMHg) was below detection in nearly all samples and was only detected at low tide, suggesting groundwater or bacteria in sediments as potential sources. At the time of the study, high tidal flushing rates and long daylight hours (that support photosynthesis) resulted in relatively high dissolved oxygen (DO) levels. This likely inhibited MMHg production by anaerobic bacteria. This study suggests that in coastal lagoons, factors influencing DO (e.g., day length, degree of eutrophication) could provide a modulating effect on MMHg production, with tidal influence being the primary driver.

Introduction

Coastal estuaries are complex aquatic environments where freshwater and seawater mix, resulting in physical and hydrographic gradients that vary in space and time. The biogeochemical effect of these factors is driven by overlaid diel and tidal cycles, longer-term seasonal cycles, site geomorphology, and surrounding land use characteristics, all of which have strong effects on the chemical and biological characteristics of the estuary. In turn, biogeochemical characteristics influence an estuary's ability to provide valuable ecosystem services, such as fish spawning habitat, reservoirs of microbial genetic diversity, and contaminant processing before they reach the coastal ocean. For example, estuarine vegetation can remove anthropogenic nutrients from the water, mitigating their impact on coastal waters[216].

Estuaries are potentially important locations to study additional types of anthropogenic contaminants, such as mercury (Hg) that accumulates in marine food webs via bioamplification, an increase in concentration within tissues of a substance as higher trophic levels are reached. Because of this capability, humans can be exposed to high levels of Hg through ingestion of organisms at high trophic levels. Hg is a potent, heavy metal neurotoxin present in the environment that derives from both natural and anthropogenic sources. Environmental Hg levels have approximately tripled over the past century[217–219] due to anthropogenic activities, such as fossil fuel combustion, mining operations, and industrial applications. The cycling of Hg is highly sensitive to physical and chemical factors. Atmospheric deposition[217,218] is the major source of Hg to aquatic habitats, where biological uptake and volatilization are major sinks[218].

Total Hg (Hg_T) includes all forms of organic and inorganic Hg in aquatic systems. In coastal and terrestrial aquatic systems, Hg exists primarily as monomethylmercury (MMHg) and divalent Hg (Hg^{2+}), where Hg^{2+} typically comprises >90% of Hg_T. Once deposited from the atmosphere, Hg undergoes oxidization from Hg^0 (gaseous) to Hg^{2+} [220]. Dissolved organic matter (DOM) is crucial for Hg phase partitioning due to its high affinity for MMHg and Hg^{2+}; DOM attenuates and reacts with light, and can generate MMHg[221]. Presence of DOM skews unfiltered Hg_T concentrations due to strong sorption affinity, where changes in temperature, salinity, and photolytic adsorption and desorption affect dissolved and colloidal (i.e., filtered) Hg_T concentrations. Sunlight-driven photodegradation of MMHg to Hg^{2+} increases Hg^{2+} in bodies of water with low turbidity and DOM[222–224].

Biologically mediated chemical transformations affect the partitioning of Hg into different chemical species, including bioaccumulative MMHg. In coastal environments, MMHg is primarily formed through methylation of Hg^{2+} by anaerobic, sulfate-reducing bacteria[225–227]. Hence processes that drive anoxic conditions play an important role in controlling the flux of MMHg into food webs. In the largest bioamplification step, MMHg enters aquatic food webs via primary producers, such as phytoplankton with an approximately 100,000-fold increase in MMHg in phytoplankton cells relative to concentrations in the ambient water[228]. Conversely, algal decay contributes to MMHg in the water column[228]. MMHg concentrations in the food web continue to increase up to ~10-fold at each subsequent trophic level[229]. As a result, MMHg concentrations in predatory fish and marine mammals can be more than one million times higher than that in the water where they live[230,231].

Hg cycling varies among different aquatic environments depending on biogeochemical and physical factors (Supplementary Table 1). In a study comparing various aquatic ecosystems, the highest MMHg concentrations were found in hypersaline environments associated with high DOC, sulfur, and low pH, while the lowest concentrations were found in freshwater wetlands with the opposite biogeochemical traits[227]. Thermally stratified, still bodies of water exhibit low surface [Hg_T] due to heavy light penetration, that triggers photodegradation of MMHg to volatile Hg^0 [232], and a lack of MMHg replenishment from the sediment-water interface to the surface[223]. High algal biomass in surface waters can limit light penetration through the water, promoting Hg_T and MMHg accumulation in deeper waters due to lack of photodegradation and anoxia[227]. Conversely, moving bodies of water experience greater mixing that diffuses MMHg from the sediments throughout the water column and the surface water[223]. MMHg concentrations in the Everglades were inversely correlated with nutrient concentrations, with eutrophic areas exhibiting the lowest concentrations of MMHg[233]. In Malibu Lagoon, elevated [MMHg] was attributed to sedimentary bacterial methylation and tidal re-suspension of sediments, while elevated Hg_T likely derived from anthropogenic sources and weathering of rocks[234].

These studies in freshwater and seawater-dominated systems demonstrate the complexity of biogeochemical Hg cycling and highlight how the balance between ambient physical, chemical, and biological processes gives rise to diel Hg transformations in freshwater and saltwater environments. However, little is known about diel Hg cycling in coastal estuaries, which share certain characteristics with freshwater and seawater environments. Specifically, the chemical characteristics of coastal estuaries, including Hg dynamics[234], are strongly influenced by tidal cycles that control the relative amounts of fresh

and saltwater. Photoperiod drives biological processes that also influence water chemistry; however, very little is known about the interactive effects of tidal and diel cycles on estuarine Hg dynamics.

In this study, we sought to (1) characterize how the biological and chemical characteristics of a Southern California coastal lagoon change over tidal and diel cycles, and (2) determine if and how these factors influence Hg cycling. We hypothesized that Hg levels, speciation, and partitioning would vary over the diel cycle depending on photoperiod and tidal cycle, as well as by factors controlling oxygen availability, such as physical mixing, water quality, photosynthesis, and respiration.

Methods

Overview of Site and Sampling Schedule.

San Elijo Lagoon is a 3.7 km^2 wetland in Southern California bordered by the cities of Encinitas, Solana Beach, and Rancho Santa Fe (fig 5.1). San Elijo is a coastal lagoon that transitions from estuarine to lagoon conditions in response to seasonal berm breach and formation, respectively. The lagoon is the terminus of the Escondido Creek that drains a 219 km^2 watershed. The mouth of the lagoon is located at Cardiff Beach, where tidal exchange occurs with the Pacific Ocean when a sand berm is not present. In this study, samples were collected within the lagoon approximately 0.7 km inland from the coast, and approximately 1.4 km upstream along the length of the main channel.

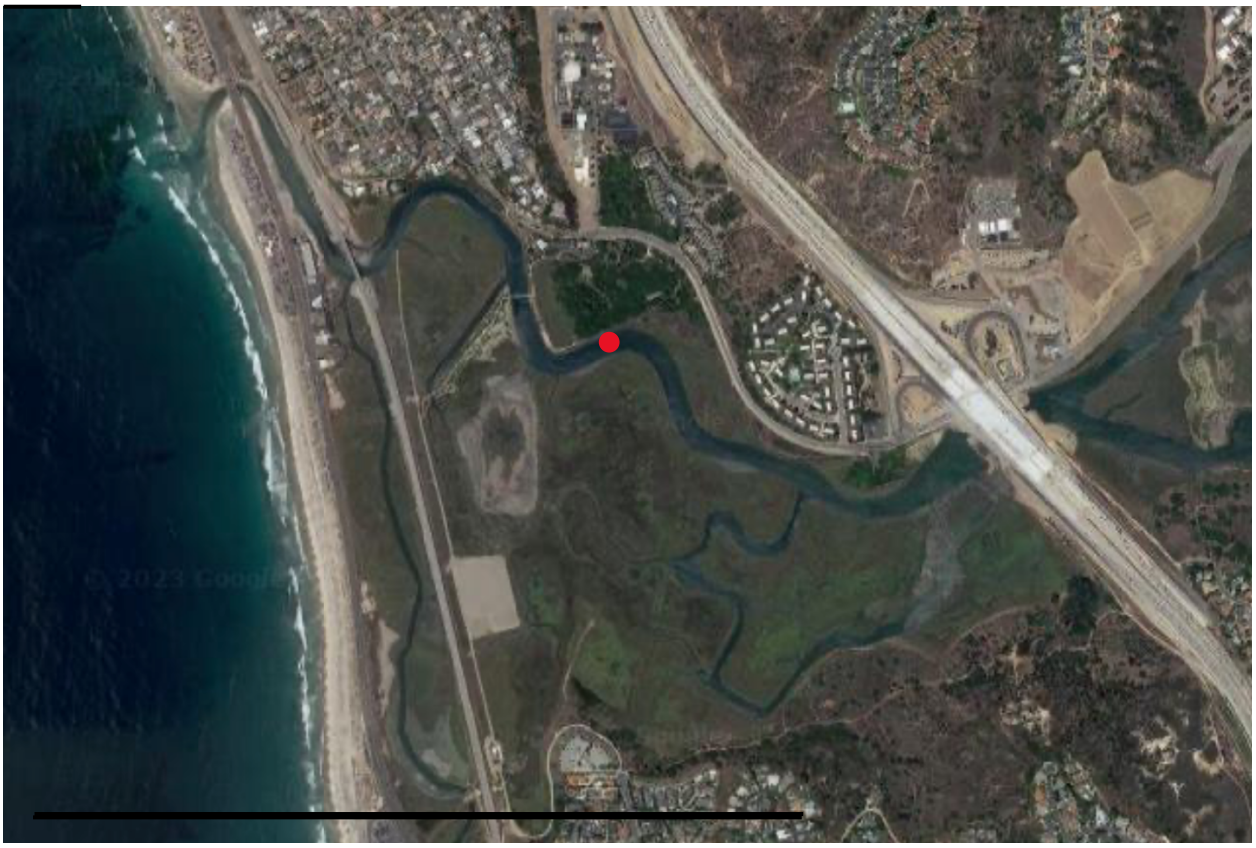

Figure 5.1. San Elijo Lagoon State Park, with sampling site marked in red. Scale bar on bottom left represents 1km.

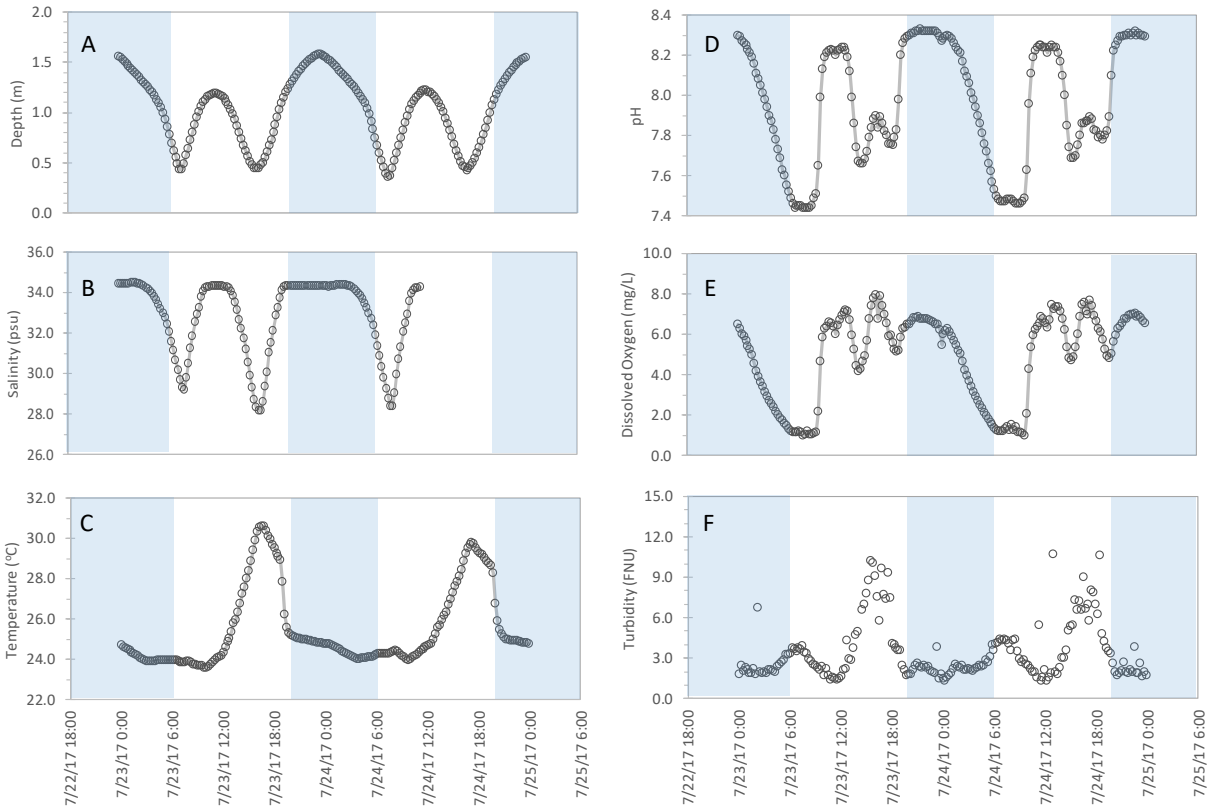

Figure 5.2: Water quality data for (A) water depth, (B) salinity, (C) temperature, (D) pH, (E) DO, and (F) turbidity measured via the permanently deployed sonde over the two-day period encompassing the diel study. Shaded regions denote nighttime hours.

Water quality probes for measuring temperature, salinity, pH, and dissolved oxygen (YSI Incorporated) were deployed along with sampling lines to collect readings from the surface and base (10cm) of the water column (Fig 5.2B). The lines were loosely tethered to a post approximately 2m from the shoreline of the lagoon to ensure they did not drift with the tides (Fig 5.3). The deep-water sampling line and probe were encased in a weighted plastic cage to ensure grounding and water flow. The cage elevated the probe and sampling line approximately 10cm above the sediment to avoid interference from excess particulates suspended during the initial placement onto the bottom sediment. The surface water sampling line and probe were attached to a buoyant foam floatation device that rose and fell

with the tide to assure measurements were consistently collected approximately 10cm below the water surface.

Figure 5.3. Photos of the sampling site and probes.

Sampling was conducted every one to two hours from 16:00 on June 23, 2017, to 12:30 on June 24, 2017. Peristaltic pumps were used to collect water through trace metal clean, Teflon sample lines coupled with in-line C-flex tubing. After collecting unfiltered samples, a 0.2µm cartridge filter (0.2 µm Supor Hydrophilic Polyethersulfone Membrane, AcroPak 200 Capsule Filter) was attached to the line to collect filtered samples.

In addition to the two water quality probes deployed with the surface and deep sampling lines, a YSI sonde was permanently deployed at the sampling site approximately 25 cm above the bottom of the lagoon. The sonde recorded water depth, temperature, salinity, dissolved oxygen, turbidity, pH, and fluorescence-based chlorophyll concentrations every 15 minutes. Data were retrieved for June 23-24, 2017.

Hg analyses

All Hg samples were collected in acid cleaned, borosilicate bottles with Teflon lined lids[235] and handled using established trace metal clean protocols[236,237]. Once collected, samples were stored in clean plastic bags on ice in the dark. Samples were then transported to the UC Irvine laboratory where they were preserved within five hours of returning from the field (0.5% BrCl (v/v) for Hg_T, and 0.2% H_2SO_4 (v/v) for MMHg.)

Samples were sent to Brooks Applied Labs to obtain Hg_T and MMHg concentrations. Total recoverable Hg (Hg_T) concentrations were determined using established protocols[238,239] following oxidation with bromine chloride, reduction with tin (II) chloride, gold trap amalgamation, and quantification by cold vapor atomic fluorescence spectrometry (CVAFS) using a Brooks Rand Instruments MERX-T CVAFS Mercury Automated-Analyzer.

The method detection limit (MDL) was 0.10 ng Hg/L and the method reporting limit (RDL) was 0.40 ng/L. The RDL is the detection limit for Hg, while the MDL is statistically calculated.

MMHg concentrations were determined on water samples preserved with trace metal grade H_2SO_4. MMHg concentrations were determined by ethylation, Tenax trap collection, gas chromatography separation, isothermal decomposition, and quantification by CVAFS following established protocols[240–242]. The method detection limit was 0.02 ng/L and the method reporting limit was 0.05 ng/L.

Principal component analysis was performed in MATLAB to determine the environmental factors that most strongly influenced filtered and unfiltered Hg_T, as well as the fraction of Hg in the dissolved phase (filtered Hg_T/ unfiltered Hg_T x 100).

Nutrient analyses

At each time point, a 0.2μm filtered sample was collected for dissolved nutrients, including nitrate (plus trace amounts of nitrite), phosphate, and ammonium analyses. Samples were collected in acid cleaned 50mL plastic Falcon tubes and were stored frozen until analysis. Ammonium was measured spectrophotometrically (640nm) using the phenol hypochlorite method[243]. Nitrate+nitrite and phosphate were measured using a QuickChem 8000 flow injection autoanalyzer (Lachat Instruments). The detection limits for these species were 0.01 μM for ammonium, 0.014 μM for nitrate+nitrite, and 0.053 μM for phosphate.

Phytoplankton identification and photosynthetic traits

Phytoplankton abundances were determined via microscopy for cells >3μm, and via flow cytometry for cells <3μm. All samples were preserved with 4% formaldehyde. For microscopy, 100mL of water were collected in glass Pyrex bottles and stored in the dark at 4^{o}C until analysis. Cells were concentrated 10- to 50-fold over 24 hours using Utermohl settling chambers. The concentrated samples were viewed using a Nikon Diaphot microscope at 40x magnification under phase contrast, and dominant cell types were identified to the level of genus. Due to extensive aggregation of cells with particulate organic material in the samples, quantitation of cells >3μm was not possible, and only qualitative assessment of dominant genera was possible.

Flow cytometry samples (1mL) were stored at -80^{o}C until analysis. Picophytoplankton cells in 50μL aliquots of sample were enumerated on a Novocyte flow cytometer (ACEA Biosciences, Inc.) based on their chlorophyll and phycoerythrin autofluorescence characteristics using the manufacturer's software.

Chlorophyll-a concentration was determined fluorometrically following acetone extraction as described previously[244]. Briefly, 70mL sample were filtered under gentle vacuum onto 25mm GFF filters (Whatman), and the filters were stored at -80^{o}C until analysis. Chlorophyll was extracted from the filters in 10mL of 90% acetone at 4^{o}C in the dark for 24 hr. Chlorophyll fluorescence was measured on a Trilogy fluorometer (Turner) using the non-acidified module. Raw fluorescence was converted to concentration using a calibration curve generated from a chlorophyll standard prepared from Anacystis nidulans.

The maximum photosynthetic efficiency of the phytoplankton community was measured on a custom designed FIRe fluorometer (M. Gorbunov, Rutgers University). The

fluorometer was operated in continuous mode for each time point. Four liters of sample were pumped via peristaltic pump into a dark de-bubbler that drained into the 25mL sample cuvette at a flow rate of ~400mL min^{-1}. Fluorescence induction from saturating single-turnover flashes of blue light were repeated 20 times and averaged to determine the maximal photochemical efficiency (Fv/F_M; variable fluorescence/maximal fluorescence) of the sample, and ~20 replicate measurements were made at each time point and averaged.

Results

Water quality analyses

Measurements for water quality were made via the permanently deployed sonde and probes for surface and bottom samples. There were negligible differences among the values from these three instruments, indicating that the lagoon was well mixed. Over the two sampling days, the water depth ranged from 0.4 – 1.6 m (Fig 1A), salinity ranged from 28.4 to 34.4 psu (Fig. 1B), and water temperature ranged from 23.6 to 30.6^{o}C (Fig.1C). The pH ranged from 8.34 at high tide during the day to 7.44 at low tide during the evening (Fig. 1D). Note that the accuracy of the pH meter is ± 0.2. DO concentrations were also dependent upon tide and time of day, with the highest levels observed at high tide during the day (7.89 mg/L) and lowest at low tide during the evening (0.99 mg/L) (Fig. 1E). Turbidity ranged from 1.31 to 10.65 FNU (Formazin Nephelometric Unit), with the highest values occurring at low tide (Fig. 1F).

Nutrient and Hg analyses.

The lack of variability between surface and bottom water chemistry characteristics in the lagoon as described above indicates that the lagoon water was well-mixed at our site. Therefore, Hg and nutrient analyses were conducted for bottom water samples only. Filtered and unfiltered MMHg concentrations were below detection (0.02 ng/L) in all but three samples (data not shown). Unfiltered Hg_T ranged from 0.32 – 1.61 ng/L, and filtered Hg_T ranged from 0.1 – 1.12 ng/L (Fig. 5.4A). The highest Hg_T concentrations occurred at low tide. The fraction of Hg_T in the dissolved phase ((filtered Hg_T/ unfiltered Hg_T) x 100) ranged from 20 – 80%, with more Hg_T present in the dissolved phase during daylight hours (Fig. 5.4B). Most nutrient concentrations reached maximum levels at low tide and minimum levels at high tide. Phosphate concentrations ranged from 0.34 to 2.85 μM, and ammonium ranged from 0.96 to 3.43 μM (Fig. 5.4C). Nitrate was below detection limit (<0.014 μM) in all samples. Dissolved oxygen and water depth measurements from the permanently deployed sonde (fig 5.2 D,E) are repeated in Fig. 5.4D,E for ease of comparison.

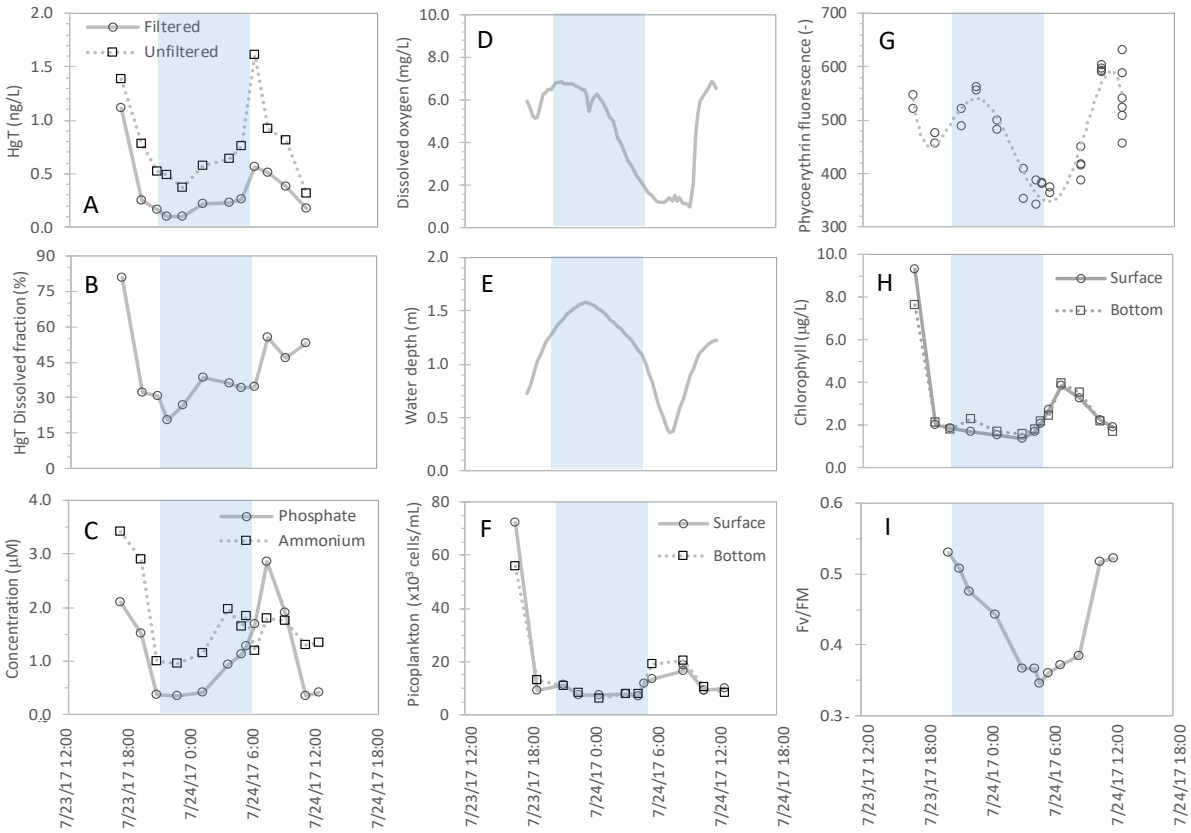

Figure 5.4: Diel measurements of (A) filtered and unfiltered Hg_T, (B) fraction of Hg_T in the dissolved phase, (C) ammonium and phosphate concentrations, (D) DO (E) water depth, (F) picoplankton concentration, (G) phycoerythrin fluorescence, (H) chlorophyll concentration, (I) Fv/Fm; collected from July 23-24, 2017. Shaded regions denote night time hours.

Principal Component Analyses

A Principal Component Analysis (PCA) is a way to compare data points by reducing the dimensionality. Samples are clustered based on similarity on a 2-dimensional plane, allowing patterns to be teased out. The analysis consists of two axes, component 1 (horizontal) and component 2 (vertical). In this analysis, component one is tidally influenced and component 2 is temporally influenced. Factors that align closely with the horizontal are tidally influenced, while factors that align closely with the vertical are temporally influenced.

From the PCA of water quality parameters and Hg variables, factors influencing Hg_T levels and speciation were identified. For filtered (Fig. 5.5A) and unfiltered (Fig. 5.5B) Hg_T

and the fraction of Hg_T in the dissolved phase (Fig. 5.5C), the first principal component in each analysis captured 68%, 66%, and 64% of the variance, respectively, and discriminated Hg_T according to salinity, turbidity, water depth, and chlorophyll. The second principal component in each analysis explained an additional 27%, 26%, and 27%, respectively, of the variation and discriminated Hg_T according to DO, temperature, and pH.

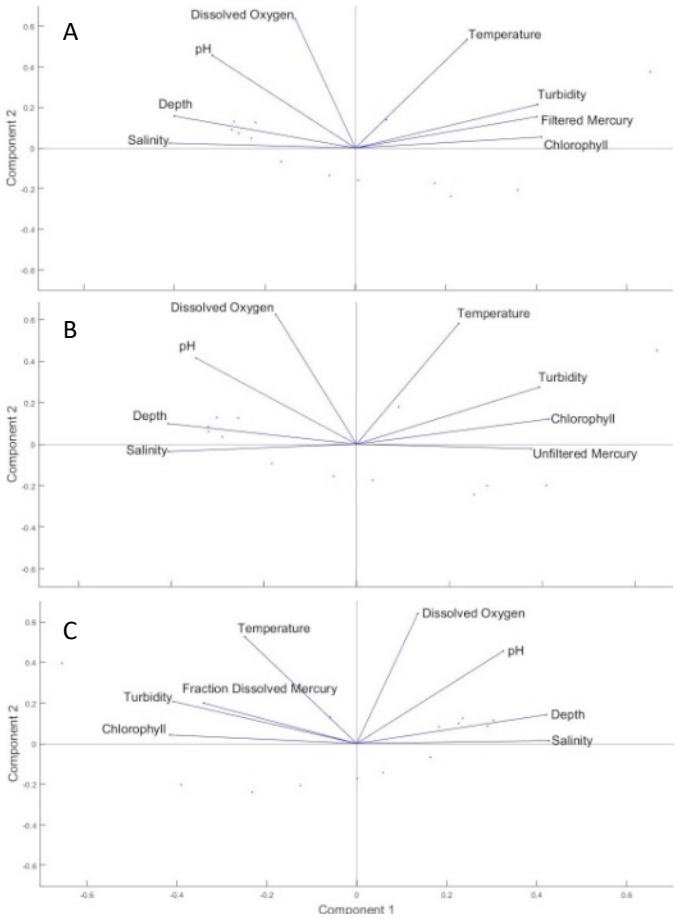

Figure 5.5: Principal component analysis for (A) filtered Hg_T, (B) unfiltered Hg_T, and (C) fraction of Hg_T in the dissolved fraction. For each parameter, PC1 captures tidal influence, and PC2 captures diel effects.

Phytoplankton identification and photosynthetic traits.

Phytoplankton (>3 μm) included cells in the following genera: Melosira, Pleurosigma, Guinardia, Nitzchia, Dictyocha, Navicula, Prorocentrum, Ceratium, and Cylindrotheca. Extensive aggregation of cells with particulate debris in the water precluded quantitation of cell concentrations for species >3μm. Picophytoplankton cell (<3μm) abundances were similar in samples collected from surface and bottom waters (Fig. 5.4F). Their abundance was highest (~$7x10^4$ cells/mL) during the day on July 23, but rapidly declined at sunset and remained relatively stable throughout the night (~$1x10^4$ cells/mL). Following sunrise, the picophytoplankton abundance briefly doubled between 6-9am, then declined back to nighttime levels by noon. The mean cellular phycoerythrin fluorescence was positively correlated with tide (Fig. 5.4G).

Chlorophyll a was measured via the permanently deployed sonde and confirmed via acetone extraction for samples collected at the sampling time points. The minimum values occurred at high tide (0.34 μg/L), and the maximum values occurred at low tide (6.21 μg/L) (Fig. 5.4H). The maximum photochemical efficiency (Fv/FM) showed diel changes with midday maximum values of 0.531 and 0.523 on July 23 and 24 respectively (Fig. 5.4I). The nighttime minima (0.346) occurred at 5:15am on June 24 just before sunrise.

Discussion

Many important biogeochemical cycles are influenced by tidal cycle and irradiance in San Elijo Lagoon. Diel monitoring at a mid-lagoon station revealed that the biological and chemical characteristics of the water were driven by the predominant water source at the time of sampling (seawater at high tide and freshwater at low tide), as well as biological

effects that resulted from diel processes like photosynthesis. The following describes how tide and irradiance influenced several important biogeochemical cycles and their effects on Hg dynamics within San Elijo Lagoon over a diel cycle in summer.

Biogeochemical characteristics over tidal and diel cycles.

Because phytoplankton can influence the partitioning of mercury in aquatic systems, we analyzed concentrations over the course of our study. Phytoplankton community structure and photosynthetic efficiency were influenced by irradiance over the diel cycle. The highest chlorophyll-a and picophytoplankton concentrations were observed during daylight hours, when photosynthetic efficiency reached maximal levels (Fig. 2I); at night the trend reversed, and concentrations decreased. This pattern is consistent with an actively photosynthesizing community that maintained high productivity during the day but declined in abundance at night due to lack of photosynthesis (hence allowing grazers to cause a net reduction in phytoplankton abundance) and dilution at high tide by seawater (that contained fewer cells). Cellular phycoerythrin fluorescence of the picophytoplankton was strongly correlated with tide (Fig. 5.4G), suggesting that the population may have comprised two types of cells, each corresponding to either the fresh water or seawater endmembers. However, it was not possible to delineate between the types, because the difference in fluorescence was not significant enough to parse them into separate groups. The nutrients ammonium and phosphate were both higher at low tide than they were during high tide, although this did not appear to affect the phytoplankton community or photosynthesis to the extent that irradiance did, suggesting the communities were nutrient replete.

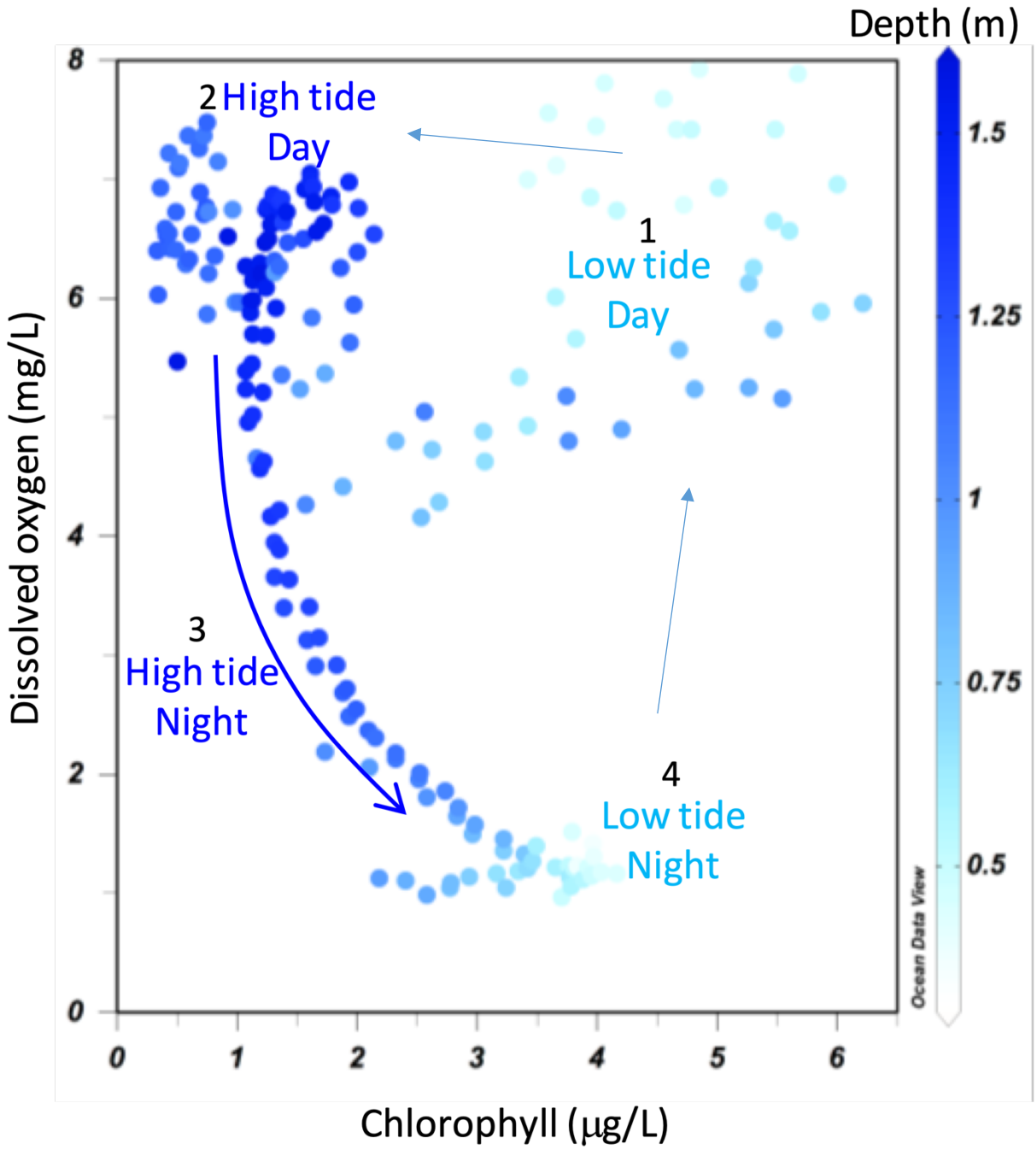

Figure 5.6: Dissolved oxygen and chlorophyll levels in San Elijo lagoon were driven by tidal and diel cycles, where photosynthesis during daylight hours maintained oxic conditions even during low tide.

The dynamic changes in phytoplankton abundance and photosynthetic activity had a pronounced effect on the lagoon's biogeochemical characteristics. In particular, DO levels in

San Elijo Lagoon were controlled by both physical and biological processes (Fig. 5.6). Higher DO levels in the seawater endmember result from the well-mixed conditions of the coastal ocean and from the low biological oxygen demand of seawater compared to lower salinity water within the lagoon. Therefore, high tides brought seawater with higher DO levels into the lagoon, while low tides brought freshwater with lower DO concentrations.

Overlaid on this physical tidal cycle was the biological effect on DO levels, which was driven by the balance between photosynthesis that generates oxygen, and respiration that consumes oxygen. During the day, photosynthesis generated oxygen, leading to relatively higher DO levels regardless of the point in the tidal cycle. At night photosynthesis ceased, and respiration became the dominant biological process. The effect respiration had on DO levels depended on the tidal cycle. Seawater with high DO content flooded the lagoon during the evening high tide, and oxygen was gradually consumed via respiration. In contrast, low tide periods flooded the lagoon with low oxygen, low salinity water, respiration further reduced DO. Therefore, the DO minimum occurred at night, but the amount of time required to draw down the DO levels depended on the tide and the initial DO levels in the water.

The lagoon DO levels were highest during the day when high photosynthetic rates coupled with increased chlorophyll content produced oxygen. At low tide, the low DO concentrations were overcome by the relatively higher concentration of phytoplankton in the lagoon water, as discussed above. Chlorophyll concentrations were approximately 10-fold higher at low tide than at high tide (Fig. 5.4H), such that oxygen was biologically generated at a faster rate at low tide than for seawater at high tide (Fig. 5.4D). Despite lower chlorophyll content, DO levels remained elevated at high tide because the seawater had higher initial DO content.

Together these overlapping tidal and diel photosynthetic cycles gave rise to four primary sets of conditions that controlled the DO conditions during our sampling (Fig. 5.6). First, when sampling began on the afternoon of July 23, the lagoon was at low tide. At this time the chlorophyll levels were high from the fresh water that dominated the lagoon, and the DO content was high due to photosynthesis. Second, as the sun set and the tide rose, seawater flooded the lagoon causing an initial spike in DO, followed by a gradual decline of DO through the dark hours as respiration dominated. Third, the tide began to ebb as sunrise approached, and the incoming freshwater brought low DO levels that were further reduced by respiration prior to daylight. Following sunrise, oxygen was generated by photosynthesis in the high-chlorophyll lagoon water, returning the system to high chlorophyll, high DO conditions.

Factors controlling Hg dynamics

Many physical and chemical factors have potential to influence Hg levels and speciation in aquatic habitats. In tidally influenced lagoons, rapid changes in redox state, DO, turbidity, and light all have the potential to affect the Hg cycle. However, the relative importance of each of these factors is likely to be site-specific and vary seasonally.

In the waters of San Elijo Lagoon, the amount of Hg_T was controlled primarily by the tidal cycle, with the diel cycle providing a secondary, moderating effect. The concentration and partitioning of Hg_T in the seawater and freshwater endmembers were likely driven by the prevailing water quality characteristics of these two endmembers. Consequently, Hg dynamics at the sampling site were predominantly driven by the relative amount of seawater

and freshwater at a given time (i.e., point in the tidal cycle), with irradiance causing a smaller influence on the system (i.e., time of day).

The concentrations of Hg_T in both the filtered and unfiltered fractions were highest at low tide, when the freshwater endmember dominated the lagoon's water content, as indicated by PC1 for each parameter (Fig. 5.5 A,B). PC1 was driven by salinity, turbidity, and chlorophyll, all parameters that were tidally influenced. Of these parameters, turbidity and salinity were more highly correlated with filtered and unfiltered Hg_T concentrations (Fig. 5.5 A,B), indicating that the lagoon was the dominant source of Hg_T, consistent with prior observations in other lagoons in Southern California[234]. PC2A presented a modulating effect that was determined by the time of day that influenced the lagoon water's DO content, pH (due to both tide and photosynthesis) and temperature (due to daytime warming).

The fraction of Hg_T in the dissolved and colloidal (filtered) phase also correlated with tidal and diel signals. Samples collected during low tide (PC1), and the daylight hours (PC2; Fig. 5.5C) contained the highest fractions of dissolved Hg_T. Filtered Hg_T concentrations showed a stronger influence from the daylight than unfiltered Hg_T (PC2; Fig. 5.5 A,B), indicating that while the freshwater endmember was a source of Hg_T, the partitioning of Hg_T between dissolved and particulate phases was influenced by photolytic processes, similar to those observed at other sites[245].

MMHg concentrations were below detection for most of the time points despite the water reaching low DO levels (Fig. 5.2E; Fig 5.4D). Low MMHg concentrations in both filtered and unfiltered fractions could indicate slow MMHg production, fast photodegradation, or a combination of both. It is unlikely that the low, dissolved MMHg levels were due to biological uptake, because most unfiltered samples were also below MMHg detection limits. We

suggest that low rates of anaerobic Hg methylation were the primary cause of the low MMHg concentrations in the lagoon, because even in pre-dawn, low tide samples, when DO levels were lowest, detectable MMHg concentrations were not observed. It is possible that the sustained high DO levels from photosynthesis and tidal flushing inhibited anaerobic microbial activity and growth, such that anaerobic bacterial populations did not appreciably affect MMHg concentrations.

Submarine ground water discharge (SGD) is a source of MMHg in other tidally influenced systems[234], due to the favorable anoxic conditions within the sediment. For example, in Malibu Lagoon, MMHg transport from SGD was most pronounced at low tide when groundwater seeped from the shallow aquifer into the lagoon[246]. It is possible that anaerobic microbial activity in the sediment could play a significant role in San Elijo Lagoon during periods of sustained anoxia. For example, during winter when day lengths are shorter (minimizing photosynthetically derived oxygen input) or following the decomposition of large algal blooms in the summer, which would deplete oxygen levels due to enhanced microbial respiration. Indeed, the few samples in which MMHg levels were above detection in this study occurred around low tide, suggesting the SGD is a potential MMHg source at this site. During our sampling period however, the rapid tidal flushing, long daylight hours, and relatively high DO levels throughout the day likely reduced MMHg levels in the water. Additionally, we have observed that while groundwater samples from this site are anoxic throughout the year, the water above the sediments is not anoxic. It is possible that the heterogeneous substrate at our sampling site that has multiple layers of sand and smaller sediment (e.g. mud), could reduce the flux of groundwater into the lagoon. Anoxic groundwater may therefore be a larger source of MMHg in estuaries with coarser sediments.

Future Work

Continued study of Hg dynamics across a variety of aquatic environments is imperative. To better understand Hg cycling, it is important to quantify the dynamics within a greater number of lagoons, for varying lengths of time, and across seasons. This study captured characteristics of a tidally influenced lagoon in the middle of summer over a two-day period. First, as demonstrated through this and previous research (Supplemental Table 1), the diel cycles of Hg species vary strongly depending on ecosystem type—wetland, estuary, coastal and open ocean environments all exhibit different biogeochemical characteristics that influence Hg cycling. For estuaries, the tidal forces and flushing time will have strong impacts. Potential effects of the photocycle on phase partitioning are obscured in environments where two bodies of water are mixing. Second, anthropogenic Hg enters coastal waters via freshwater transport. Because Hg^{2+} is a precursor to bioaccumulative MMHg, understanding the biogeochemical characteristics that favor MMHg production will help determine ecosystem hotspots and drive mitigation efforts towards relevant areas.

The aquatic Hg cycle comprises a highly complex set of interrelated reactions and processes, each influenced by a range of physical, chemical, and biological factors. In contrast to other aquatic systems, in which these factors have comparatively less variability in space and time, predicting Hg levels and speciation in tidally influenced estuaries is challenging due to the interplay between spatial and temporal characteristics of each site. In San Elijo Lagoon, Hg dynamics were driven primarily by physical processes, where the concentration of Hg_T was determined by tidal mixing, and Hg partitioning was influenced by mixing and the diel cycle. The biologically mediated effect of photosynthesis and respiration on DO levels, although expected to influence the production of MMHg via regulation of anaerobic

microbial activity, was not observed at this site at the time of our sampling. However, anoxia could support MMHg production at the sediment interface at other sites within the lagoon, or at other times of year (e.g., shorter days, following decomposition of algal blooms, etc.). This process may be more pronounced in other estuaries that have sustained periods of anoxia (e.g. eutrophication). Future investigations on estuarine Hg dynamics should investigate sites with different characteristics (geomorphology, watershed land use type, residence time), along with seasonal sampling to capture the effects of algal blooms, variable photoperiod, and precipitation events.

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Table 5.1: Summary of studies on diel mercury dynamics in aquatic systems.

Site Description	ΔMeHg	ΔHg	ΔDGM	Driving Processes	Citation
Surface water from 2 contrasting lakes in Kejimkujik, Nova Scotia, Canada Big Dam West Lake: 44°46′25′′ W 65°29′50′′ N Puzzle Lake: 44°32′25′′ W 65°23′08′′ N	25% increase from nighttime minimum to 12PM peak			DOM-dependent photoproduction of MeHg in water column	Siciliano, S.D., O'Driscoll, N.J., Tordon, R., Hill, J., Beauchamp, S., Lean, D.R.S., 2005. Abiotic production of methylmercury by solar radiation. Environ. Sci. Technol. 39, 1071–1077.
Incubated lake water from 4 different lakes (2 logged, 2 not logged) in Lac Berthelot, Quebec, Canada 48.5217° N 76.1591° W	Puzzle Lake incubated water experienced 25% increase from night minimum to 12PM peak			Size and concentration of DOM determines whether or not FW lake can generate MeHg DOM < 5 kDa or 30 kDa < DOM < 300 kDa produced MeHg when exposed to sunlight Lakes in logged watersheds produce MeHg, while those in unlogged watersheds do not	Siciliano, S.D., O'Driscoll, N.J., Tordon, R., Hill, J., Beauchamp, S., Lean, D.R.S., 2005. Abiotic production of methylmercury by solar radiation. Environ. Sci. Technol. 39, 1071–1077.
Surface lake water in Experimental Lakes Area (ELA) Northwestern Ontario, Canada 49.7833°N 93.8158°W	Decrease in incubated lake water exposed to PAR No change when bottles are in the dark			Abiotic photodegradation; first order rate (directly proportional) with respect to [MeHg] and solar radiation intensity Abiotic photodegradation	Sellers, P., Kelly, C. A., Rudd, J. W. M. & MacHutchon, A. R. Photodegradation of methylmercury in lakes. Nature; London 380, 694 (1996).

				350x more than biological demethylation	
Silver Creek in Helena, Montana N48°44′58.8″ W112°15′17.1″ Drains gold mine basin	68% increase in filtered [MeHg] from 0900h minima to 1500h peak	24% increase in unfiltered Hg from 0600h minimum to 2100h peak 7% increase in filtered Hg from 0600h minimum to 2100h peak		Filtered MeHg followed photo cycle which indicates: 1. Biotic methylation dependent upon photosynthesis 2. Temperature-dependent methylation: increase temp, increase methylation by humic compounds pH decrease causes methylation rate increase because bacterial uptake is better at lower pH Unfiltered Hg responded to changes in suspended particle concentrations because of strong sorption affinity Filtered Hg responds to changes in temperature, pH, photolytic adsorption-desorption Biogeochemical influence, not hydrological, because diel cycle of Hg species not in phase with diel cycle of stream flow	Nimick, D.A., McCleskey, R.B., Gammons, C.H., Cleasby, T.E., Parker, S.R., 2007a. Diel mercury-concentration variations in streams affected by mining and geothermal discharge. Sci. Total Environ. 373, 344–355.
Madison River in Yellowstone National Park	93% increase in			Filtered MeHg followed photo	Nimick, D.A., McCleskey, R.B., Gammons, C.H.,

N 44º39'25.46" W 111º04'04.67" Drains geothermal basin	filtered [MeHg] from 0445h-0800h minima to 1400h-2000h peak		cycle which indicates: 1. Biotic methylation dependent upon photosynthesis 2. Temperature-dependent methylation: increase temp, increase methylation by humic compounds pH decrease, methylation rates increase because bacterial uptake is better at lower pH	Cleasby, T.E., Parker, S.R., 2007a. Diel mercury-concentration variations in streams affected by mining and geothermal discharge. Sci. Total Environ. 373, 344–355.
Wetland in Salt Lake City, Utah N 41.1100° W112.1400°	Peaked between 0100h and 0200h; minimum at 1200h MeHg: Hg_T increase by 10% during non daylight and decrease with increased sunlight duration	[Hg_T] peaked at 0200h; minimum at 1100h	Photodegradation of MeHg	Naftz, D.L., Krabbenhoft, D.P., Cederberg, J.R., Beisner, K.R., Carling, G.T., 2009. Diurnal trends in methylmercury concentration in a wetland adjacent to Great Salt Lake, Utah, USA. Geol. Soc. Am. Abstracts with Programs 41, 200. Naftz, D. L. et al. Diurnal trends in methylmercury concentration in a wetland adjacent to Great Salt Lake, Utah, USA. Chemical Geology 283, 78–86 (2011).
Agricultural and Non-agricultural Wetlands in Yolo Bypass, CA 38.4999° N 121.6011° W	Increase at night by 100%	[$Hg(II)_R$] and [Hg_T] higher in agricultural wetlands than in non-agricultural	Microbial activity dominated Hg-methylation Conditions that favor microbial sulfate reduction contribute to high	Marvin-DiPasquale, M. et al. Methylmercury production in sediment from agricultural and non-agricultural wetlands in the

				MeHg production potential rates Rice has the capacity to accumulate mercury, and then re-releases after harvest back into sediment	Yolo Bypass, California, USA. Science of The Total Environment 484, 288–299 (2014). Fleck, J.A., Downing, B.D., Saraceno, J.F., Gill, G., Stephenson, M., Alpers, C.N., Bergamaschi, B.A., 2009. Diurnal trends in methylmercury concentration and organic matter photo-reactivity in agricultural wetlands of the Yolo Bypass. California. Geo. Soc. Am. Abstracts with Programs 41, 200
Everglades, Florida Rubber Tree Head 80º23 W 26º18 N	Diel variation tracks photo cycle with noon maxima and midnight minima	Diel variation tracks photo cycle, with afternoon peaks and nighttime minima	Diel trend: peaks at noon with levels 3-7x higher than minima before dawn	[MeHg] not linked to rainfall or photolysis reactions; generally high levels of MeHg due to lack of photodegradation at depth Hg_T / Hg_R controlled by photolytic sorption and desorption; rainfall input DGM production indirectly controlled by photolysis (reductive species or electron transfer-induced reduction)	Krabbenhoft, D. P., Hurley, J. P., Olson, M. L. & Cleckner, L. B. Diel variability of mercury phase and species distributions in the Florida Everglades. Biogeochemistry 40, 311–325 (1998).

				No DGM production at night	
Big Dam West Lake Annapolis, Subd. D, NS, Canada 44°46′25′′ W 65°29′50′′ N June 6-8 2001			Peak at 1800h Minimum between 2000-0600h	Oxidation-reduction potential has opposite trend (peak at 0600h, minimum between 1200-1800h) Dependent upon photoreduction/ photooxidation and microbial oxidation/ reduction	O'Driscoll, N. J., Siciliano, S. D. & Lean, D. R. S. Continuous analysis of dissolved gaseous mercury in freshwater lakes. Science of The Total Environment 304, 285–294 (2003).
Cane Creek Lake, Cookeville, TN, USA (Reservoir Lake) 36º09.73'N 85º32.64'W June 2003- May 2004			<u>July</u>: peak at 1300h, minima at 0900 <u>September</u>: peak at 1400h, minima 2000-0900h <u>January</u>: peak at 1300h, minima at 1200h <u>May</u>: peak at 1300h, minima at 1700h	Correlation of [DGM] with global solar radiation and UVA radiation → recognizable trends Influence of water temperature on [DGM] trend was found to be weak Weak correlation with sunlight was only found on cloudy and overcast days; during these times, solar radiation did not vary but DGM did → indicates other factors are at play (biological, non photochemical abiotic)	Dill, C., Kuiken, T., Zhang, H. & Ensor, M. Diurnal variation of dissolved gaseous mercury (DGM) levels in a southern reservoir lake (Tennessee, USA) in relation to solar radiation. Science of The Total Environment 357, 176–193 (2006).
Malibu Lagoon, California (Brackish Lagoon) 34.0336° N, 118.6804° W July 23 to 24, 2009	<u>Filtered</u> and <u>unfiltered</u> concentrations were similar throughout the study [MMHg] were higher in	Groundwater: <u>Filtered</u> concentrations were constant throughout the cycle <u>Unfiltered</u> concentrations fell with low tide and		MMHg is produced onshore in sediments or in subsurface groundwater Microbial mercury methylation likely explains why	Ganguli, P.M., Conaway, C.H., Swarzenski, P.W., Izbicki, J.A., and Flegal, A.R. Mercury Speciation and Transport via Submarine Groundwater

	groundwater than seawater Groundwater concentrations were slightly higher at high tide (1200h) Seawater concentrations were higher at low tide (0700h) Surface Water <u>Unfiltered</u> [MMHg] was constant throughout the tidal cycle [MMHg] was highest in surface waters than in groundwater and seawater adjacent to lagoon	remained low throughout low tide <u>Unfiltered</u> concentrations were always less in groundwater than in seawater Seawater: <u>Filtered</u> concentrations remained relatively constant throughout high and mid-tide but increased after low tide due to groundwater <u>Unfiltered</u> concentrations fell with the tide but became variable at low tide Surface Water: <u>Unfiltered</u> concentrations were higher at high tide than low tide [HgT] was highest in surface waters than in groundwater and seawater adjacent to lagoon	MMHg was highest in surface waters [HgT] similarities between groundwater and seawater during high and mid-tide due to mixing of water masses through subsurface sediment Anthropogenic inputs and geologic weathering upstream contribute to highest [HgT] in surface waters	Discharge at a Southern California Coastal Lagoon System. Environ. Sci. Technol. 46, 1480-1486 (2012).

DISSERTATION SUMMARY

In this work, I explored three important human impacts on marine and coastal ecosystems: anthropogenic nutrients, plastic, and mercury cycling.

In the near coastal ecosystem within the Southern California Bight off the coast of Orange County, I analyzed seasonal biogeochemical cycles and primary productivity in the context of nutrients and chlorophyll concentrations. I observed Spring upwelling that is typical of the region, in addition to strong summertime stratification and nutrient drawdown. Chlorophyll concentrations tracked nutrient concentrations, with higher levels observed during the Spring. In addition, I observed how a heavy rain event can contribute a significant pulse of nutrients to an already anthropogenically-influenced region, leading to a punctuated increase in chlorophyll concentrations.

I also demonstrated that the influences of natural and anthropogenic nutrients are difficult to distinguish. With overlapping effects, I saw clear nutrient pulses from both seasonal coastal upwelling and isolated rain events. In addition, there is a constant and steady influx of anthropogenic nutrients from the wastewater treatment plant operated by Orange County Sanitation District. Initially I drew a conclusion that there is little to no impact on the coastal environment, because there was no significant difference in chlorophyll or nutrient concentrations between seawater collected off the effluent pipe and at our designated “reference” site. However, upon closer examination of bathymetry and nearshore current dynamics, it is likely that the reference site chosen is not far enough away from the effluent site to make a true comparison. The cyclonic eddy that exists for much of the year leads to a high retention time of water within the bight and a recirculation and distribution

of nutrients throughout the coastal region. Future studies examining the impacts of wastewater effluent on this region should choose multiple reference sites further up and down the coast, as previous examinations of other waste water treatment plants demonstrate significant influence on local biogeochemistry.

I examined the impacts of microplastic on marine invertebrates on a small scale through laboratory incubation experiments and globally through a synthesis paper. In the laboratory, I found that Artemia salina ingests virgin microplastic beads immediately upon exposure, and that ingestion is concentration dependent. Through quantitative analyses, I found that ingestion rate plateaus after 90 minutes of exposure, and that more plastic was ingested with increasing concentration in seawater. These findings are, however, specific to A. salina, as ingestion rates are highly dependent on feeding style and preferences. A. salina is a passive filter feeder that will ingest particles it encounters as it swims. However, other zooplankton that are selective feeders may reject particles based on physical or chemical properties. Further studies are needed to assess differences among zooplankton species groups.

Through my investigation of the impacts of microplastic on marine invertebrates, I found great variety depending on a matrix of variables including plastic type and species type. The impact of microplastic on an organism depends on its size, shape, color, chemistry, presence of biofilm, density, and location within the water column. Further, the effects of microplastic will differ depending on the feeding style and habitat preference of an organism. In addition, there is not yet a consensus among scientists on sampling and experimental methods, therefore cross-comparison between studies remains challenging. Some studies have found that both particles and the chemicals contained within plastic can transfer to

higher trophic levels after ingestion, while other studies demonstrate small or no impact. Ingestion of microplastic can influence fecal pellet sinking rate that can have large repercussions on carbon export. Further, the rafting of marine species on plastic debris influences habitat and distribution of invasive species. I have found that microplastic carries marine pathogens, which can have devastating effects on coral reef systems, and that the chemicals embedded within plastic are classified and restricted toxins by the Stockholm Convention. Microplastics are contaminants of emerging concern and will require much more research to understand the current and future impacts it will have on marine ecosystems.

Mercury (Hg) is a potent neurotoxin that is both naturally and anthropogenically sourced into marine environments. Its cycling is highly dependent upon local biogeochemistry, therefore studies across a variety of ecosystems are necessary to understand the dynamics. I examined a tidally influenced estuary in Southern California over a 24-hour period to capture influence of both the photoperiod and tides. Anoxic bacteria in the soil convert Hg into mono methylmercury (MMHg), and therefore concentration of dissolved oxygen is a large determinant of MMHg concentration relative to Hg. MMHg enters the food web in its largest bioaccumulative step via phytoplankton, and this can be exacerbated in the presence of microplastic particles. In our study, most of our samples were below detection limit for MMHg, and those that were above detection were found during low tide. Therefore, I hypothesize that submarine groundwater discharge could be a likely source for MMHg, however further studies at this site are required to verify.

Though the study was inconclusive for MMHg cycling, I was able to capture the layered dynamics of biogeochemical cycles within the estuary, notably the influence of tide

and time of day on phytoplankton abundance and dissolved oxygen (DO) concentration. I found peak DO concentration during the day at high tide and conversely the lowest DO concentration at night during low tide. In addition, the highest phytoplankton concentrations were observed at low tide, and concentrations peaked during the daytime high tide. These observations were correlated to the end members, with the ocean providing high DO concentration and the river providing high phytoplankton concentrations.

Similar studies of MMHg should be repeated in diverse aquatic regions that include estuaries with different flushing rates to determine the effect on MMHg in different ecosystems. In this estuary, I observed higher DO concentrations linked to the ocean water input and higher chlorophyll concentrations in the river water input. This is likely not true across all estuaries and other freshwater-saltwater confluences. The biogeochemical characteristics of the environment have a huge influence on mercury cycling; therefore, a diverse range of study sites is necessary. Finally, including submarine groundwater discharge rates are important, as many previous studies have demonstrated their mercury contribution.

Humans are increasingly impacting marine ecosystems in negative ways. There is no shortage of pollutants or contaminants that can be highlighted and examined. Hg is a well-known heavy metal contaminant that has been under analysis, yet there is still much to learn about the cycling of bioavailable MMHg within the context of coastal biogeochemical dynamics. Wastewater effluent is mostly monitored for its interaction with humans in the near coastal environment, with efforts to maintain low levels of fecal indicator bacteria and other human-related impacts. However, its influence on bottom-up control coastal ecosystems merits further study, as it becomes difficult to distinguish consistent background

anthropogenic nutrients from natural seasonal dynamics. Finally, microplastics are the latest pollutant to garner attention, and there is relatively little known about the impacts of the particles themselves and the chemicals imbibed within on all aspects of the foodweb, from zooplankton to top marine predators and humans. Unification and standardization of methodologies is crucial for cross-comparison of studies so that we can truly investigate the consequences of this human-made pollutant. Humans created this mess and it's up to us to fix it.

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