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From Human Threats to Human Solutions: Impacts of Freshwater Runoff Pollution on Rocky
Shores and a New Approach to Training Environmental Problem Solvers

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Raechel Jasmine Hill

Dissertation Committee:
Professor Matthew E. S. Bracken, Chair
Assistant Professor Joleah Lamb
Assistant Professor Celia Symons

2024

DEDICATION

To

myself

for staying strong and persevering

and to

my friends and chosen family

for their unconditional love and support

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Ch. 1 CRediT author statement: **Raechel Hill**: Conceptualization, Methodology, Visualization, Data curation, Writing- Original draft preparation, **Matthew Bracken**: Supervision, Visualization, Writing- Reviewing and Editing

Ch. 2 CRediT author statement: **Raechel Hill**: Conceptualization, Methodology, Visualization, Data curation, Writing- Original draft preparation, **Matthew Bracken**: Supervision, Visualization, Writing- Reviewing and Editing, **Nathan Sinn**: Investigation, Methodology, Writing- Reviewing and Editing, **Natalie Strasburg**: Conceptualization, Methodology

Ch. 3 CRediT author statement: **Raechel Hill**: Writing- Original Draft, Reviewing & Editing, Supervision, **Matea A. Djokic**: Writing- Original Draft, Reviewing & Editing, Supervision, **Andrea Anderson**: Data Curation, Writing- Original Draft, Visualization, **Kristin Barbour**: Writing- Original Draft, **Amanda M. Coleman**: Data Curation, Writing- Original Draft, Visualization, **Alexis D. Guerra**: Writing- Original Draft, **Courtney Hunt**: Conceptualization, Data Curation, Supervision, Project Administration, Writing- Original Draft, **Amber Jolly**: Writing- Original Draft, **Jennifer J. Long**: Conceptualization, Methodology, Data Curation, Supervision, Project Administration, Funding Acquisition, **Kyle T. Manley**: Data Curation, Writing- Original Draft, Writing- Reviewing & Editing, Visualization, **Jonathan L. Montoya**: Writing- Original Draft, **Carl A. Norlen**: Writing- Original Draft, **Andie Suratt**: Writing- Original Draft, **Kameko Washburn**: Writing- Original Draft, **Weber**: Writing- Original Draft, **Allison Welch**: Writing- Original Draft, **Cynthia Wong**: Writing- Original Draft, Visualization, **Steven D. Allison**: Conceptualization, Methodology, Data Curation, Supervision, Project Administration, Funding Acquisition, Writing- Original Draft, Writing- Reviewing & Editing

VITA

Raechel Jasmine Hill

EDUCATION

Ph.D. in Biological Sciences, University of California, Irvine	2019 - Present
M. S. in Biological Sciences, University of California, Irvine	2019 - 2023
B.A. in Environmental & Ocean Sciences, University of San Diego	2015 - 2019

PUBLICATIONS

Hill, R. J. & Bracken, M. E. S. 2024. "Runoff-associated subsidies of inorganic nitrogen pollution are associated with shifts in rocky intertidal community structure". *In prep.*

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TEACHING

Limnology and Freshwater Ecology + Lab	2024
Introduction to Biological Research Analysis Lab	2024
Processes in Ecology & Evolution	2020, 2023
Global Sustainability III	2021
Organisms to Ecosystems	2021

MENTORING

Tracy Le, Psychology, UCI	2023-2024
Nathan Sinn, Biological Sciences, Mt. SAC	2023
Calvin Huang, Biological Sciences, UCI	2022-2023
Joshua Lu, Biological Sciences, UCI	2021-2023
Marcus Lira, Civil & Environmental Engineering, Mt. SAC	2021
Natalie Strasburg, Ecology & Evolutionary Biology, UCI	2020-2021
Nathan Green, Philosophy, UCI	2020

OUTREACH

Project GAIA: Undergraduate Art + Ecology Retreat	2023
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Collaboration with Claire Trevor School of the Arts	2020 – 2023
Ridge2Reef Summer Institute	2023
Ridge2Reef Art Gala	2022

SERVICE

ARDEI Council Member	2023 - 2024
Ridge2Reef Student Representative	2022 - 2023
Graduate Student Representative	2020 – 2022

CERTIFICATIONS

Research Justice Workshop: Community-Based Research Methods	2020 - 2021
Inclusive Excellence Program	2020 - 2021
Mentoring Excellence Program	2020

AWARDS & HONORS

Ridge2Reef NRT Fellowship	2021 – 2023
Eugene Cota-Robles Fellowship Recipient	2019 - 2024
UC Irvine Provost PhD Fellowship Recipient	2019
McNair Scholar, Ronald E. McNair Scholars Program	2017 - 2019
Kiwanis Club Foundation Scholarship	2017 - 2019
Torero Renaissance Scholar	2016 - 2019
Dean’s List, First Honors	2016 - 2019

ORAL PRESENTATIONS

“Modification of Nutrient Inputs Along OC Coastlines.” Oral Presentation for Orange County Marine Protected Area Council’s Continuing Education Workshop. May 2021.

“Human Modification of Land-Sea Nutrient Inputs Along a Coastal Mediterranean Ecotone.” Oral Presentation for Western Society of Naturalists. November 2020.

“Human Mediated Changes in the Timing and Magnitude of Nutrient Inputs to Nearshore Systems.” Oral Presentation for University of California, Irvine Summer Research Symposium, CA. August 2019.

POSTER PRESENTATIONS

“Environmental STEAM: Collaboration in Practice.” Poster Presentation for Winter Ecology & Evolutionary Biology Graduate Student Symposium. March 2023.

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ABSTRACT OF THE DISSERTATION

From Human Threats to Human Solutions: Impacts of Freshwater Runoff Pollution on Rocky Shores and a New Approach to Training Environmental Problem Solvers

By

Raechel Jasmine Hill

Doctor of Philosophy in Biological Sciences

University of California, Irvine 2024

Professor Matthew E. S. Bracken, Chair

The magnitude and scale of current threats to ecosystems requires interdisciplinary approaches to both science and training. For example, urbanization has resulted in increased runoff from communities into coastal habitats, necessitating work across the marine-terrestrial interface. This runoff holds myriad pollutants which can impact vulnerable ecosystems, such as coastal rocky reefs. I evaluated two pollutants in domestic runoff, nitrogen-based nutrients and glyphosate-based herbicides, and their effects on rocky intertidal biodiversity and ecosystem health. Biweekly measurements were taken from runoff-affected tidepools, runoff-unaffected tidepools, drain sources, and the ocean to assess levels of both nutrients and glyphosate at our Southern California field site. Further, I experimentally added nutrients to tidepools and dosed the green seaweed *Ulva* sp. with glyphosate to determine the extent of effect these pollutants would exert on the ecosystem. In chapter 1, I showed that nitrate + nitrite concentrations varied over time, with runoff-affected tidepools exhibiting significantly higher concentrations than control tidepools. Internal nitrogen concentrations in *Ulva* correlated positively with nitrate +

nitrite concentrations, and total grazer biomass increased with higher internal nitrogen in *Ulva*. Experimental nitrogen additions enhanced *Ulva* cover compared to control pools. In chapter 2, I showed elevated glyphosate concentrations exceeding EPA safe drinking-water limits in the field, with substantial pH reductions observed at low glyphosate concentrations. These findings indicate that increased nutrients lead to bottom-up effects on nutrient cycling, and that reduction in pH associated with glyphosate has the potential for appreciable reduction in local pH. My findings underscore the need for regulatory consideration of these pollutants in coastal management, especially in protected areas, to mitigate risks to marine ecosystems and human health. However, environmental challenges, such as polluted freshwater runoff, are global in both scope and scale. Interdisciplinary and collaborative approaches to research can be used to better address these complex issues, and in chapter 3, I describe a graduate training program that centers these approaches. The Ridge 2 Reef National Science Foundation Research Traineeship Program was developed to provide transferable and cross-disciplinary training to graduate students of various academic backgrounds. The program was successful in improving the communication skills, career knowledge, and understanding of global perspectives in research of the trainees. The interdisciplinary and collaborative approaches to training were also successful in increasing trainee confidence and preparedness for environmental career paths.

INTRODUCTION

Current global environmental threats impact both social and ecological systems across scales, from individuals to the biosphere (Vitousek et al., 1997). The complexity of these effects calls for correspondingly complex approaches to research and training. However, despite the scope, scale, and interactive nature of these threats, they are often studied separately (Halpern et al., 2008; Sala et al., 2000). In addition to focusing on one ‘challenge’, research is often siloed by ecosystem, regardless of the impact or connection to other locations or habitat types. This separation of environments and issues is also reflected in the literature. For example, research in terrestrial systems rarely references similar work from aquatic and marine studies, despite clear similarities and important connections between ecosystems (Menge et al., 2009). The lack of inter-ecosystem study and communication creates a dearth of information on the effects of these global environmental challenges on critical ecotones as well as on broad, well-informed solutions to these challenges. The continued lack of interdisciplinary and collaborative environmental research renders these anthropogenic threats understudied and creates too large a gap in the research to implement effective environmental regulation and management.

Ecotones – boundaries between ecosystems (Kolasa & Zalewski, 1995) – highlight the need for interdisciplinary research. For example, understanding cross-system subsidies from land to water (or vice-versa) can benefit from input from scientists studying aquatic and terrestrial systems (Knight et al., 2005; Raffaelli et al., 2005; Stapp et al., 1999). For example, 2.5 million kilometers of global coastline occur at the boundary of terrestrial and marine systems (Liu et al., 2020), and the connections between these systems remain poorly understood. Anthropogenic freshwater runoff, e.g., excess wastewater from coastal communities, increasingly impacts marine ecosystems and has increased in frequency and concentration of pollutants, e.g. bacteria,

heavy metals, nutrients, synthetic residues, and herbicides (McPherson et al., 2005; McPherson et al., 2002; Noble et al., 2003; Toor et al., 2017). Not only does urbanization and associated runoff threaten coastal habitats (Fabricius, 2005; McCarthy & Incardona, 2008; McPherson et al., 2005), but there is a lack of government regulation regarding domestic effluent and coastal runoff. Most regulations pertaining to anthropogenic runoff focus on agricultural, industrial, or urban effluent and its effects on freshwater or terrestrial ecosystems (Kayhanian et al., 2012; Kochan, 2005; Roesner & Traina, 1994; Tsihrintzis & Hamid, 1997). Where effects of polluted runoff on marine systems are considered, they focus on offshore outfalls up to 20km away or events such as flooding and associated large-scale impacts (Sutula et al., 2021). Fewer studies acknowledge the effects of runoff throughout the year, although dry weather runoff can account for a substantial fraction of total annual runoff into coastal systems (McPherson et al., 2002). Research into the impacts of runoff on shallow, coastal ecosystems is limited, and the corresponding pollutants remain potentially major threats, especially in habitats such as estuaries, beaches, reefs, and rocky shores at the land-sea margin (Gunes et al., 2021; Palla et al., 2017).

In chapter 1, I evaluate how rocky shorelines can act as short-term catchment sites at low tide, where tide pools can retain runoff for hours to days at a time, contributing to a long-term pattern of acute, daily spikes of polluted freshwater inputs. In Southern California, where rocky shores are abundant and essential coastal habitats, freshwater input is especially pertinent, as the region is characterized by a Mediterranean climate – hot, dry summers, mild winters, and low overall annual rainfall – and nutrient-poor nearshore ecosystems (Cooper et al., 2013; Martiny et al., 2016; Noe & Zedler, 2001). Further, there are over 270 storm drain discharges across various marine protected areas in Southern California (Schiff et al., 2011), contributing significant levels

of runoff to ecosystems that historically experienced freshwater in infrequent, seasonal spikes. There is a clear need to quantify the magnitude and effects of polluted freshwater runoff and its subsequent effects on the biodiversity and fitness of rocky shore systems (Polis et al., 1997).

Of the various pollutants associated with freshwater runoff in marine systems, nutrients, particularly nitrogen-based nutrients, have transformed ecosystems worldwide as a result of human alteration (Vitousek, Aber, et al., 1997). Coastal marine ecosystems are generally nitrogen poor, which limits overall productivity as there is high competition for essential nutrients (Elser et al., 2007; Howarth & Marino, 2006; Vitousek & Howarth, 1991). However, the effects of nutrient additions on a system depend on the degree of nutrient limitation (Worm et al., 2002), which can determine the effect of added nutrients on diversity and productivity (Bracken & Nielsen, 2004; Worm et al., 2002). These anthropogenic inputs are further exacerbated by coastal development. In Southern California, the development of coastal scrublands into irrigated gardens has led to near-constant flow of nutrient-laden freshwater from the land (Toor et al., 2017), through the intertidal zone (Martin et al., 2022; Schiff et al., 2011; Whitaker et al., 2010), to the ocean (Howard et al., 2014; Sutula et al., 2021). My work demonstrates the impact of runoff-associated nitrogen, which acts as a spatial subsidy of nutrients into Southern California intertidal systems, enhancing growth and internal nitrogen concentrations of seaweed and altering the diversity and abundance of consumers.

Another understudied pollutant associated with runoff from irrigated lawns, gardens, and golf courses is glyphosate ($C_6H_{17}N_2O_5P$), also known as N-(phosphonomethyl) glycine. Glyphosate is the most widely used herbicide in the United States (Duke & Powles, 2008) and in global agriculture (Benbrook, 2016; Duke, 2018) and is also used to control weeds in residential and commercial landscaping (Matozzo et al., 2020). In addition to continual debate on its

classification as a carcinogen, (Davoren & Schiestl, 2018; Meftaul et al., 2020; Paumgarten, 2019), glyphosate-based products have been found to be toxic in terrestrial and aquatic environments and to a variety of taxa (Annett et al., 2014; J. P. K. Gill et al., 2018; Tsui & Chu, 2003). However, the presence and effects of glyphosate in marine ecosystems is understudied, although it has recently been documented in global watersheds, agricultural runoff, marine sediments, and wetlands (Edwards et al., 1980; Feltracco et al., 2022; Kim et al., 2010; Lima et al., 2023; Lupi et al., 2019; Skeff et al., 2015). Glyphosate poses a significant potential threat to marine ecosystems as it is a non-selective herbicide that inhibits the shikimic acid pathway, an essential enzyme pathway in photosynthesis, present not only in plants but also algae (Holländer & Amrhein, 1980). In chapter 2, I document high levels of glyphosate – exceeding U.S. Environmental Protection Agency safe drinking water limits – in runoff from drains and creeks along the Southern California coastline. My work highlights the prevalence and potential impacts of this herbicide. Importantly, the seaweed *Ulva*, which is often associated with runoff, was only affected by glyphosate at concentrations substantially higher than those I quantified. However, glyphosate runoff remains a potential concern to both human and ecosystem health.

Freshwater runoff is a global problem and affects all coastlines, regardless of climate, nutrient availability, or human presence. Such large-scale environmental problems require equally large, complex, and involved solutions. However, the interdisciplinary approaches necessary to evaluate and mitigate these problems are hampered by the organization of typical academic training programs, where the scholars and scientists studying these challenges are divided by disciplinary boundaries (Duderstadt, 2012; O’Neill et al., 2019; Postel, 2000; Schenk et al., 2009; Van Den Beemt et al., 2020). Mitigating multi-faceted environmental threats requires collaboration across disciplines such as engineering, ecology, and law, as well as

engagement with stakeholders including government agencies, private entities, universities, and the public (A. M. Gill & Stephens, 2009). Further, university programs are often designed to train students for a future in academia, but 57% of Ph.D. recipients pursue careers outside of academia (Early Career Doctorates Survey, 2017). Without sufficient training in communication, project and team management, budgeting, leadership, stakeholder engagement, and other transferrable skills, graduate students may be ill-equipped to address and resolve real-world problems that span multiple disciplines and require solution-based research (McGunagle & Zizka, 2020). To better prepare the next generation of scientists to tackle both global challenges and non-academic realms, graduate education needs to fill the gaps between disciplines, integrate transferable skills training, and prioritize interdisciplinary, collaborative methods.

In chapter 3, I describe a training program established to achieve this goal of training graduate students for non-academic, environmental management. The Ridge 2 Reef (R2R) Program, a National Science Foundation Research Traineeship, was established at the University of California, Irvine (UCI), in 2017. The program sought to accomplish five main goals; (1) provide students the training and opportunities to develop interdisciplinary skills; (2) promote transferable, career-relevant skills; (3) build partnerships in and out of academia; (4) broaden participation in environmental careers; and (5) institutionalize successes of the program throughout the university. These goals were accomplished through a mix of interdisciplinary curriculum and training, requiring collaborative internships, and offering professional development activities. Program success was evaluated via quantitative and qualitative methods, using annual surveys and assessments of student perceptions and success in various criteria, such as knowledge of global environmental affairs, confidence in their abilities, and level of soft skills.

The success of current and future environmental leaders depends on interdisciplinary and collaborative training that reflects the complexity and interconnectedness of global environmental issues. Despite substantial research into anthropogenic threats worldwide, most studies remain siloed within both discipline and ecosystem. For example, studies in terrestrial systems rarely cite similar studies in aquatic systems, despite aquatic studies' integration of terrestrial processes in their research (Menge et al., 2009). This unbalanced approach in environmental research limits both applicable findings and the scope of regulatory management, increasing the amount of work and time needed to successfully resolve global issues. For environmental science and management to successfully evolve, higher education must form active connections between disciplines. Further, incorporating cross-disciplinary training will encourage a better understanding of the linkages and subsidies between ecosystems, such as the similar and linked threats to both aquatic and marine environments (Halpern et al., 2008; Sala et al., 2000). Integrating collaborative practices in graduate education provides the foundation for future professionals to conduct successful and innovative multi-ecosystem work. Cross-disciplinary training and subsequent research is necessary to evaluate and properly respond to our current environmental challenges.

CHAPTER 1

Runoff-associated subsidies of inorganic nitrogen pollution are associated with shifts in rocky intertidal community structure

INTRODUCTION

Human alteration of nitrogen availability is transforming ecosystems worldwide (Vitousek, Aber, et al., 1997). In coastal marine systems, where nitrogen availability often limits productivity (Elser et al., 2007; Howarth & Marino, 2006; Vitousek & Howarth, 1991), excess nitrogen can lead to eutrophication and low-oxygen conditions (Altieri & Witman, 2006; Ryther & Dunstan, 1971; Scavia et al., 2003), transforming marine systems. Most work on anthropogenic nutrient subsidies to marine systems has focused on the negative impacts of eutrophication, which occur when excess nitrogen is added to nutrient-replete systems (Schindler, 2006). However, the effects of nutrient additions on a system depend on whether that system is nutrient-limited (Worm et al., 2002). Whereas adding nutrients to a nutrient-replete system has the potential to result in overgrowth, competitive exclusion, and decrease biodiversity, adding nutrients to a low-nutrient system can enhance diversity and productivity (Bracken & Nielsen, 2004; Worm et al., 2002).

Natural patterns in nutrient availability have been disrupted by coastal development. For example, along the coastline of Southern California, USA, the transformation of coastal scrublands into irrigated gardens has led to near-constant flow of nutrient-laden freshwater from the land (Toor et al. 2017), through the intertidal zone (Martin et al., 2022; Schiff et al., 2011; Whitaker et al., 2010), to the ocean (Howard et al., 2014; Sutula et al., 2021). Most studies on the bottom-up effects of nutrient polluted runoff focus on coastal zones up to 20 km offshore and

look only at large rain, climate, or outfall events across several years (Sutula et al., 2021). Fewer studies acknowledge the effects of runoff throughout the year, although dry weather runoff can account for 10-30% of the total annual runoff from that area (McPherson et al., 2002). There is a clear need to quantify the magnitude and effects of nutrient subsidies and their subsequent effects on community diversity and nutrient cycling in rocky shore systems at the transition between land and sea (Polis, Anderson, and Holt 1997).

Occupying the boundary between land and sea, rocky shores and intertidal zones are essential, dynamic habitats of the California coastline. This is particularly so in Southern California, which is characterized by a Mediterranean climate of dry, hot summers, mild winters, low overall annual rainfall, and nutrient-poor coastal zones (Cooper et al., 2013; Martiny et al., 2016; Noe & Zedler, 2001). Seasonal increases in nitrogen availability in Southern California coastal waters can be attributed to two main seasonal events; upwelling in the spring and summer (Barth et al., 2007; Howard et al., 2014) and runoff in the fall and winter as a result of seasonal rains (Bograd et al., 2015; Sutula et al., 2021). Although these seasonal inputs are mirrored in shallow, coastal waters at the land-sea interface, nutrient levels remain relatively low year-round (see *Results*). However, over 250 storm drain discharges now occur across fourteen marine protected areas in Southern California, allowing runoff to flow directly into tidepools and rocky intertidal zones (Schiff et al., 2011). Although rocky intertidal organisms are resistant to natural fluctuations in temperature, salinity, and nutrient availability (Braby & Somero, 2006; P. Fong et al., 1996), the introduction of anthropogenic runoff now simulates and magnifies the effects of seasonal rain events on a near daily basis (Leong et al., 2018; Roemmich, 1989).

Here, we evaluated the potential for a spatial subsidy of nitrogen – the alteration of freshwater flow into the intertidal zone associated with adjacent coastal development – to modify

a Southern California rocky shore ecosystem. By monitoring drain sources and experimentally adding nutrients to unaffected tidepools, we were able to determine the biological baseline of runoff-affected versus unaffected tide pools at our field site as well as the long-term effect of added nutrients on tide pool invertebrate grazer biodiversity and internal nitrogen concentrations (%N) in the ephemeral green alga, *Ulva* sp., a seaweed genus often associated with runoff-impacted coastal areas (Bews et al., 2021; Mourad & El-Azim, 2019; Yauchi et al., 2004). This species is characterized by rapid growth and nutrient uptake rates despite variable conditions (Choi et al., 2010; Hiraoka et al., 2020; Luo et al., 2012). We hypothesized that freshwater runoff input from drains into tide pools would result in increased water-column inorganic nitrogen concentrations, higher %N in *Ulva*, altered invertebrate grazer biodiversity and biomass, and greater seasonal nutrient fluxes in comparison to unaffected tide pools. We also conducted a nutrient-addition experiment in tide pools that were unaffected by runoff, predicting that adding nitrogen would enhance *Ulva* growth.

METHODS

This study was conducted on mid-upper rocky intertidal reefs at Corona del Mar State Beach (33.59°N, 117.87°W) between October 2019 and December 2021. Gaps in the data set were a result of the inability to conduct field work due to COVID-19 restrictions, closures associated with a nearby oil spill, and/or severe weather. Within the study site, which is an Area of Biological Significance, there are seven areas of runoff discharge along a 600m stretch of rocky beach, primarily associated with residential runoff from Corona del Mar and the homeowners in Buck Gully (“State Water Resources Control Board” 2017.).

Water samples were taken biweekly from runoff-affected tidepools, experimental addition tidepools (during the December 2020-February 2021 nutrient addition experiment), control tidepools that were unaffected by runoff or nutrient addition, drain sources, and the ocean. Runoff sources ($n = 7$) included concrete storm drains, channelized creeks, and private PVC pipes from beach-side homes. Ocean samples were acquired from lower intertidal areas with sufficient wave action. Runoff-affected tide pools ($n = 5$) were exposed to frequent freshwater discharge and spanned tidal elevations from 0.5 to 1.5 m above MLLW.

At each biweekly sampling event, four types of measurements were taken, including water samples, algal samples, invertebrate diversity surveys, and water-quality measurements. A 50mL water sample was collected directly from each water source and frozen prior to spectrophotometric analysis of nutrient concentrations. Concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) (N + N) were analyzed using a nutrient autosampler (Lachat, Loveland, CO, USA). Up to 50mg of the ephemeral, green seaweed *Ulva* sp. was collected from each water source and analyzed for internal nitrogen concentrations (%N) using an elemental analyzer (CE Elantech, Lakewood, NJ, USA).

Invertebrate biodiversity surveys were conducted in runoff-affected, experimental addition, and control pools and included six categories of consumers: turban snails (*Tegula* sp.), littorine snails (*Littorina* sp.), chitons (*Cyanoplax hartwegii* and *Nuttalina californica*), limpets (*Lottia* sp.), hermit crabs (*Pagurus* sp.), and striped shore crabs (*Pachygrapsus crassipes*). The average mass of these invertebrates has been previously reported and was used to estimate biomass in the pools (Bedgood et al., 2023; Bracken, Oates, et al., 2018). If enough water was present, temperature and salinity readings were taken at all water sources using a multiparameter digital water quality meter (YSI Incorporated, Yellow Springs, OH, USA).

We identified 30 tide pools that were isolated from anthropogenic runoff by drainage channels. Control ($n = 15$) and experimental nutrient-addition ($n = 15$) pools were randomly chosen across tidal elevations (0.5m, 1.0m, and 1.5m above MLLW), with volumes ranging from 5 to 43L. Experimental pools contained a nutrient dispenser that released sodium nitrate (NaNO_3^-) and potassium phosphate ($\text{K}_3\text{PO}_4^{3-}$) at levels similar to the averages found in outflow from the drains. The dispensers were anchored in the pools one week before the experiment began in December 2020. Dispensers were changed every 7-10 days to ensure constant input of nutrients into the addition pools. The experiment ran until February 2021, when a late-winter heat wave caused mortality of invertebrates and algae.

All water quality, %N, and invertebrate survey data between time points analyses were conducted using linear mixed effects models with the “lmer” function in the lme4 package (Bates et al., 2015) in R v.4.1.2 (R Core Team, 2021). Correlative relationships between variables were analyzed using basic linear models. Experimental effects of nutrient additions on average, post-manipulation *Ulva* cover were analyzed using ANCOVA with the “anova_test” function in the rstatix package (Kassambara, 2023) after accounting for initial *Ulva* cover as a covariate. Full reproducible code are described in Appendix A. Responses with non-normally distributed residuals were transformed using the inverse hyperbolic sine transformation ($\log(y + (y^2 + 1)^{1/2})$).

RESULTS

Nitrate (NO_3^-) + nitrite (NO_2^-) concentrations (N + N) in drain outflow water varied over time ($F_{27,167} = 2.8$, $P < 0.001$) and were higher than in ocean water ($F_{1,7} = 10.4$, $P < 0.05$) (Fig 1a). This difference was more pronounced on some dates than on others, e.g., during heavy rainfall in April 2020 (time x source interaction: $F_{25,167} = 1.6$, $P < 0.05$) (Fig 1a). N + N

concentrations also varied over time in runoff affected tide pools ($F_{24,364} = 11.4$, $P < 0.001$) and were higher than in control tide pools ($F_{1,22} = 28.7$, $P < 0.001$) (Fig 1b). This difference was also more pronounced on some dates than on others (time x source interaction: $F_{11,364} = 4.9$, $P < 0.001$) (Fig 1b). Salinity was lower in runoff-affected pools than in control pools ($F_{1,19} = 42.4$, $P < 0.001$) and N + N concentrations declined as tide pool salinity increased ($F_{1,281} = 64.4$, $P < 0.001$). Internal nitrogen concentration (%N) in *Ulva* sp. was higher in tide pools with higher concentrations of dissolved inorganic nitrogen ($F_{1,209} = 8.0$, $P = 0.005$) (Fig 2a). Total grazer biomass was higher in pools with greater %N in *Ulva* spp. ($F_{1,293} = 4.9$, $P = 0.028$) (Fig 2b).

Tegula sp. were more prevalent in runoff-affected pools than in control pools ($F_{1,20} = 35.6$, $P < 0.001$) and were found in higher abundance in pools with higher concentrations of %N ($F_{1,267} = 6.4$, $P < 0.05$). *Littorina* sp. were also more prevalent in runoff-affected pools than in control pools ($F_{1,20} = 5.5$, $P < 0.05$) and were found in higher abundance in tide pools with higher concentrations of %N ($F_{1,267} = 4.1$, $P < 0.05$). There was a significant difference in invertebrate community structure between runoff-affected and control pools ($SST = 10.9$, $P < 0.05$). The Shannon Diversity index for consumers was higher in runoff-affected pools than in control pools ($F_{1,22} = 4.4$, $P < 0.05$) as was the Shannon Equitability Index ($F_{1,23} = 5.2$, $P < 0.05$) (Fig 3a). Consumer biomass was higher in runoff-affected pools than in control pools ($F_{1,28} = 29.1$, $P < 0.001$) (Fig 3b).

Experimental nutrient additions resulted in enhanced cover of *Ulva* within three months of nutrient additions. After accounting for initial *Ulva* cover in the pools as a covariate ($F_{1,27} = 102.4$, $P < 0.001$), average *Ulva* cover in nutrient addition pools was more than twice as high as cover in control pools ($F_{1,27} = 6.5$, $P = 0.017$) (Fig 4).

DISCUSSION

We predicted that increases in N + N availability from the introduction of freshwater runoff would result in increased %N in *Ulva*, altered invertebrate biodiversity and biomass, and greater seasonal fluxes in nutrient concentration. We found that control pools, on average, had similar ambient levels of N + N to that of lower intertidal ocean water (Fig 1). However, runoff from storm drains contained nearly 50 times as much N + N as either the control pools or the ocean, which resulted in a nearly 20-fold increase in N + N concentrations in runoff-affected tidepools (Fig 1). The elevated levels of ambient N + N, in contrast to unaffected control pools, support our prediction that polluted runoff would increase the concentration of nutrients in tidepools. These increases in N + N were paralleled with substantial seasonal nutrient fluctuations in both runoff and runoff-affected tidepools (Fig 1). Control pools and ocean samples showed some seasonal variation, but only in the range of 10 μ mol/L N + N, while storm water and runoff-affected pools experienced variations of upwards of 30-100 μ mol/L N + N (Fig 1). The significant seasonal variation observed in March, April, July, and August 2020 for runoff-affected pools highlights the increased seasonal variation of nutrient levels in comparison to control pools. Although there were rain events during March and April of that year, there were none in July or August (University of California, Division of Agriculture and Natural Resources, 2024). The large increase in N + N input during the summer months may be attributed to dry weather flow (McPherson et al., 2002), as such runoff is consistent year-round in Southern California and the high concentrations of N + N detected in it has the potential to adversely affect marine organisms post-discharge. While sampling, we observed that dry weather runoff was released, at minimum, twice daily at each of the seven drain sources at CDM, in contrast to storm events which happen only a few times per year. This is in direct contrast with wet weather

flow which can contribute 77% of the average total nitrogen load annually in a similar estuarine study (McPherson et al., 2002). However, the influence of dry weather runoff, and of runoff overall, on rocky intertidal habitats is further supported by the inverse relationship between N + N and salinity. Even when rain events were discounted, there was still a negative correlation between N + N concentration and salinity of both control and runoff-affected pools, suggesting that the salinity of tidepools in runoff-affected areas could be potentially used as an indicator for nutrient loading (Page et al., 1995). Further, the decreases in salinity associated with freshwater runoff adds additional stress to organisms an already extreme ecosystem (Garrity, 1984).

The presence of constant, increased nutrient levels was also correlated with changes in nutrient cycling and biomass within the tidepools. We found a significant, positive correlation between N + N concentrations and %N in *Ulva* (Fig 2a), suggesting that nutrient loading is associated with bottom-up effects on nutrient cycling within tidepools. Grazer biomass also increased significantly with increases in %N of *Ulva*, (Fig 2b), however it was not directly related to N + N concentration. This finding highlights nutrient-associated enhancement of *Ulva* quality (i.e., nitrogen content) as the driver of higher grazer abundances (Dickman et al., 2008; Moorthi et al., 2016; Nixon et al., 1986). Further, both the Shannon Diversity and Equitability Index were significantly higher in runoff-affected pools than in control pools (Fig 3a), highlighting the effect of bottom-up nutrient subsidies on community structure. Total invertebrate grazer biomass was also higher in tidepools with greater N + N concentrations (Fig 3b). Our nutrient addition experiment resulted in a doubling in *Ulva* cover in nutrient-addition tide pools relative to control pools (Fig 4), further supporting the N-limited status of this ecosystem (Elser et al., 2007).

Changes in nutrient availability have the potential to cause bottom-up effects on algal growth, diversity, and biomass; herbivore abundances; and community composition in marine systems (Howard, Kudela, and McLaughlin 2017; Menge 2000; Kraufvelin et al. 2006; Bracken and Nielsen 2004). However, with a few exceptions (e.g., (Littler & Murray, 1975), despite frequent demonstrations that local-scale nutrient inputs are important determinants of the diversity and abundance of organisms on rocky shores (Aquilino et al., 2009; Bracken & Nielsen, 2004; K. J. Nielsen, 2001; O'Connor et al., 2015; Pfister, 2007), these perspectives have not been effectively applied to anthropogenic nutrient runoff effects in those systems. Our observations and experiments demonstrate that nutrient discharge associated with irrigation runoff onto rocky shores has the potential to appreciably alter nutrient availability and community structure. This finding has important ramifications for understanding human impacts on rocky shores.

Despite being an Area of Special Biological Significance, a State Beach, and a State Marine Conservation Area, unmitigated discharge of nutrient-laden wastewater is transforming this habitat. The daily input of freshwater runoff to this rocky intertidal zone should be of major concern to environmental management as the effects observed with increased nutrient concentration is likely to be magnified as time goes on and urbanization increases. The increase in invertebrate biodiversity and biomass, and the increase in internal nitrogen levels of *Ulva*, are important indicators of increased nutrient responses, but are also likely just some of the major ecological shifts that are associated with such anthropogenic input (Carstensen et al., 2011; C. R. Fong & Fong, 2018). Further, protected species are often at higher risk from anthropogenic harm (O'Hara et al., 2021; Powles et al., 2000). Ultimately, the lack of consideration of domestic sources into runoff and pollutant calculations and regulatory goals is a not insignificant oversight

in coastal ecosystem management. Although anthropogenic nutrient inputs do not have largely negative effects on this rocky-intertidal ecosystem, the associated shifts in producer and consumer relationships are still cause for further investigation.

FIGURES AND TABLES

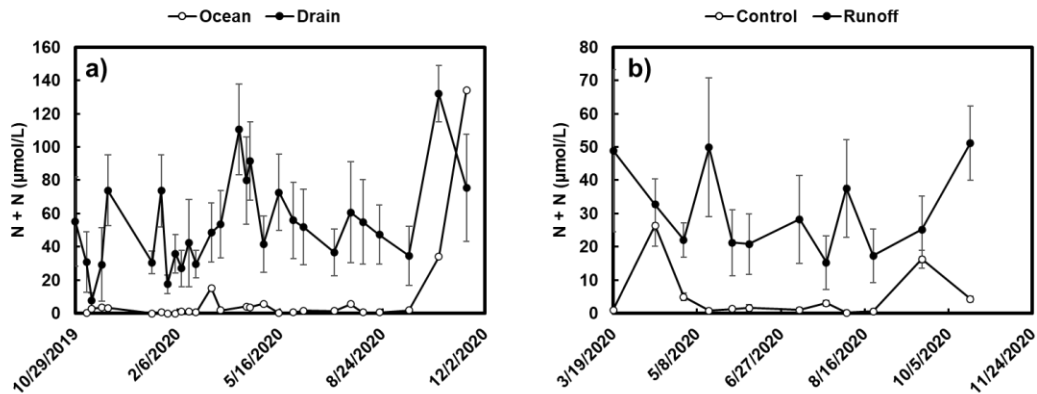


Figure 1. Nitrate (NO_3^-) + nitrite (NO_2^-) concentrations ($\text{N} + \text{N}$, $\mu\text{mol/L}$) for each sample type over the course of the study. Each point is the average concentration on that date with corresponding standard error. a) Drain outflow samples show significantly higher values of $\text{N} + \text{N}$ than in the ocean ($F_{1,7} = 12.3$, $P < 0.05$). Seasonal peaks in $\text{N} + \text{N}$ concentration in drain outflow can be seen in April, October, and November 2020 ($F_{24,152} = 4.4$, $P < 0.001$). b) Runoff-affected tide pool samples show significantly higher values of $\text{N} + \text{N}$ than in control tide pools ($F_{1,24} = 33.7$, $P < 0.001$). Seasonal peaks in $\text{N} + \text{N}$ concentration in runoff-affected pools can be seen in March, April, July, and August 2020 ($F_{24,397} = 2.6$, $P < 0.001$).

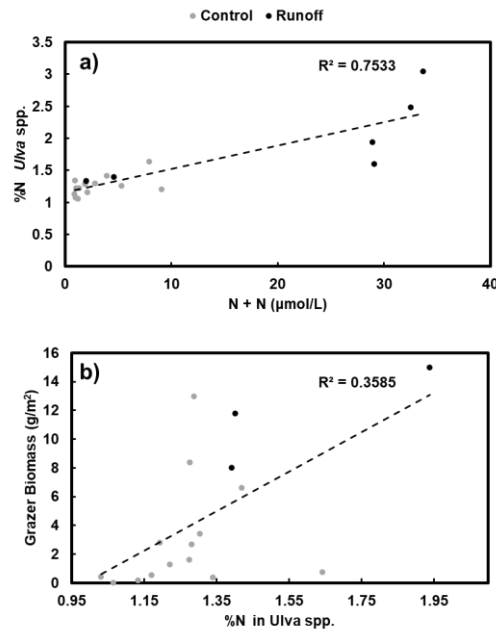


Figure 2. The average relationship between the %N in *Ulva* sp. and grazer biomass (g/m^2) and Nitrate (NO_3^-) + nitrite (NO_2^-) concentrations ($\text{N} + \text{N}$, $\mu\text{mol/L}$). a) There is a significant positive correlation, $R^2=0.7533$, between grazer biomass (g/m^2) and %N in *Ulva* spp. ($F_{1,15} = 8.3$, $P < 0.05$). b) There is a significant positive correlation, $R^2=0.3585$, between %N in *Ulva* spp. and $\text{N} + \text{N}$ ($F_{1,18} = 54.9$, $P < 0.001$).

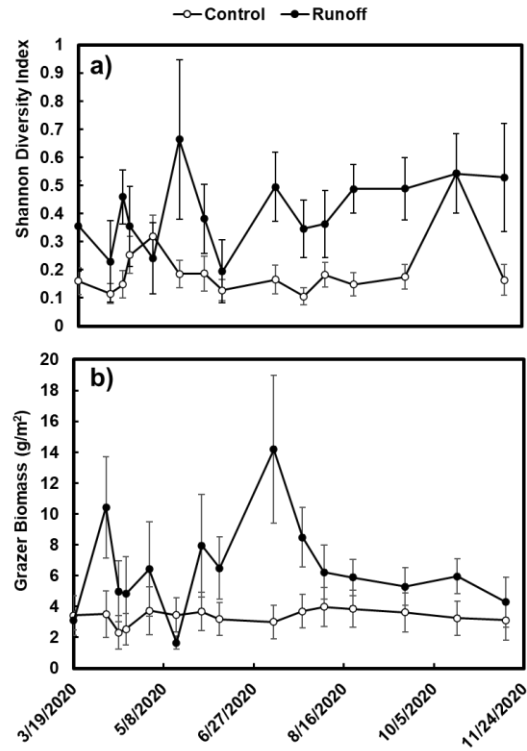


Figure 3. Shannon Diversity Indices and grazer biomass (g/m²) for runoff-affected and control tide pools over time. Each point is the average concentration on that date with corresponding standard error. a) The Shannon Diversity Index for runoff-affected pools was significantly higher than in control pools ($F_{1,22} = 17.2, P < 0.001$). There was a peak in diversity for both runoff-affected (April) and control pools (October) ($F_{25,409} = 1.87, P < 0.05$). b) Grazer biomass (g/m²) was significantly higher in runoff-affected pools than in control pools ($F_{1,28} = 29.1, P < 0.001$). There was a peak in grazer biomass for runoff-affected pools in June-August ($F_{25,409} = 13.2, P < 0.001$).

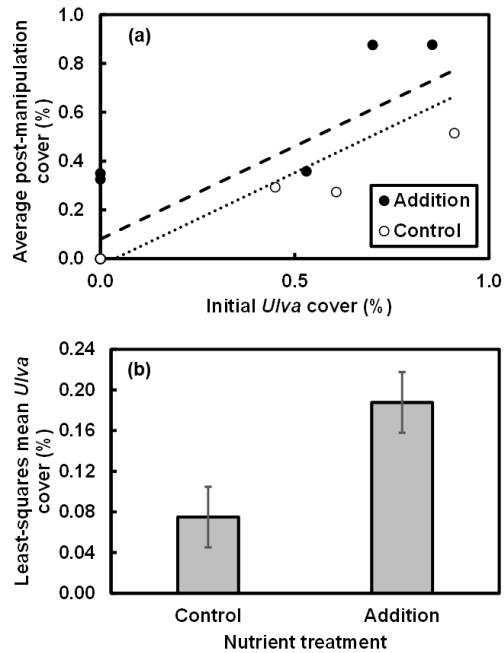


Figure 4. Experimental nutrient additions enhanced cover of the seaweed *Ulva* in tide pools. (a) Average post-manipulation cover of *Ulva* was closely correlated with initial cover ($P < 0.001$), and after accounting for initial *Ulva* cover as a covariate, average cover in nutrient-addition pools was more than twice as high as in control pools ($P = 0.017$). (b) Least-squares means of average *Ulva* cover after accounting for initial cover. Values are means \pm SEs.

CHAPTER 2

Effects of runoff-associated glyphosate in rocky shore habitats on photosynthetic function of an intertidal seaweed

INTRODUCTION

The increasing frequency and concentrations of anthropogenic runoff into coastal habitats threaten the integrity of associated ecological communities (Fabricius, 2005; McCarthy & Incardona, 2008; McPherson et al., 2005). Further, regulations that seek to limit runoff impacts primarily focus on agricultural, industrial, or urban effluent and its effects on freshwater or terrestrial ecosystems (Kayhanian et al., 2012; Kochan, 2005; Roesner & Traina, 1994; Tsihrintzis & Hamid, 1997). Research and corresponding policy regarding domestic effluent and coastal runoff are far more limited, rendering these understudied threats especially impactful in habitats at the land-sea margin, including estuaries, beaches, reefs, and rocky shores (Gunes et al., 2021; Palla et al., 2017). Rocky shores in particular can act as short-term catchment sites at low tide, where tide pools can retain runoff for hours to days at a time, contributing to a long-term pattern of acute, daily spikes of polluted freshwater inputs. Associated pollutants include bacteria, heavy metals, nutrients, synthetic residues, and herbicides (McPherson et al., 2005; McPherson et al., 2002; Noble et al., 2003; Toor et al., 2017). For example, glyphosate ($C_6H_{17}N_2O_5P$), also known as N-(phosphonomethyl) glycine, is the most widely used herbicide in the United States (Duke & Powles, 2008) and a potential pollutant of concern in coastal systems.

Due to its low cost and high effectiveness, glyphosate has become the most commonly used herbicide in global agriculture (Benbrook, 2016; Duke, 2018), and it is available in a variety

of commercial forms, including RoundUp® (Bayer Crop Science LP, St. Louis, Missouri, USA), a broad-spectrum organophosphate herbicide that is commonly applied to control weeds in residential and commercial landscaping (Matozzo et al., 2020). Despite a decade-long debate about its classification as a probable carcinogen (Davoren & Schiestl, 2018; Meftaul et al., 2020; Paumgarten, 2019), Roundup® is readily available in a variety of formulations at local retailers. Furthermore, numerous studies have highlighted the toxicity and impacts of glyphosate in terrestrial and aquatic environments and on a variety of taxa (Annett et al., 2014; Gill et al., 2018; Tsui & Chu, 2003). Despite its documented presence in global watersheds and agricultural runoff (Edwards et al., 1980; Lima et al., 2023; Lupi et al., 2019), the presence and effects of glyphosate in coastal runoff have only recently been investigated.

Through over-application (Benbrook, 2016; Duke, 2018) and a half-life of up to ten months (Mercurio et al., 2014), glyphosate has been increasingly found in marine sediments and wetlands globally (Feltracco et al., 2022; Kim et al., 2010; Skeff et al., 2015). This potentially represents a major threat to coastal ecosystems, as glyphosate is a non-selective herbicide that inhibits the shikimic acid pathway, an essential enzyme pathway in autotrophs, present not only in plants but also algae (Holländer & Amrhein, 1980). Glyphosate can cause significant decreases in enzymatic activity and overall total protein activity in common blue-green algae species (Salman, 2016), decrease leaf area and biomass in seagrasses (Wyk et al., 2022), and reduce chlorophyll absorbance and photosynthetic yield in tropical macroalgae and seagrasses as well as rockweeds (Cruz de Carvalho et al., 2022; Falace et al., 2018; Kittle & McDermid, 2016; Pang et al., 2012). However, most studies on photosynthetic organisms are short-term (around one-week maximum), and at effective concentrations as low as 250 µg/L or 1.48 µmol/L (Cruz de Carvalho et al., 2020; Lam et al., 2020; Matozzo et al., 2020). Only one study has looked at

consistently higher concentrations of glyphosate (up to 36 g/L or 2.13×10^5 $\mu\text{mol/L}$) and its effect on marine macroalgae (Kittle & McDermid, 2016), and long-term studies (up to 56 days) have focused solely on marine invertebrates (Matozzo et al., 2020).

There is growing evidence that glyphosate can cause deleterious effects in a variety of marine organisms at low concentrations and over a short amount of time, but assays are not often included in regular water quality and environmental monitoring (Mercurio et al., 2014). This omission results in a major blind spot in coastal research and management. Further, the impacts of glyphosate have the potential to be particularly strong in ecosystems at the land-sea interface, where proximity to coastal development, the application of herbicides, and extensive irrigation results in year-round runoff to adjacent shorelines (McPherson et al., 2002). For example, the development of the Southern California coastline – where the climate is characterized by hot, dry summers – has resulted in near-constant runoff from irrigated coastal properties via creeks, drains, and seeps onto local beaches, including rocky reefs and tide pools (Ackerman & Schiff, 2003; Littler, 1979; McPherson et al., 2002). Despite being a common model system for experimental ecology (Bracken et al., 2017; Sousa, 1979; Underwood, 2000), rocky shores have been largely ignored in pollution studies and regulations, and the impacts of runoff-associated pollutants remain largely unknown. This issue is of great importance even in otherwise protected habitats like Marine Protected Areas (MPAs) or Areas of Special Biological Significance (ASBS). In Southern California alone, over 270 storm drains have been identified across 14 ASBS sites, contributing significant amounts of anthropogenic runoff (Schiff et al., 2011). In preliminary samples of runoff from creeks, seeps, and drains that flow onto rocky shores in Corona del Mar (CDM) State Beach in Newport Beach, California, we found remarkably high concentrations of glyphosate (see *Results*). These high levels of glyphosate are particularly

concerning given that this shoreline is protected as an ASBS, a State Marine Conservation Area, and a State Beach.

To evaluate the presence of glyphosate and its potential impacts, as well as fill in the gap on high-concentration research, we conducted routine water sampling and quantified the effects of glyphosate on *Ulva* sp., a common seaweed species growing at CDM. This seaweed genus seemed a likely candidate for measuring the potential impacts of glyphosate because of its use in similar studies (Cruz de Carvalho et al., 2022; Kittle & McDermid, 2016), its resilience and quick growth (Bews et al., 2021; Mourad & El-Azim, 2019; Yauchi et al., 2004), and its constant presence at our study location. Further, several studies have documented differential gene expression and increased developmental abnormalities in mussels oysters, and sea urchins (Asnicar et al., 2020; Matozzo et al., 2020; Milan et al., 2018; Séguin et al., 2017) as well as increased stress responses and decreased photosynthetic activity in micro- and macroalgae (Cruz de Carvalho et al., 2020, 2022; Stachowski-Haberkorn et al., 2008) at concentrations lower than what has been observed at CDM. We predicted that the photosynthetic efficiency and chlorophyll-a content of *Ulva* would be reduced at concentrations similar to those observed in the runoff at CDM (see *Results*). Further, due to glyphosate's acidic nature (Devkota & Johnson, 2020), we predicted that glyphosate-associated reductions in pH would result in even greater declines in photosynthetic efficiency and chlorophyll-a.

METHODS

To evaluate these predictions, we placed *Ulva* in a series of concentrations of glyphosate herbicide (RoundUp Super Concentrate®) for 96 hours and measured relative chlorophyll-a using spectrophotometry and photosynthetic efficiency using pulse amplitude modulated (PAM)

fluorometry. Environmental water samples were collected monthly at Corona del Mar State Beach, Newport Beach, California, USA (33.59°N, 117.87°W) for seven months from a variety of runoff sources, including streams, concrete storm drains, and PVC pipes associated with private residences. Two 50mL water samples were taken from each drain-source when runoff was present. Samples remained unfiltered and were frozen within one hour of collection. When both samples were defrosted for analyses, one of them was reserved to assess baseline orthophosphate levels. The pH of the other sample was adjusted to 5 using 0.5M NaOH, 1mL 30% H₂O₂ was added, and the sample was boiled down to a solid using a water bath at 95°C (Glass, 1981). Cool, dry solid samples were rehydrated with 20mL 0.1M HCl until the solid had dissolved, then the total volume was increased to 50mL with artificial seawater. Colorimetric determination via a Lachat nutrient analyzer (Hach, Loveland, Colorado, USA) was used to measure the total phosphate concentration of the transformed sample, from which the natural phosphate concentration of the untransformed sample was subtracted to determine the total glyphosate concentration. Recovered phosphate concentrations of oxidized glyphosate samples were closely related to initial glyphosate concentrations in the samples ($R^2 > 0.99$; Fig. S1).

This experiment aimed to determine how glyphosate, in its form as an isopropylamine salt in the herbicide RoundUp®, would affect the photosynthetic efficiency and chlorophyll-a concentration of the common green alga *Ulva* (collected from runoff-free areas). However, glyphosate, as both a pure isopropylamine salt and the main ingredient in RoundUp Super Concentrate®, is acidic and can lower the pH of artificial seawater-based solutions even at low concentrations (Devkota & Johnson, 2020) (see *Results*). The artificial seawater was made with deionized water, which had a pH of 9 when mixed with aquarium salt.

Therefore, three types of trials were conducted to test both the effect of glyphosate and the compounding effect of pH on *Ulva*. Each trial consisted of $n = 6$ replicates with no glyphosate and $n = 3$ replicates each of 17 concentrations of glyphosate ranging from 4.14 to 51,280 $\mu\text{mol/L}$ for a total of $n = 57$ replicates per trial. The first trial consisted of RoundUp-spiked aquaria with unadjusted pH levels, which resulted in pH levels as low as 4.16 ± 0.01 at the highest glyphosate concentrations. The second trial consisted of RoundUp-spiked aquaria with pH levels adjusted to 8-8.5, using 0.5M NaOH, for all concentrations. A total of 18 concentrations in the range of 0-51,280 $\mu\text{mol/L}$ glyphosate, in the form of RoundUp Super Concentrate®, were chosen to add to the aquaria for the first two trials. The minimum, maximum, and other key concentrations were chosen for the following reasons: 4.14 $\mu\text{mol/L}$ (0.7 mg/L) is the United States Environmental Protection Agency safe drinking water limit for glyphosate (Environmental Protection Agency 2023), 10 $\mu\text{mol/L}$ is the average concentration measured in runoff at the field site (see *Results*), 45 $\mu\text{mol/L}$ is the maximum concentration measured in the runoff (see *Results*), 30,815 $\mu\text{mol/L}$ is the recommended concentration to be sprayed on general weeds and annuals, and 51,280 $\mu\text{mol/L}$ is the recommended amount to be sprayed on tough weeds and perennials. Additional concentrations were used as intervals (i.e., 100; 200; 300; 600; 900; 1,200; 1,500; 3,000; 3,500; 4,000; 10,000; and 20,000 $\mu\text{mol/L}$). The third and final trial did not contain any glyphosate and instead contained seawater at pH levels between 4.3 and 7.6 (i.e., corresponding to the pH effects associated with glyphosate addition), adjusted using 0.5M HCl.

In each trial, aquaria were filled with 1L of Instant Ocean® based seawater (salinity 30-35ppt) and contained 1-1.5g of *Ulva*. *Ulva* individuals were collected from CDM 24 hours before each trial, cleaned of sediment and epiphytes using artificial seawater, acclimated in natural

seawater aquaria at 19°C, and blotted dry before the initial mass was recorded. The aquaria were placed in growth chambers with an average temperature of 22.1±1.3°C, which corresponded to typical field temperatures at the time of collection. Aquaria received an average of 338.3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ of irradiance during the 13-hour daily light cycle. Aquaria were not aerated to better simulate still-water tide pool conditions during low tide. At CDM, the mean duration of tide pool emersion is 73 hours, and some pools are isolated for as long as 9 days during periods of neap tides (Bracken et al., 2018). Photosynthetic characteristics were measured using a pulse amplitude modulated fluorometer (Walz Diving PAM II, Heinz Walz GmbH, Heinz Walz GmbH, Effeltrich, Germany) at five different points over 96 hours: pretrial or 0 hours, 4 hours, 24, 48, and 96 hours. The maximum potential quantum efficiency of Photosystem II, Fv/Fm, was used as an indicator for photosynthetic efficiency (Beer et al., 2000; Burdett et al., 2012; Falace et al., 2018; Kittle & McDermid, 2016; Ralph & Gademann, 2005; Schreiber, 2004). The algae were taken out of the aquaria and placed into small containers with uncontaminated artificial seawater while the PAM was in use. For chlorophyll-a determination, extra *Ulva* samples from each collection period as well as all experimental samples at the end of each trial were placed in 10mL 90% methanol and refrigerated for 48 hours before the extracted liquid was read on a spectrophotometer at 664nm (Kittle & McDermid, 2016).

Environmental glyphosate data was analyzed using ANOVA in the dplyr package (Wickham et al., 2023) in R v.4.1.2 (R Core Team, 2021). The relationship between photosynthetic responses (Fv/Fm and relative chlorophyll-a) and glyphosate concentration was represented by an inverted S-curve that relates performance to stress and includes the concentration corresponding to a 50% reduction in performance as a parameter (Van Genuchten & Gupta, 1993). The model was optimized using the nonlinear least-squares function in the

nlstools package (Baty et al., 2015). The concentration of glyphosate at which there was a 50% reduction in Fv/Fm and relative chlorophyll-a was compared between trial types using an unpaired t-test. pH-associated analyses were conducted using linear mixed effects models with the “lmer” function in the lme4 package (Bates et al., 2015). Full reproducible code is included in Appendix B.

RESULTS

The concentration of glyphosate was measured in runoff from various sources, in tidepools that regularly received runoff effluent, and in the ocean at CDM for 7 months. The average concentration of glyphosate in runoff from creeks and drains was $8.19 \pm 2.71 \mu\text{mol/L}$ (Fig 1a), and the maximum concentration recorded was $45 \mu\text{mol/L}$ (Fig 1b). There were no significant differences in glyphosate concentration between groups ($p = 0.0923$). We found that glyphosate greatly reduced the pH of artificial seawater, even at environmentally relevant concentrations (Fig 2). However, we also found that the presence of *Ulva* mitigates the reduction in pH by glyphosate by up to 2.05 pH units at glyphosate concentrations $\leq 4,000 \mu\text{mol/L}$ ($p = 0.0233$) (Table S1).

The maximum potential quantum efficiency of Photosystem II (Fv/Fm) of *Ulva* was significantly reduced by addition of glyphosate in both the adjusted-pH and unadjusted-pH trials. In the adjusted- and unadjusted-pH trials at zero hours (i.e., prior to glyphosate exposure), there was no change in Fv/Fm, reflecting the fact that individuals had not been exposed. However, by the end of the trials at 96 hours, the glyphosate concentration at which there was a 50% reduction in Fv/Fm was $7,830 \pm 1,807 \mu\text{mol/L}$ glyphosate ($p < 0.001$, $df = 54$, $F = 4.332$) for the adjusted-pH trial and $3,580 \pm 105.9 \mu\text{mol/L}$ glyphosate ($p < 0.001$, $df = 54$, $F = 33.8$) for the unadjusted-pH trial

(Figs. 3a,3b). There was a significant difference in the 50% Fv/Fm values between the two trials at 24 hours ($p=0.010$, $df=112$, $t=2.618$) and 96 hours ($p=0.021$, $df=112$, $t=2.3$) (Fig 4). Because of the number of replicates, we were not able to conduct all trials simultaneously. This resulted in six replicate 0 $\mu\text{mol/L}$ concentrations, which when added to the three replicates of the other 17 concentrations, resulted in 57 total samples. This is, of course, doubled when looking at both the pH adjusted and unadjusted trials, $n = 114$. However, comparisons between the different sets of incubations indicated there was no difference in initial Fv/Fm values between them (Bonferroni adjusted $p=0.091$, $df=7$, $F=23.7$).

The relative chlorophyll-a concentration of *Ulva* was significantly reduced in both the adjusted pH and unadjusted pH trials. In the adjusted- and unadjusted-pH trials at zero hours (i.e., before the experiment), there was no significant concentration at which relative chlorophyll-a would be reduced by 50%. However, by the end of the trial at 96 hours, the glyphosate concentration at which there was a 50% reduction in relative chlorophyll-a was 3414 ± 1300 $\mu\text{mol/L}$ glyphosate ($p=0.0112$, $df=54$, $f=2.625$) for the adjusted-pH trial and 2916 ± 156 $\mu\text{mol/L}$ glyphosate ($p<0.001$, $df=54$, $f=18.615$) for the unadjusted-pH trial (Figs. 5a,5b). Because of the number of replicates, it was not possible to run all trials simultaneously, but there was no difference in initial chlorophyll-a values between sets of trials ($p=0.3314$, $df=12$, $F=1.0245$). Trials in which only the pH of artificial seawater was adjusted yielded no significant differences in Fv/Fm or chlorophyll-a of *Ulva* (Figs. S2,S3).

DISCUSSION

We predicted that Fv/Fm and relative chlorophyll-a would be reduced at concentrations similar to those observed in the runoff at CDM, e.g. we would see a 50% reduction in Fv/Fm and

relative chlorophyll-a at $<50 \mu\text{mol/L}$ glyphosate. Glyphosate can induce a range of effects on various marine organisms at concentrations even lower than those tested; glyphosate-based herbicides decreased cell density, caused membrane damage, and induced various stress responses in marine diatoms at $1.35 \mu\text{mol/L}$ (Cruz de Carvalho et al., 2020), caused disturbances to marine microbial communities at $0.006 \mu\text{mol/L}$ (Stachowski-Haberkorn et al., 2008), and inhibited photosynthetic activity at $1.48 \mu\text{mol/L}$ in *Ulva lactuca* (Cruz de Carvalho et al., 2022). We confirmed that preliminary observations of glyphosate concentrations at CDM exceeded EPA safe drinking-water limits (Fig 1). However, our tests of algal photosynthetic performance indicated that - at least for our target species, *Ulva*, impacts are likely to occur only at much higher concentrations (Figs. 3,5). Similar results are seen for other macroalgal species, with significant reductions in both Fv/Fm and chlorophyll-a at concentrations of $>1,330 \mu\text{mol/L}$ glyphosate (Table 1). Despite these findings, the detection of glyphosate at average concentrations exceeding $8 \mu\text{mol/L}$, twice the EPA safe limit for drinking water, at CDM is still of import to marine organisms, ecotoxicological research, and environmental management (Fig 1).

In addition to the direct effects of glyphosate on organismal performance, its ability to substantially reduce pH even at low concentrations is cause for concern. Low pH levels, in the absence of glyphosate, had no effect on *Ulva*'s photosynthetic performance (Figs. S2, S3). However, low pH enhanced the effect of glyphosate effect on photosynthetic performance, with unadjusted pH trials showing a 50% reduction in performance at concentrations much lower than the adjusted pH trial (Figs. 3,5). Further, adding $45 \mu\text{mol/L}$ glyphosate reduced the pH of seawater from 8.96 ± 0.05 to 8.56 ± 0.10 , a 0.4-unit decline, caused larger drops in pH at higher concentrations (Fig 2). These results may be difficult to discern in the field as pH values in 10

tide pools on the California coast ranged from 7.53 to 8.87 depending on pool characteristics and isolation time (Bracken, Silbiger, et al., 2018). Regardless, the effect of glyphosate on pH is concerning, particularly in the context of climate-mediated changes in ocean pH (i.e., ocean acidification), which have emerged as a major threat to marine ecosystems (Guinotte & Fabry, 2008; Hendriks et al., 2010; Zunino et al., 2021). For comparison, average ocean pH has declined by 0.1 units since the beginning of the Industrial Revolution (IPCC, 2023). CO₂-mediated changes in pH are predicted to result in up to a 1.4-unit decline in mean ocean pH over the next 80 years (Bao et al., 2012; Caldeira & Wickett, 2005), and those changes are predicted to cause reductions in growth, survival, calcification, and reproduction of marine life (Kroeker et al., 2010, 2013; Ross et al., 2011). Our unexpected finding that glyphosate substantially reduces pH, even at the concentrations we observed, could contribute to ocean acidification, albeit by a different mechanism (Bao et al., 2012; Caldeira & Wickett, 2005), highlighting the potential for runoff-associated impacts. Studying effects of glyphosate on pH is particularly important given that it acts as an acidic salt instead of by altering carbonate chemistry, as is typically seen with CO₂-associated ocean acidification. In contrast to CO₂-associated reductions in pH, which can enhance photosynthesis (Bracken, Silbiger, et al., 2018) adding acid to seawater can cause reduced chlorophyll-a content, negatively impact photosynthesis, and reduce algal growth rates (Hinga, 2002; Li et al., 2017).

However, when *Ulva* was added to the glyphosate-spiked seawater, there was a significant reduction in the effect of glyphosate on the pH by up to 2.05 pH units (Fig 2). *Ulva* substantially mitigated the effects of glyphosate on pH at concentrations of $\leq 4,000$ $\mu\text{mol/L}$. This effect is associated with photosynthesis: as *Ulva* removes CO₂ from the water, pH increases due to a reduction in carbonic acid (Björk et al., 2004). The ability of *Ulva* to mitigate low-pH situations

has numerous implications. First, glyphosate is more effective in the lower salinities associated with freshwater runoff, both in the reduction of pH in a less buffered solution and in affecting the shikimic acid pathway (Devkota & Johnson, 2020; Holländer & Amrhein, 1980). *Ulva*'s resistance to changes in pH and salinity (Björk et al., 2004; Ichihara et al., 2013) likely contributes to its dominance in runoff-affected areas where other macroalgal species cannot thrive. Second, *Ulva* mitigates glyphosate-mediated changes in pH by increasing alkalinity, reducing the impact of glyphosate (Fig 2). Whereas these patterns have not been verified in the field, *Ulva* may enhance the resilience of marine communities to both glyphosate and ocean acidification via a mechanism similar to that associated with other coastal vegetation, such as seagrass and kelp (Bracken, Silbiger, et al., 2018; K. Nielsen et al., 2018).

Inputs from storm drains, homeowner-installed drainpipes, and nonpoint source unrestricted runoff can have significant effects on the biodiversity of the ecosystem but are not included in current regulations – including its status as a State Beach, Area of Special Biological Significance, and State Marine Conservation Area – that protect Corona del Mar State Beach. Although we found that the amount of glyphosate required to reduce photosynthetic performance in *Ulva* is almost 1,000 times the EPA safe drinking limit and 100 times the concentrations found in runoff at our study location, these results provide important benchmarks for the regulation of glyphosate in wastewater. Furthermore, observed levels of glyphosate are still a cause for concern, given that the maximum measured concentration of 45 $\mu\text{mol/L}$ (Fig 1), and the highest levels overall were recorded in the freshwater effluent streams at the beach, where the public often interacts with this water through walking, playing, or – especially in the case of small children – swimming. Given the potential health risks of glyphosate, the presence of this pollutant is potentially concerning (Dwight et al., 2007).

In addition, a majority of environmental models and regulations that address the presence of runoff and its water quality only include stormwater effluent and spill events. The constant flow of irrigation-fed streams and the frequent high-concentration bursts from residential pipes are not taken into consideration when determining runoff remediation and policy. Therefore, the introduction of low-pH, glyphosate-contaminated freshwater to coastal habitats via anthropogenic runoff may be an unaccounted-for contributor to ocean acidification. Research into runoff-based pollution and its effects on marine systems is essential for creating frameworks to inform practical environmental policy. The lack of regulation for pollutants like glyphosate, which can impact macroalgae and marine invertebrates, has the potential to cause lasting detrimental effects on marine ecosystems (Asnicar et al., 2020; Falace et al., 2018; Gill et al., 2018; Kilbride & Paveglio, 2001; Lam et al., 2020). The potential for glyphosate to hinder algal growth and photosynthetic efficiency depends on local environmental concentrations, and its unexpected ability to reduce the pH of freshwater runoff represents an additional threat. The inclusion of glyphosate in water quality assessment and regulations is essential for better coastal management, but it is especially pertinent in protected areas such as our study location, as protected species are typically at higher risk of harm from anthropogenic activities (O'Hara et al., 2021; Powles et al., 2000). Overall, the presence and effects of a low-pH carcinogen, like glyphosate, should be given more consideration, as it poses a significant risk to both coastal ecosystem and human health.

FIGURES AND TABLES

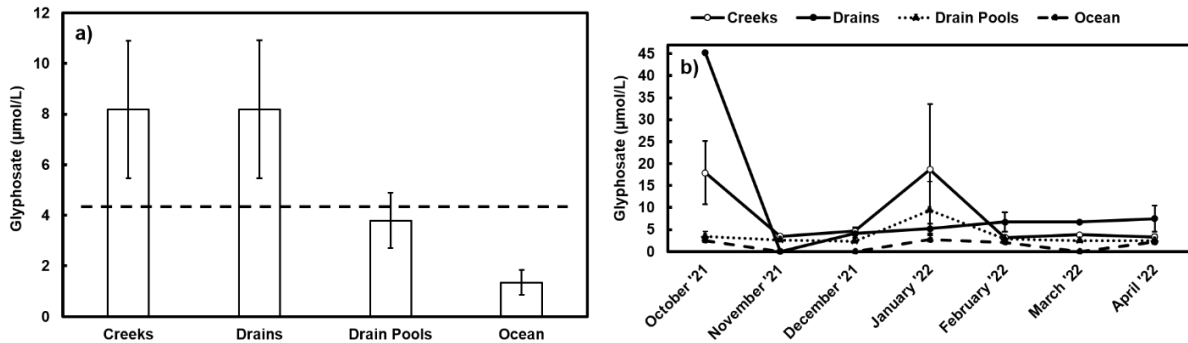


Figure 1. Environmental concentrations of glyphosate associated with various runoff sources. Sources include creeks ($n = 2$), drains ($n = 3$), runoff-affected tide pools ($n = 8$) and the ocean. (a) Overall mean concentrations were highest in creeks and drains and lowest in the ocean. (b) Values were highly variable over time, with maximum values of 45 $\mu\text{mol/L}$ in storm drains in October 2021 and an overall mean concentration across locations of $8.19 \pm 2.71 \mu\text{mol/L}$. The dotted line shows the EPA safe drinking water limit for glyphosate, which is 4.14 $\mu\text{mol/L}$. There were no significant differences in glyphosate concentration between groups ($p = 0.0923$). Values are means \pm SEs.

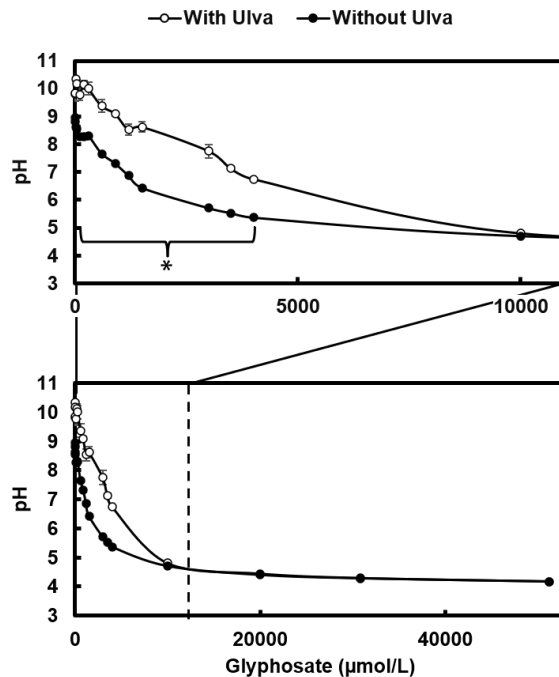


Figure 2. The pH of glyphosate isopropylamine salt solutions after a four-day incubation with and without the seaweed *Ulva*. Glyphosate, when added to artificial seawater, quickly reduced the pH even at low concentrations. For example, at 0 $\mu\text{mol/L}$ glyphosate, the pH was 8.96 ± 0.05 , but it declined to 8.56 ± 0.1 pH at 45 $\mu\text{mol/L}$. When *Ulva* was added, the pH remained significantly higher at all measured glyphosate concentrations $\leq 4,000 \mu\text{mol/L}$ ($p = 0.0233$).

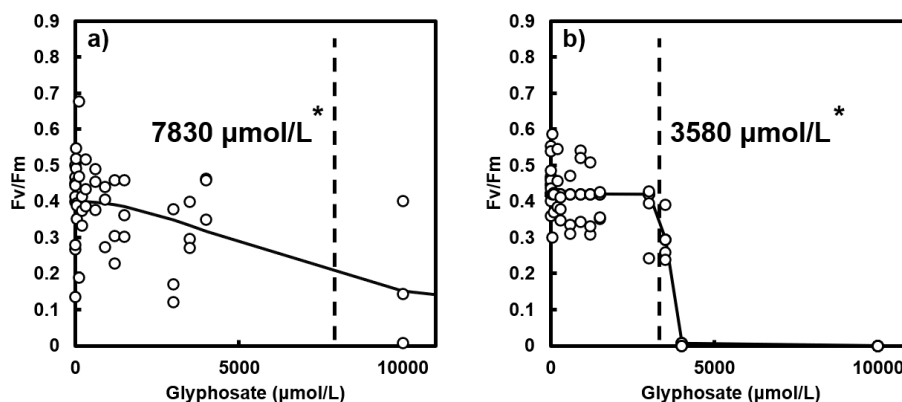


Figure 3. Non-linear least squares models describing the relationship between F_v/F_m and glyphosate concentrations. (a) F_v/F_m values for the adjusted pH trial at 96 hours. (b) The F_v/F_m values for the unadjusted pH trial at 96 hours. The dotted lines represent the glyphosate concentration at which there was a 50% reduction in F_v/F_m : $7,830 \pm 1,807 \mu\text{mol/L}$ in the adjusted pH trial ($P < 0.001$) $3,580 \pm 105 \mu\text{mol/L}$ in the unadjusted pH trial ($P < 0.001$).

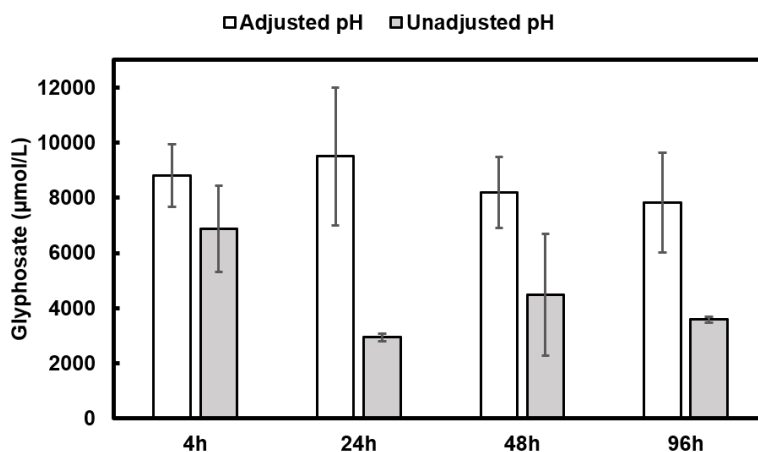


Figure 4. Glyphosate concentrations corresponding to a 50% reduction in F_v/F_m for adjusted-pH and unadjusted-pH trials. The glyphosate concentration at which there was a 50% reduction in F_v/F_m was significantly higher for the adjusted pH trials at 24 hours ($P = 0.010$) and 96 hours ($P = 0.021$). Values are means \pm SEs.

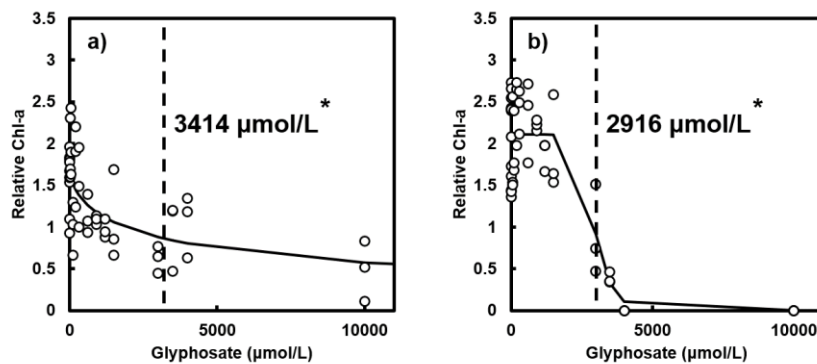


Figure 5. Non-linear least squares models describing the relationship between relative chlorophyll-*a* levels and glyphosate concentrations. (a) Relative chlorophyll-*a* values for the adjusted pH trial at 96 hours. (b) The relative chlorophyll-*a* values for the unadjusted pH trial at 96 hours. The dotted lines represent the value at which there was a 50% reduction in relative chlorophyll-*a*: 3,414±1,300 µmol/L in the adjusted pH trial ($P = 0.011$) and 2,916±156 µmol/L glyphosate in the unadjusted pH trial ($P < 0.001$).

Table 1. Glyphosate concentrations at which there is a 50% reduction in performance for various marine macroalgal species. Performance was measured via F_v/F_m and relative chlorophyll-*a* and compared among species from different studies.

Species	F_v/F_m (µmol/L)	Chlorophyll- <i>a</i> (µmol/L)	Duration	Study
<i>Ulva</i> sp.	3,580	2,916	4 days	This study
<i>Ulva intestinalis</i>	8,872	2,306	5 days	(Kittle & McDermid, 2016)
<i>Gayralia oxysperma</i>	4,613	5,323	5 days	(Kittle & McDermid, 2016)
<i>Rhizoclonium riparium</i>	10,173	5,323	5 days	(Kittle & McDermid, 2016)
<i>Pterocladia capillacea</i>	1,330	2,662	5 days	(Kittle & McDermid, 2016)
<i>Neosiphonia savatieri</i>	8,872	NA	5 min	(Pang et al., 2012)

CHAPTER 3

From Ridge 2 Reef: An Interdisciplinary Model for Training the Next Generation of Environmental Problem Solvers

INTRODUCTION

Research universities are responsible for training scholars who can apply knowledge to address societal challenges (Duderstadt, 2012; O'Neill et al., 2019), including environmental issues. These institutions are also positioned to address the need for an interdisciplinary workforce as they train students in all fields. Yet science-based solutions to environmental problems have historically been limited by disciplinary boundaries that inhibit systems-level analysis (Postel, 2000; Schenk et al., 2009; Van Den Beemt et al., 2020). Graduate programs often reflect this partitioning, providing students with in-depth training and education in a single discipline. By neglecting interdisciplinary training in curricula and degree requirements, traditional programs make it difficult and time-consuming for students to seek collaborations or skill development outside their explicit field of study (Campbell et al., 2005).

Whereas many job sectors are needed to solve environmental problems, graduate programs have typically focused on preparing students for careers in academia. This narrow focus does not reflect the post-graduate reality, in which 57% of Ph.D. recipients pursue careers outside of academia, according to a 2017 survey by the National Center for Science and Engineering Statistics (Early Career Doctorates Survey, 2017). Without sufficient training in communication, project and team management, budgeting, leadership, stakeholder engagement, and other transferrable skills, graduate students may be ill-equipped to address and resolve real-world

problems that span multiple disciplines and require solution-based research (McGunagle & Zizka, 2020).

To fill these gaps in the graduate education of environmental problem solvers, the Ridge 2 Reef (R2R) training program was established at the University of California, Irvine (UCI), in 2017. R2R was specifically designed to train a new generation of graduate students with the skills to tackle environmental challenges, especially in regions heavily impacted by human activities. For example, the wildland-urban interface in Southern California faces threats from wildfire, invasive plants and animals, drought, extreme heat, and pollution (Jenerette et al., 2022). Mitigating these threats requires collaboration across disciplines such as engineering, ecology, and law, as well as engagement with stakeholders including government agencies, private entities, universities, and the public (A. M. Gill & Stephens, 2009).

Compared to traditional graduate programs that focus on disciplinary training and academic career paths, R2R was designed to address the need for a 21st century workforce that bridges disciplines in and out of academia (Fig 1). The program also aimed to train students with transferrable skills, such as interdisciplinary communication, project management, and quantitation, while offering opportunities to pursue projects that traditional graduate programs do not have the resources to support. Specifically, R2R sought to accomplish five goals:

- Goal 1 – *Develop interdisciplinary skills* and scientific knowledge to facilitate management of terrestrial and aquatic ecosystems experiencing environmental change.
- Goal 2 - *Promote transferable, career-relevant skills* through curriculum that emphasizes quantitation, communication, and professional development.

- Goal 3 - *Build partnerships* in and out of academia to enhance trainee career placement and effective knowledge transfer.
- Goal 4 - *Broaden participation* in the pipeline of graduates pursuing environmental careers.
- Goal 5 - *Institutionalize success* by incorporating effective elements of the R2R program including courses, partnerships, collaborations, and professional development activities into other graduate programs while disseminating the training model to other institutions.

The aim of this paper is to describe the R2R program and assess its effectiveness in achieving Goals 1 through 5 with quantitative and qualitative methods. After briefly introducing the program structure, we discuss the program evaluation approach and outcomes. Finally, we discuss lessons learned that may aid other institutions seeking to build programs in transdisciplinary graduate education.

Program Description

R2R was funded by a \$3-million grant to UCI from the National Science Foundation (NSF) Research Traineeship (NRT) program. The program began in 2017 and ended in 2023, ultimately enrolling a total of 54 graduate students (“trainees”), including 3 Masters and 51 PhD students. These trainees were enrolled in five cohorts, with Cohort 1 beginning in 2017, Cohort 2 in 2018, Cohort 3 in 2019, Cohort 4 in 2020, and the final cohort, Cohort 5, beginning in 2021.

Governance

The governance structure of R2R included a faculty director, an academic coordinator, and an executive committee with oversight from an external advisory board. The director was the lead

principal investigator on the NRT grant and chaired the executive committee. The academic coordinator built partnerships with external entities and managed daily program operations including scheduling, communications, finances, logistics, staffing, and assisting students. The executive committee advised the director and included nine UC Irvine faculty members who were well recognized scholars in ecology and evolutionary biology, Earth system science, civil and environmental engineering, epidemiology, and political science. Two trainee representatives served on the executive committee to communicate trainee needs and feedback. The trainee representatives were nominated by their peers and served staggered 2-year terms to promote institutional memory. The four-member external advisory board included representatives from two outside academic institutions, a conservation non-profit, and a joint powers research agency. These external advisors were consulted to provide a real-world perspective in designing the program curriculum and training activities.

Program Phases

The R2R program consisted of four different phases: 1) pre-curricular activities including recruitment, admission, and orientation; 2) structured curricular activities including two years of formal coursework; 3) a partnership/internship experience; and 4) optional opportunities (e.g., annual multi-day workshops called Summer Institutes, student-led and social events, funding opportunities, and career-related skills training). The program structure changed over time in response to feedback from trainees, faculty, and external advisors. The final program timeline is shown in Fig 2 and described in detail below.

The first-year curriculum aimed to improve and expand environmental research knowledge, interdisciplinary collaboration, and communication skills (Goal 1: Interdisciplinary

Skills & Goal 2: Promote Transferable Skills) with a Communication Skills course and series of seminars. In the Communication Skills course, trainees received instruction from a professional communication and presentation consultant to communicate effectively with audiences outside of their disciplines and in non-technical language. All courses incorporated visiting speakers from sectors such as education, research, government, policy, non-profit, religion, public agencies, science communication, and industry who discussed career options and the skills and qualifications necessary to pursue those positions.

A three-course curriculum aimed to equip second-year trainees with leadership skills and opportunities for professional and career development, public engagement, professional certification, and career placement (Goal 2: Promote Transferrable Career-Related Skills). The first course, Professional Workshop, included informational interviews, certifications, and pertinent training for professional development. The second Inclusion and Team Science course explored the benefits and challenges of working in a collaborative environment and provided trainees with strategies to succeed in collaborative work. Finally, the Project Management course culminated in a collaborative project designed by the enrolled students.

At a time that was convenient to the trainees in Cohorts 3-5, students both designed and participated in a partnership or internship, applying their training from the two years of courses to real-world issues (Goal 3: Build Partnerships). Partnership/internship requirements were flexible so the experience could be most effective in preparing an individual trainee for their specific field and career aspirations. The disciplinary focus of internships or partnerships varied based on trainees' career and research goals.

Each program year, R2R hosted a themed Summer Institute to reinforce trainee technical skills, identify knowledge gaps in research fields, interact across disciplines and cohorts, and help

develop trainees' identities as researchers. Overall, R2R held four multi-day institutes each with a unique theme: Climate and Life (2018), Microbes and Global Change (2019), Environmental Data Science (2021) and Program Success and Sustainability (2022). The 2020 Summer Institute was canceled due to the COVID-19 pandemic. The Microbes and Global Change and Environmental Data Science Summer Institutes were focused on building data analysis skills through writing code and completing analyses of ecological datasets in small groups.

Trainee Support

To free up student time for training activities and encourage program participation (Goal 4: Broaden Participation), R2R offered financial support through competitive fellowships. After completing one year in R2R, trainees were eligible to apply for a one-year R2R fellowship that covered tuition and stipend support. Annual stipend levels were set according to NSF guidelines at \$34,000 until the 2022-2023 academic year when they rose to \$37,000. R2R fellowships were awarded by the program executive committee to trainees who demonstrated the potential to create new opportunities for research and training, support interdisciplinary collaborations and/or internship experiences, disseminate research outcomes broadly, and achieve academic or career goals.

Mentoring groups were established to provide professional support and promote program cohesion. R2R mentoring groups consisted of three or four trainees across different cohorts and one R2R faculty member. Mentoring groups were coordinated by senior trainees and met quarterly to discuss topics relevant to professional or academic development such as internship applications, grant writing techniques, and career progression.

METHODS

Ethics Statement

The formal evaluation of R2R was approved by the UCI Institutional Review Board (human subjects number 2017-3722). All participants provided informed verbal consent. As a low-risk, exempt study, the Institutional Review Board did not require or recommend obtaining written consent from participants. Verbal consent was deemed sufficient and reduced the administrative burden on participants and program staff. Prospective participants were provided with a study information sheet describing the evaluation process and goals. Consent to participate in the study was witnessed by project leaders and documented in a participant tracking sheet maintained by the project external evaluator. Consent was voluntary and could be revoked at any time with no penalty. No minors were involved in the study. The recruitment period for this study began on November 2, 2017, and ended on August 31, 2023.

External Program Evaluation

The R2R executive committee contracted The Mark USA, Inc. (“The Mark”) to lead formal program evaluation. Based in Irvine, California, The Mark is a professional evaluation firm with over 10 years of experience evaluating hundreds of academic and private sector programs. At the beginning and end of each academic year, The Mark administered quantitative evaluation surveys to assess progress toward achieving program goals. Assessment tools also included qualitative interviews of current trainees as well as faculty and internship partners. Note that there was no “control” group consisting of students who did not participate in the program, so analyses and inferences are limited to R2R participants. Findings from surveys and interviews were used in

formative assessments to adjust program goals and guide development of training activities. Results were also used in summative assessments to evaluate progress toward achieving R2R's five main goals. Formative and summative assessment results are discussed in the next section and raw data from the evaluation instruments are accessible in (Manley et al., 2023).

Interview Methodology

To understand the impact of different aspects of the program, program leaders conducted semi-structured interviews with trainees regarding their experience in the R2R program. Interviews took place at the end of each academic year. In the first year (2018-2019) of interviewing, each trainee was interviewed separately. In the following years, trainees were interviewed in cohort-based focus groups, which varied in size from two to five trainees (see Table S1 for the number of participants by cohort in each academic year). Interviewers audio-recorded and transcribed the interviews. Transcripts were separated into idea units corresponding with the interview questions. The idea units were coded to identify themes related to program strengths, challenges, and recommendations for program improvements.

Survey Methodology

Annual pre- and post-surveys were developed by The Mark in consultation with the R2R executive committee. The surveys included Likert scale items and open-ended questions to assess goal areas and collect feedback on participants' experiences in the program. Participants rated all Likert scale questions on five- or seven-point scales. The pre-survey asked questions that measured trainees' knowledge and skills (including environmental policy knowledge and data analysis, communication, leadership, and mentoring skills), career interest and knowledge, and

collaborations in research and education-related activities at the beginning of program year 1 for each cohort. At the end of each academic year, participants were asked in the post-survey to rate their knowledge and skills again to assess annual changes in these areas, to report their collaborations over the year as well as their current career interest and knowledge, and to provide feedback on courses and program activities.

Of the 41 trainees who participated in the R2R evaluation process at the end of Spring 2022, one was in Cohort 1, 10 were in Cohort 2, five were in Cohort 3, 19 were in Cohort 4, and six were in Cohort 5. Analyses were conducted for all respondents to assess changes in knowledge and skills (Cohorts 1, 2, 3, 4, and 5 combined). Analyses performed on “matched trainee respondents” include those respondents who completed their respective pre-survey and the 2022 post-survey. In total, there were 25 matched trainee respondents representing five cohorts (Table S2).

Data Analysis

We analyzed quantitative results using means, response frequencies, ranges, and parametric tests (two sample t-tests) to assess the statistical significance ($p < 0.05$) of changes in trainees’ reported knowledge and skills. Survey items to measure growth areas were categorized by concept and composite means were calculated.

The Mark’s survey data were used to assess changes between cohorts in different skillsets throughout the timeframe of the program. Each skillset has multiple questions within the overall theme. For example, interdisciplinary interaction and collaboration included 10 unique questions. To get an overall understanding of dynamics within each cohort, we annually averaged respondents’ answers for each cohort within each unique skillset. To ensure uniformity, we

normalized the data on a scale of 0 - 1 $((\text{data} - \text{minimum value}) / (\text{maximum value} - \text{minimum value}))$). Because cohorts consist of different trainees with different backgrounds, experiences, and skillsets, each individual and cohort has a different baseline for each skillset coming into the program. To best control for this variable baseline between cohorts, we standardized the data by subtracting each cohort's mean pre-survey answers for each skillset from their post-survey means for each year. We then combined Cohorts 1 and 2, and Cohorts 3, 4, and 5 to analyze trends and assess how evolution of the program could have influenced differences between initial cohorts and later cohorts (Fig 3) and ran two-sample t-tests for combined cohorts to determine statistical significance of changes to surveyed skills.

However, there was no control group of graduate students of which to compare the R2R cohorts' results. This is due to many different factors. First, the proposed control group would have to complete the annual surveys, across their full time at UCI, on a voluntary basis. Further, program leaders would have to identify students from similar disciplines across campus and in different stages of degree completion. New students would have to be interviewed each year of the program as well, to further simulate the incoming cohorts. This posed too difficult a task to accomplish, however, it is recommended to integrate a control group in the assessment of future programs.

RESULTS

Quantitative Evaluation

Quantitative evaluations based on annual survey responses generally showed improved interdisciplinary and transferable skills and success in building partnerships and broadening participation. Goal 5, Institutionalizing Success, was not evaluated in the quantitative evaluation.

Goal 1: Develop Interdisciplinary Skills

Cross-disciplinary knowledge significantly increased ($p < 0.001$), but evaluation surveys did not show marked improvement in collaboration skills (Fig 4) In 2020, there was a clear decrease in interdisciplinary interaction and collaboration ($p < 0.001$) (Fig 4).

Goal 2: Promote Transferable Skills

Leadership skills, which aid in project management, consistently improved year-to-year, with an average improvement of $4.8 \pm 2.9\%$ ($p < 0.001$) (Fig 4). Similarly, mentorship skills improved consistently, with an average annual improvement of $8.6 \pm 2.3\%$ ($p < 0.001$) (Fig 4). Trainees' data analysis skill improved in years following the Year 2 (Microbiomes and Global Change) and Year 4 (Environmental Data Science) Summer Institutes by 13% and 19%, respectively ($p < 0.001$) (Fig 4). Students' self-reported skill level and confidence with data analysis consistently improved year to year, with an average annual improvement of $15.2 \pm 2.8\%$ ($p < 0.001$) (Fig 4). Trainees reported improved communication skills and important strides in career knowledge, with an average annual improvement of $14.8 \pm 6.3\%$ ($p < 0.001$) and $27.8 \pm 4.3\%$ ($p < 0.001$), respectively (Fig 4).

Goal 3: Build Partnerships

The disciplinary focus of internships or partnerships varied from research to education, policy, communication, and fine arts across over 40 experiences with 37 different entities. These internships and collaborations included participation with eight non-profit organizations (e.g., Irvine Ranch Conservancy), 14 government agencies (e.g., National Aeronautics and Space

Administration) from city to federal levels, 11 educational groups or institutes (e.g., Coastal Ocean Environment Summer School in Ghana), and four industry groups (e.g., IQ Air). Within these internships and collaborations, the areas of focus were diverse with trainees working on projects ranging from communication, education, and research to conservation and policy. These experiences are reflected in large increases in trainees' knowledge and understanding of global perspectives of research, with an average annual improvement of $28.8\% \pm 5.7\%$, post Cohort 1 ($p < 0.001$) and a smaller, non-significant increase in Cohort 1 ($p = 0.25$) (Fig 4).

Goal 4: Broaden Participation

R2R cohort sizes ranged from 6-19 trainees, and disciplines varied between years (Fig 5). Most trainees came from STEM fields such as ecology and evolutionary biology, earth system science, and civil and environmental engineering, while a smaller number of trainees came from outside STEM fields, including history and education. Program targets for broadening participation were set to reflect national demographics, with a goal of >50% identifying as females or non-binary and >32% identifying as underrepresented minorities (URM). By the end of the program, 69% of trainees responding to evaluation surveys were female and 46% were URM. As of 2022, 15 trainees had graduated from the program. Of graduated survey respondents ($n = 11$), all held professional or academic positions in STEM.

Qualitative Evaluation

Qualitative evaluations focused on Goals 3-5 and generally supported the results of the quantitative evaluation. Challenges noted by R2R trainees in surveys were also expressed in qualitative evaluation interviews.

Goal 3: Build Partnerships

In the early program years, networking with professionals in science beyond UCI was limited to individuals who could meet in person. Trainees expressed that they felt limited in their ability to find internships with partner organizations. Both graduate students and alumni lacked formal avenues to stay connected, for example through an alumni or research conference. The external evaluator recommended that a directory of former alumni could benefit R2R students by creating new opportunities for communication and mentorship. Information such as post-graduate career pathways and job openings could be posted through the directory, allowing R2R participants to make connections with alumni in different fields within and outside of academia. In response to these needs, the R2R program launched a LinkedIn page in 2021.

After the R2R core curriculum had been redesigned, and with a rise in the number of disciplines represented by trainees, from four departments to seven, there were larger increases in some skills and knowledge for Cohorts 3-5 as compared to Cohorts 1-2 (Fig 3). PhD trainees provided feedback highlighting a need for flexibility with R2R program requirements to align with specific graduate program requirements and individual dissertation research needs. As a result, starting with Cohort 2, internship requirements became more flexible and were re-branded as a partnership requirement. This requirement was intentionally broadened so trainees and their faculty advisors could align internship experiences or collaborations with specific needs of each trainee.

Benefits of external partnerships and collaborations were not only limited to students and the university. Interviews show that external partners expanded their capacity for solving environmental problems by working with R2R trainees and faculty. Interviewees reported

organizational benefits such as building a stronger connection with the university and an increased capacity to conduct research. Three partners noted that R2R internships brought individuals with a great deal of high-quality research experience into their organizations.

Goal 4: Broaden Participation

R2R began an internal mentoring program in program Year 3, which was intended to address student feedback that highlighted a lack of faculty involvement, low diversity in faculty mentors, and limited interaction between R2R cohorts and across departments. During the initial roll-out of the mentorship program, mentoring groups included one faculty mentor, one peer mentor, and 2-3 additional trainees. Trainees and faculty were assigned to mentoring groups by the R2R academic coordinator. In its first year, the mentoring program received mixed reviews with suggestions that more structure and/or guidance for mentoring groups was needed. In its second year (program Year 4) the mentoring program had a similar format, with added guidance on quarterly discussion topics provided by the academic coordinator to provide structure. The mentoring program continued to receive mixed reviews and feedback, with some mentoring groups being very satisfied with the program while others were dissatisfied. In year three of the mentoring program, the academic coordinator sent out a survey to ask trainees about their goals for the mentoring groups and then assigned trainees to groups accordingly to improve mentorship compatibility and productivity within the groups.

Further, the perceived lack of disciplinary diversity within the R2R faculty (most were affiliated with ecology or Earth system science) posed a problem throughout the entirety of the program, leading to some trainees not feeling supported and a subsequent decline in both staff and student involvement within and across cohorts. Despite a dynamic combination of student trainees,

the combined effect of low social interaction and lack of disciplinary representation initially limited trainees' ability to participate in collaborations with other researchers.

Evaluation results confirmed that fellowship support facilitated participation in R2R training activities as well as buy-in from faculty mentors. One trainee expressed that being on an R2R “fellowship [gave them] cushion to focus on research and expand [their] network,” and 18% of the program Year 5 post survey respondents indicated being awarded a grant or fellowship as their greatest achievement in the R2R program. Aside from funding for their students, faculty also realized benefits from R2R's training model. The R2R curriculum was successful at developing students' technical and professional skills, enabling faculty to focus on other trainee needs.

Goal 5: Institutionalize Success

Trainees provided feedback that R2R courses were too time intensive and were not manageable with other demands from their graduate programs. Additionally, trainees expressed a desire to develop skills more applicable to communicating with the public and interdisciplinary colleagues. In response to trainee surveys, the program was revamped to address the concerns and desires voiced by trainees. This curriculum redesign, which dropped two disciplinary-focused courses and revamped the second-year courses, resulted in the program structure that was described in the Introduction. Trainee responses to curriculum redesign were positive overall, and they appreciated R2R leaders' ability to continue making substantive changes to the program over time. The six required courses taken by R2R Cohorts 2-5 were maintained until the end of the program, indicating a shift towards a consistent and sustainable curriculum.

Early cohorts of trainees encountered issues with conflicting time commitments depending on each student's need to balance research, coursework, and teaching assistantships – a primary

source of funding for several trainees. Additionally, requirements of the R2R program were sometimes confusing to trainees, who sought more clarity on what was expected of them.

After program Year 1, trainees shared feedback with the executive committee on their confusion about expectations and opportunities for funding from the program. The first requests for applications to the program included statements about which departments could participate and receive funding from R2R, confusing applicants. Further, early R2R cohorts felt that funding applications were ambiguous in their guidelines for the statement of interest. As a result, application prompts were clarified and improved over time based on student feedback. Students also expressed that program expectations appeared unstructured and unclear after joining the program. Although issues from past years were addressed, lack of clarity and communication resulted in persistent confusion about program goals, expectations, and progression. These problems were ultimately alleviated by program leaders creating an R2R handbook and hosting an orientation for new trainees.

DISCUSSION

The R2R program was designed to provide transferrable and interdisciplinary skill training, which is often not addressed in traditional graduate programs, to better prepare a diverse group of graduate students to face the inherently complex challenges that come with environmental problem solving. The program successfully trained students in communications, project management, and quantitative skills among others as evidenced by survey and interview responses. Although evaluation sample sizes were limited by the number of students who chose to enroll in the program and participate in surveys and interviews, we obtained enough responses to detect changes as small as 5% in skill levels based on annual pre- versus post-surveys.

Goal 1: Develop Interdisciplinary Skills

The R2R evaluation plan focused on interdisciplinarity but we acknowledge that transdisciplinary problem solving is needed to approach complex problems in the environmental sciences. Transdisciplinary approaches go beyond interdisciplinarity to generate outcomes that are greater than the sum of their disciplinary parts, such as the founding of a new field or concept (Lennon et al., 2023; Salazar et al., 2012). Interdisciplinary training from R2R has helped students move towards transdisciplinary thinking, and R2R trainees are becoming ambassadors for transdisciplinarity. For example, a team of trainees designed and hosted the inaugural *Art + Ecology: Stories that Build Connections* Gala in spring 2022, bringing together artists and ecologists to re-envision ways of thinking about solutions to local environmental challenges. Related to that vision, the students spearheaded an effort to create an interdisciplinary art space at the Burns-Piñon Ridge Reserve, a University of California natural reserve in the Western Mojave Desert. This effort has continued into the undergraduate sphere via a weekend of workshops in the new art space as well as a graduate course in art and ecology co-led by an R2R trainee. These outcomes demonstrate that R2R training is driving an institutional shift toward cultural attitudes and practices that support transdisciplinary scholarship.

Goal 2: Promote Transferable Career-Related Skills

The Communication Skills and Project Management courses, as well as internships, collaborative projects like this paper, and opportunities to put skills into practice during R2R events, helped trainees increase their knowledge and confidence in transferable skills not offered in their home programs. The communications component of the R2R program was very successful,

with students reporting a significant boost in their ability to communicate effectively in oral and written contexts to lay audiences and professionals in and out of a student's field of study. Program trainees reported an increase in overall confidence in skills and clarity in their career trajectory.

R2R provided opportunities for trainees to receive structured, consistent mentoring and skills training outside of the traditional academic apprenticeship model under which the responsibility for training falls predominantly on the faculty advisor. These mechanisms are important because faculty vary substantially in their backgrounds, experience, and availability for mentoring (Austin et al., 2007; Morgan et al., 2022). R2R training filled these gaps in faculty mentorship capacity, benefiting both the student and the advisor.

Goal 3: Build Partnerships

Throughout R2R, trainees developed partnerships with each other through coursework, workshops, and collaborative projects and with external partners through internships and other collaborations. These relationships increase UC Irvine's visibility in multiple communities and create opportunities for future career placement as well as collaboration. Partnerships also boost the relevance and impact of university research by ensuring that it addresses community needs. The R2R course on Inclusion and Team Science equipped trainees with the tools to center ethics and equity in their relationships with partners. Such training is crucial for avoiding extractive research practices and prioritizing communication and research co-design with partners from the very beginning of a project (Strand, 2003). These principles of research justice will carry forward in the new programs stemming from R2R (Table 1).

Partnerships built during the R2R program were maintained throughout the program, regardless of external factors including the COVID-19 pandemic. Although there was a substantial

decline in social interactions among trainees from Fall 2020 to Spring 2021 in all cohorts, these scores ultimately improved, surpassing pre-pandemic values following 2021. This return to pre-pandemic levels suggests that R2R built sufficiently strong partnerships that external collaborations and internships could resume as soon as pandemic restrictions were lifted.

Goal 4: Broaden Participation

Expanding diversity in both disciplines and participant demographics was key to achieving program goals. From program Year 2 onwards, R2R met its diversity goals focused on inclusion of underrepresented groups. Although survey data only tracked participation by trainees self-identifying as URM, the recruitment and pedagogical practices adopted by R2R could also promote participation by students identifying as low-income, first generation, or people of color (POC). The R2R program did face some challenges with few trainees enrolling initially due to a lack of knowledge about the program on the UCI campus. The R2R website was still being developed and finalized which might explain some of these growing pains while interest from students, faculty, and departments was still being established.

Aside from achieving its goal of >32% URM participation, retention of all trainee groups—including URM students—exceeded 90%. As part of the recruitment strategy, R2R program leaders decided early on to emphasize building a supportive, inclusive community to which URM trainees would feel a sense of belonging. R2R prioritized high-quality training, community-building, and engagement with diverse partners to create an inclusive space for graduate education. Trainees appreciated these priorities during their time in the program, commenting positively on the importance of having a diverse community in creating a supportive environment and conducting interdisciplinary research. The emphasis on community building provided clear

retention benefits for URM students and the institution. Fellowship support and the prestige associated with participation in NSF's flagship traineeship program may have also contributed to high retention rates. Going forward, these outcomes will likely attract URM recruits into new programs developed from R2R.

Goal 5: Institutionalize Success

Graduate students contribute to the research and educational mission of universities, so benefits they experience can have positive impacts on institutions (Sampson et al., 2018). R2R aimed to institutionalize successful elements and disseminate its training model to other institutions (Goal 5: Institutionalize Success). Program leaders recognized graduate students as essential for making connections across research teams, providing a foundation for transdisciplinary research and education. A highly skilled and confident community of graduate trainees tackling environmental grand challenges is a tremendous asset for any university. By fostering this community, R2R added value to UCI's human capital as well as the workforce into which trainees enter. Moreover, programs like R2R that set and achieve goals in minority representation can help create a more diverse environmental workforce.

The term "culture of improvement" was coined during focus groups with R2R trainees to describe how trainee feedback informed program decision-making. We define culture of improvement as the willingness and ability of a higher education program to directly integrate assessment evidence into decisions on program and curricular structure and teaching practices (Stanny, 2018; Suskie, 2014). For R2R, the culture of improvement was particularly strong in the intentional use of feedback and (re)design of program elements. This culture of improvement

likely contributed to gains in skill development observed for later cohorts (e.g., communication skills, $p = 0.05$; leadership skills, $p = 0.007$; and mentoring skills $p = 0.02$) (Fig 3).

There is emerging evidence that R2R has begun to shift institutional attitudes toward interdisciplinary scholarship. Some of this shift is occurring through new and ongoing programs that have adopted R2R curriculum and ideas (Table 1). One of the NRT program's main goals was to develop bold new models of graduate education with benefits that extend beyond individual grants in time and scope. At UCI, R2R has expanded the capacity for research and education through multiple avenues. Trainees have generated over 40 publications in 34 different journals and given dozens of presentations on their research. Papers which trainees authored were published in disciplines ranging from social ecology to hydrology and marine biology in journals including *Nature Sustainability*, *Proceedings of the National Academy of Sciences*, and *Journal of Problem Based Learning in Higher Education*. The full list of publications can be accessed on the NSF Public Access Repository (<https://par.nsf.gov/search/term:1735040>). Beyond these traditional metrics of research output, teams of faculty have been successful in leveraging R2R ideas and training elements to build new programs, including several that are externally funded (Table 1). In all cases, these programs have included R2R program faculty and adopted elements of the R2R training model such as courses, research ideas, and recruitment plans.

Lessons Learned from R2R

1) Focus on student outcomes—It was imperative to keep the program focused on student outcomes, particularly regarding required courses. Initial classes were not program-specific but were drawn from existing course offerings of program faculty. Those courses left some program and student needs unmet. Consequently, the R2R curriculum was revised to be more flexible and

student-driven, allowing trainees to apply their knowledge and skills in new contexts, further developing their career adaptability and confidence.

2) Conduct rigorous program evaluation—It was very beneficial to have multiple mechanisms for program evaluation. A mix of biannual surveys, interviews, and feedback from the trainee representatives on the executive committee ensured that program leaders quickly learned which elements of the program were not working well. Regular assessment is key to identifying specific challenges and making programmatic changes, ultimately fostering the “culture of improvement” and adoption of more effective courses or activities.

3) Prepare students for collaboration—Students should be trained in collaboration and team research before expecting them to engage in partnerships or internships. Early cohorts that jumped into such partnerships without sufficient training had a harder time succeeding in projects with partners. Also, additional training responsibilities were pushed onto the partner, reducing the time available for research and collaboration with the trainee.

4) Diversify learning outcomes—Events such as the Summer Institutes should be structured around broad learning outcomes that go beyond traditional research and technical skills. Workshops proved more successful and well-received by trainees when time for socializing among peers, recreation, and networking was allotted in the schedule. Building inter-personal connections and self-confidence is crucial for trainee success in the program. Evaluations also revealed that throughout the program, trainees developed partnerships with each other through collaborative projects, coursework, and workshops including the Summer Institutes.

5) Build community—Graduate school can feel isolating, and especially during the Covid pandemic, social events were an invaluable aspect of the program for trainees. R2R not only organized program social events but also to supported trainees in designing their own events.

Social events varied in size and form, ranging from pastry and coffee hours to symposia, lunches, seminars, and the *Art + Ecology* Gala. Providing a space and structure for socializing allowed trainees to build community, resulting in new collaborations and partnerships while building teamwork skills. The trainees also gained perspectives on different fields, collaborations, and ways of thinking that they may not have otherwise encountered.

Implications for Graduate Education

Overall, R2R was largely successful in achieving its goals, with a student-centered culture of improvement emerging from the program. Real-time student feedback was solicited, driving change in the R2R training program as well as lasting institutional change. Students benefited from a supportive R2R community—trainees indicated that they felt safe asking for help and making mistakes without fear of judgment. There were positive feelings about the culture of improvement, and the community aspect of the program was particularly impactful for cohorts that began during virtual instruction necessitated by the Covid pandemic as it supported meaningful connections during an otherwise isolating time. The program also directly benefited faculty mentors, departments, and the institution by providing additional training, financial support, and interdisciplinary collaborations which helped build connections between the university and external communities.

Any graduate program, regardless of focus, can benefit from the student-focused culture of improvement that emerged from the R2R training model. When implemented as the foundation of a program, this educational perspective can strengthen training for students and positively impact their careers. The commitment to academic, professional, and cultural diversity throughout all aspects of R2R ensured an atmosphere of consideration for multiple perspectives and ideas. The

integration of surveys and rapid, feedback-driven changes to program structure ensured that students' needs were respected and aligned with the program goals.

A dynamic program like R2R is rare within academia, which is often bogged down by constraints of traditional graduate programs including institutional legacies, outdated measures of student outcomes, lack of willingness to change, and lack of interdisciplinary training (Abelha et al., 2020; Leshner & Scherer, 2018; Wells, 2013). Intentional collection of qualitative and quantitative data on stakeholder engagement within the program, with the intention to listen and respond, allowed R2R to foster a culture of improvement. This dynamism is likely a significant reason why many R2R students reported increases in multiple skills over the course of the program and why many described being greatly satisfied with their experiences in the program (Fig 3).

Graduate programs that establish a culture of improvement will better prepare the next generation of researchers to address complex societal problems. Many trainees enter graduate school with an aim to address the difficult problems that society faces; for our trainees, that often means climate change and other environmental problems (Alkahrer & Goldman, 2018; Brundiers et al., 2021). Societal problems like climate change are, by definition, interdisciplinary in scope. For academic research to remain relevant in a complex world, graduate training must reflect the interdisciplinary and constantly changing nature of the world's problems (Leshner & Scherer, 2018).

Other programs can implement practices to promote a culture of improvement, thereby addressing some of the shortcomings in traditional graduate education. R2R demonstrated that soliciting feedback from current students, faculty, and staff is essential for making effective changes to curriculum and training activities. Whether it be the redesign of offered courses, building new partnerships on and off campus, or creating opportunities for students and faculty of

different disciplines to interact, graduate programs can benefit from staying student-focused and constantly striving to improve.

FIGURES AND TABLES

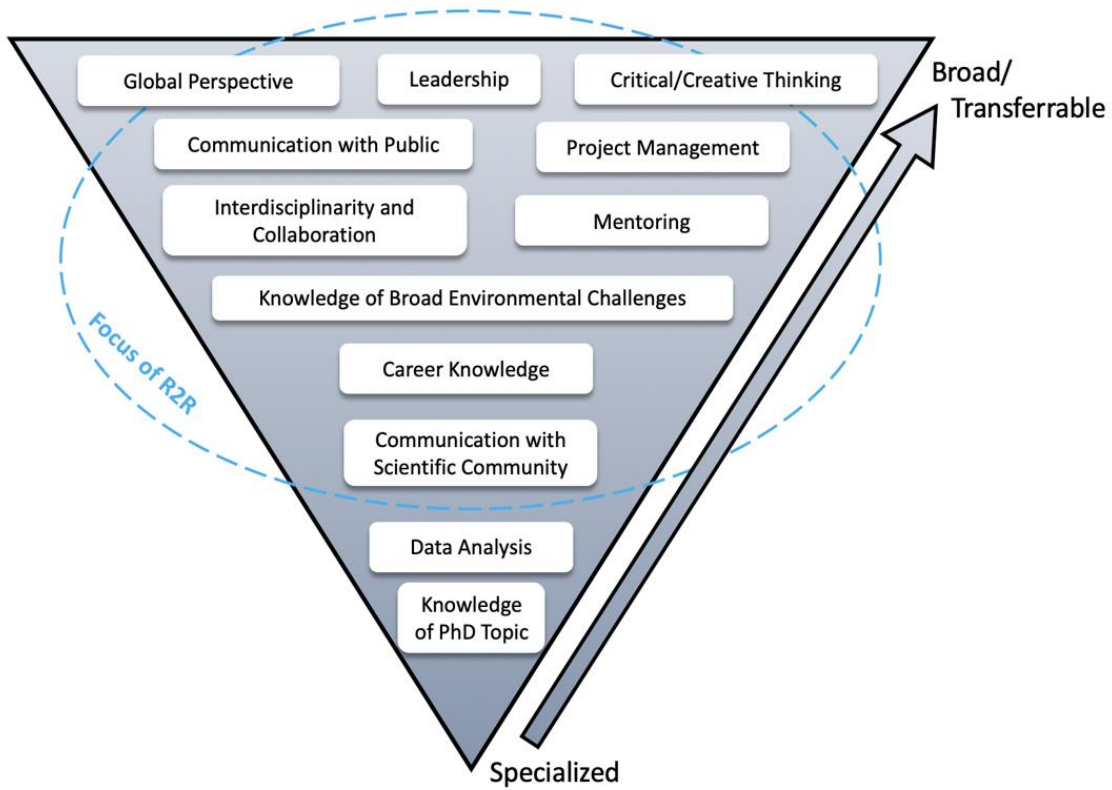


Figure 1. Conceptual framework for Ridge 2 Reef goals, adapted from the T-framework (Barile et al., 2012).

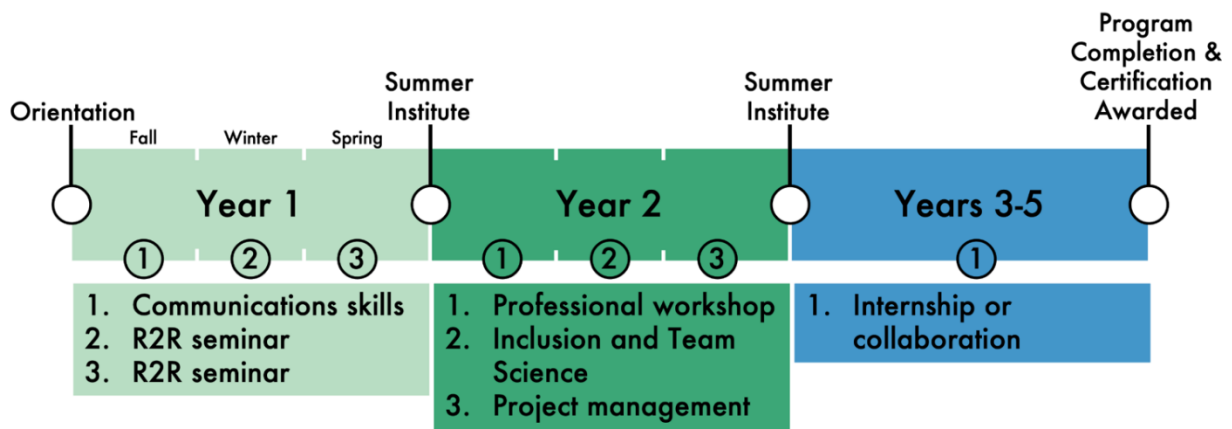


Figure 2. Final Ridge 2 Reef program timeline.

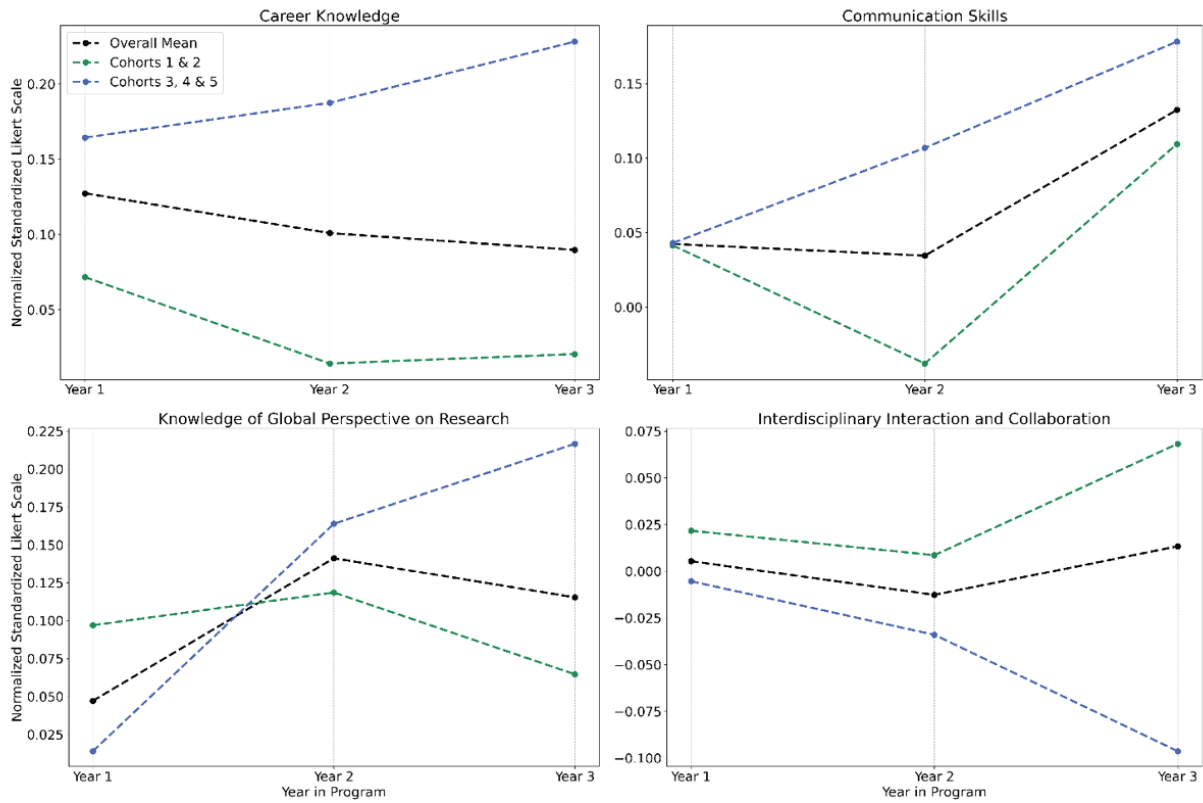


Figure 3. Plots of normalized and standardized annual survey data on four R2R themes. Data were standardized by subtraction of pre-academic year data. Combined cohort data (Cohorts 1 & 2 and Cohorts 3, 4, & 5) and overall mean plotted for years 1-3 within the R2R program.

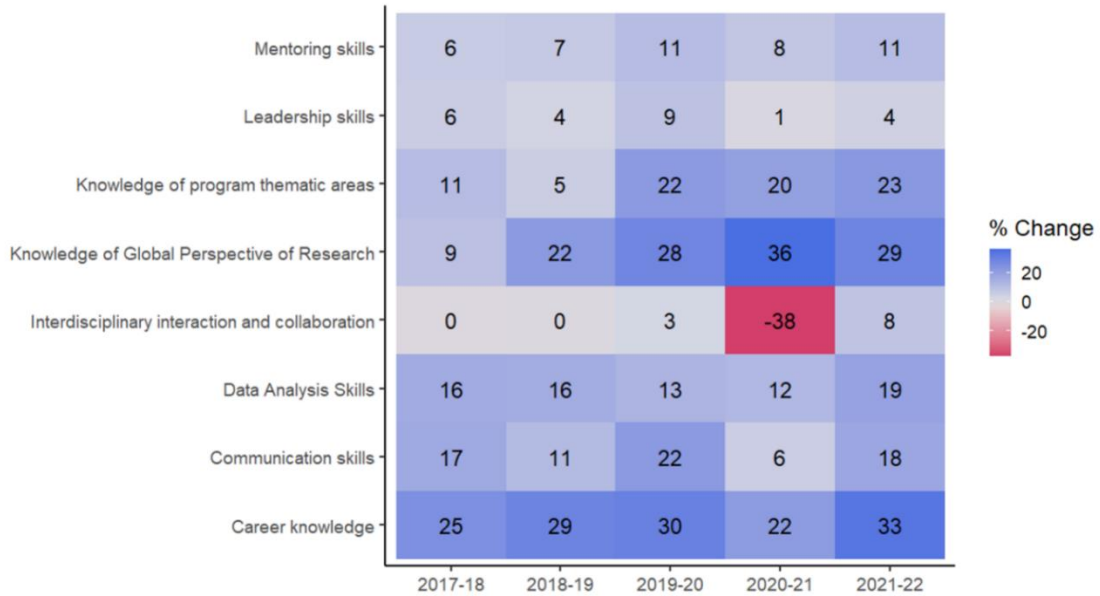


Figure 4. Average percent change in skills and/or knowledge in R2R goals year-to-year. Self-reported by trainees in standardized surveys collected at the beginning and end of each academic year.

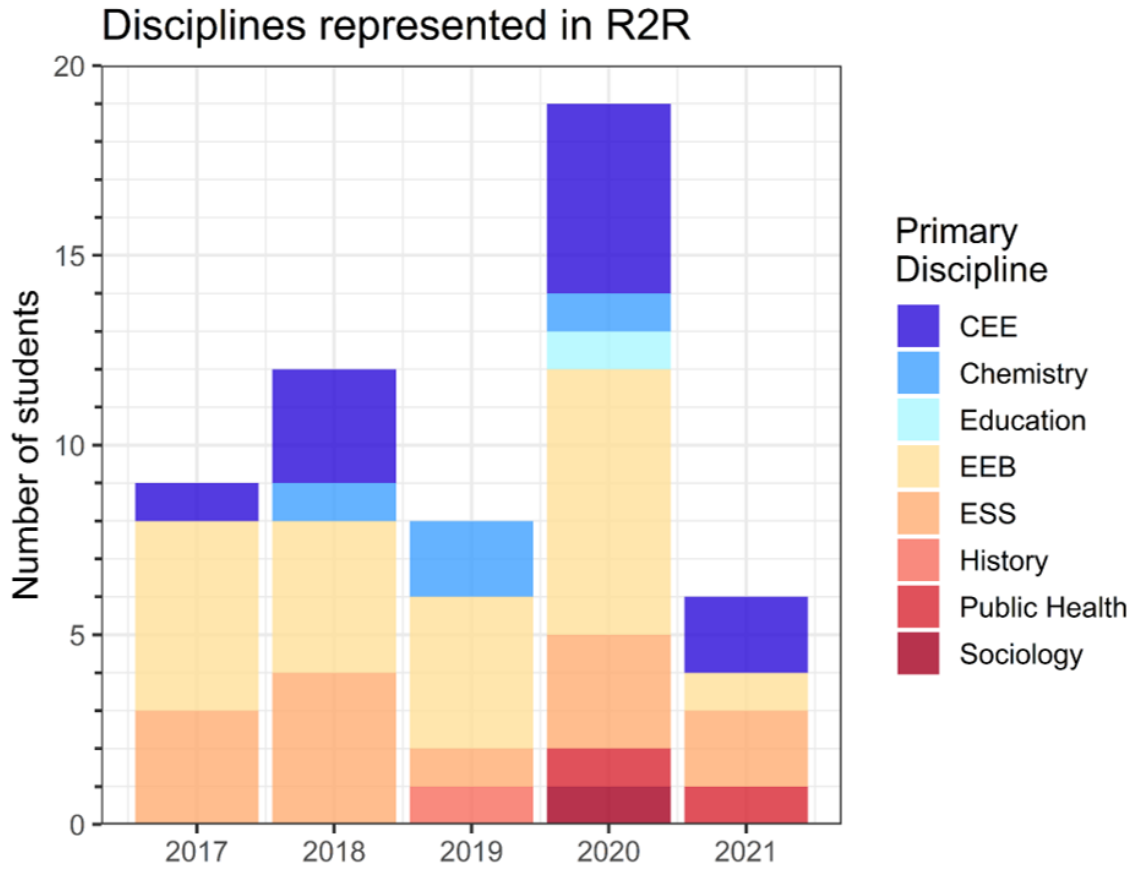


Figure 5. Ridge 2 Reef trainee cohort sizes and disciplines represented. CEE is civil and environmental engineering, EEB is ecology and evolutionary biology, and ESS is Earth system science.

Table 1. UC Irvine programs that have been supported by advances from the R2R NRT training model.

Program	Funding	Description	Connection to R2R
Center for Ecosystem Climate Solutions (2019-2023)	California Strategic Growth Council (\$4.6M)	Supports state environmental management needs through data-driven science and technology with partners from government, nonprofit, and private sectors	Center Director Michael L. Goulden is R2R co-PI
Graduate Recruitment Cluster in Environmental Racism and Health Equity (2022)	UCI Graduate Division and matching funds from schools (\$325K)	Recruitment and training of 20 graduate students in community-based research practices related to addressing environmental racism and health disparities	Adopts R2R communication skills course and recruitment plan; R2R PI Steven D. Allison is a co-PI on the cluster
CLIMATE Justice Initiative (2023)	NSF Cultural Transformations in the Geoscience Community (\$7.5M)	Supports graduate student and post-baccalaureate training to center diversity, equity, and environmental justice in climate change research	Adopts R2R curriculum model; R2R PI Steven D. Allison is a co-PI on the project; PI Kathleen R. Johnson is an R2R faculty mentor
Masters in Conservation and Restoration Science (2017)	Fee-based professional master's program	Trains masters' students in the practice of conservation and restoration science across terrestrial and marine habitats	Shared program coordinator with R2R; shared courses for some trainees; research training seminar adopted from R2R
Newkirk Center for Science and Society (2001)	Endowment from the Newkirk family (~\$250K/year)	Supports research, training, and events that explore the interface between the scientific community and societal needs	R2R PI Steven D. Allison is Director of the Newkirk Center since 2021; R2R recruitment practices and curriculum are helping to shape the Newkirk Graduate Fellows program

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APPENDIX A: Supplemental Information – Chapter 1

R Code

Figure 1, 3 and associated analyses:

Packages: lme4

X = variable (e.g. nitrate-nitrite concentrations, grazer biomass, grazer biodiversity)

Pooltype = water source (e.g. ocean, drain, runoff pool, control pool)

Date = sample time, Pool = individual tidepool or drain source

```
lmer(X ~ Pooltype*Date + (1 | Pool), data, REML = FALSE)
```

```
summary(model)
```

```
anova(model)
```

Figure 2 and associated analyses:

X= variable (e.g. % internal nitrogen values, NN = nitrate-nitrite concentrations)

Y = response value (e.g. % internal nitrogen values, grazer biomass)

```
lm(X ~ Y, data)
```

```
summary(model)
```

Figure 4 and associated analyses:

Packages: rstatix

Pooltype = Runoff and control pools

```
model1 %>% anova_test(FinalUlvaCover ~ InitialUlvaCover + Pooltype)
```

```
get_anova_table(model1)
```

```
model2 <- model1 %>% emmeans_test(FinalUlvaCover ~ Pooltype, covariate =  
InitialUlvaCover, p.adjust.method = "bonferroni")
```

```
model2
```

```
get_emmeans(model2)
```

APPENDIX B: Supplemental Information – Chapter 2

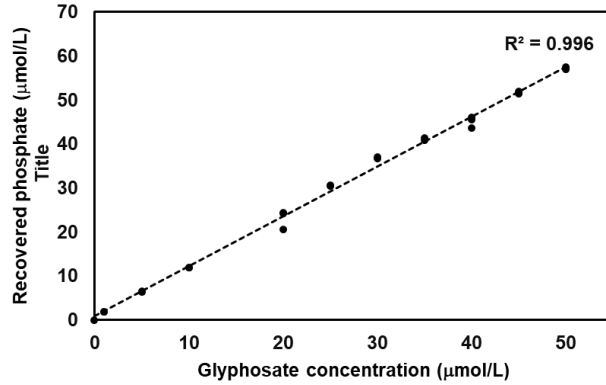


Figure S1. Effectiveness of colorimetric analyses of glyphosate based on oxidation of glyphosate to orthophosphate. Recovered phosphate concentrations were closely related to initial glyphosate concentrations in the samples ($p < 0.001$, $F_{1,18} = 9,461.5$; $R^2 = 0.996$).

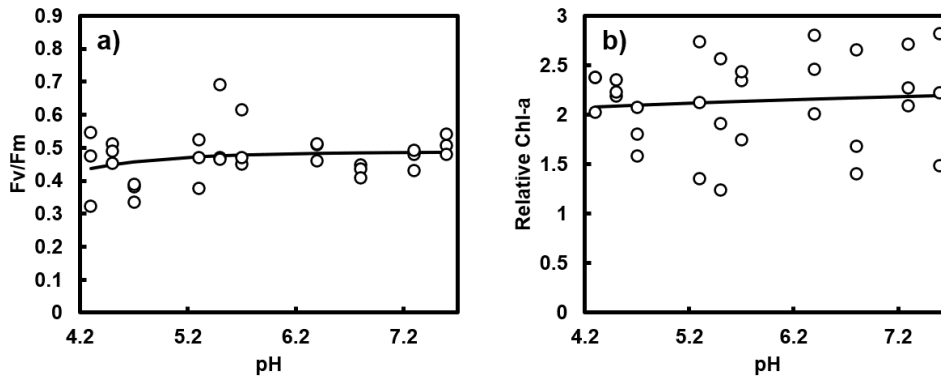


Figure S2. Non-linear least squares models of the relationship between Fv/Fm and relative chlorophyll-a and glyphosate concentration. a) The relative Fv/Fm values for the pH only trial at 96 hours. b) The relative chlorophyll-a values for the pH only trial at 96 hours. There was no significant value at which the Fv/Fm or relative chlorophyll-a were reduced by 50%.

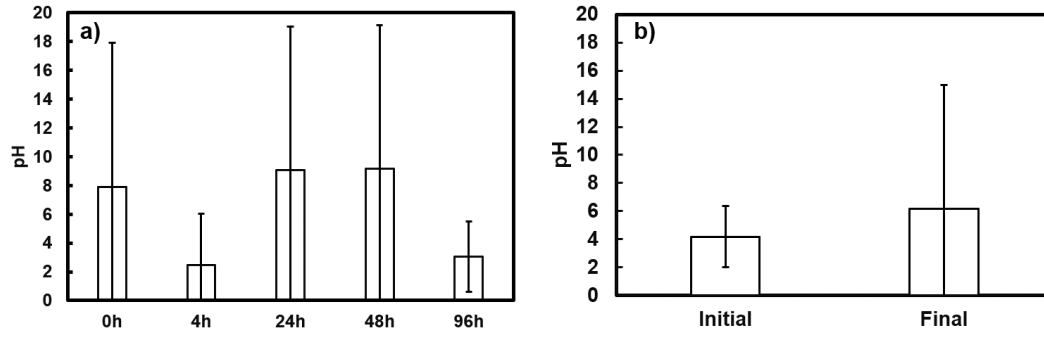


Figure S3. Average and standard error of the pH at which there was a 50% reduction in Fv/Fm and relative chlorophyll-a for the pH only trial. a) There was no significant pH at which 50% of Fv/Fm was reduced and no significant differences between time points. b) There was no significant pH at which 50% of relative chlorophyll-a was reduced and no significant differences between time points.

Table S1. pH values associated with various concentrations of glyphosate in seawater after 96 hours, with and without the presence of *Ulva* sp. Values are means \pm SEs.

Glyphosate ($\mu\text{mol/L}$)	pH with <i>Ulva</i>	pH without <i>Ulva</i>	DF	<i>p</i> -value
0	9.82 \pm 0.08	8.96 \pm 0.05	10	<0.0001
4	9.84 \pm 0.29	8.8 \pm 0.01	4	0.0233
10	10.34 \pm 0.04	8.62 \pm 0.04	4	<0.0001
45	10.19 \pm 0.15	8.56 \pm 0.1	4	0.007
100	9.78 \pm 0.2	8.27 \pm 0.12	4	0.0027
200	10.13 \pm 0.15	8.28 \pm 0.12	4	0.0006
300	10.01 \pm 0.23	8.31 \pm 0.04	4	0.0018
600	9.37 \pm 0.23	7.66 \pm 0.04	4	0.0017
900	9.1 \pm 0.08	7.31 \pm 0.06	4	<0.0001
1,200	8.53 \pm 0.21	6.87 \pm 0.07	4	0.003
1,500	8.62 \pm 0.18	6.42 \pm 0.07	4	0.0004
3,000	7.75 \pm 0.24	5.7 \pm 0.02	4	0.001
3,500	7.13 \pm 0.08	5.52 \pm 0.04	4	<0.0001
4,000	6.75 \pm 0.07	5.36 \pm 0.03	4	<0.0001
10,000	4.8 \pm 0.06	4.7 \pm 0.03	4	0.1606
20,000	4.44 \pm 0.02	4.4 \pm 0.03	4	0.5203
30,815	4.29 \pm 0.01	4.28 \pm 0.01	4	0.4354
51,280	4.17 \pm 0.003	4.17 \pm 0.02	4	0.7676

R Code

Figure 1 and associated analyses:

```
Packages: dplyr, TukeyHSD
aov(Glyphosate Concentrations ~ Runoff Sources, data)
summary(model)
TukeyHSD(model)
```

Figure 2 and associated analyses:

```
pH = pH value, Treatment = with or without Ulva, Glyphosate = glyphosate concentration
glm(pH~Treatment + Glyphosate + Treatment*Glyphosate, family = poisson (link = "log"), data)
summary(model)
```

Figure 3, 5, and associated analyses:

Packages: nlstools

x = glyphosate concentrations, y = relative chlorophyll-a values

a = maximum chlorophyll-a observed, b = half maximum glyphosate concentration, c = constant

```
nlsLM(y ~ a / (1 + (x / b)^c), start = list(a = 1.442333333, b = 25640, c = 1))
```

```
summary(model)
```

Figure 4 and associated analyses:

Packages: lme4

FvFm = pseudo-LC50 Fv/Fm values, Time = sample time (hours), Treatment = adjusted or unadjusted pH, Unit = replicate number

```
lmer(FvFm~Time*Treatment + Time + Treatment + (1 |Unit), data, REML = FALSE)
```

```
summary(model)
```

```
anova(model)
```

APPENDIX C: Supplemental Information – Chapter 3

Table S1. Ridge 2 Reef Survey response by year.

Year	2017-2018	2018-2019	2019-2020	2020-2021	2021-2022
	# Trainees	# Trainees	# Trainees	# Trainees	# Trainees
Cohort 1	10	10	6	5	1
Cohort 2		12	11	12	10
Cohort 3			7	6	5
Cohort 4				19	19
Cohort 5					6
Total	10	22	24	42	41
Response Rate	60%	50%	38%	57%	61%

Table S2. Number of paired trainee survey respondents by academic year.

Academic Year	Pre-Survey	Post Survey	Paired
2017-2018	7	7	7
2018-2019	9	6	6
2019-2020	6	4	2
2020-2021	13	13	12
2021-2022	7	6	6