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Establishing the catchment to sea connection: spatial and temporal patterns of terrestrial pollution sources and impacts to herbivorous fish

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Highlights

- We developed a research agenda that facilitated and promoted equitable, diverse, and inclusive partnerships through close collaboration and focused training with project partners.

- Knowledge products from this study were co-developed and co-produced with local and federal partners throughout the project.
- This project is the first study in American Samoa to integrate sediment and nutrient monitoring in streams with detailed investigation of the effects of these water quality parameters on both benthic and herbivorous reef fish assemblages
- Our findings highlight the factors underpinning the genesis, transport, and fates of terrestrial runoff and provide mechanistic links to ecological processes that determine the organization and composition of the benthos, and the patterns and magnitude of herbivorous fish recruitment on nearshore coral reefs. These measures of reef health have the potential to directly impact ecosystem services such as the continued provisioning of micronutrients from reef fishes essential to the nutrition of the people of American Samoa
- We build on existing ridge-to-reef efforts in American Samoa and leverage strong collaborations to critically examine and quantify reef health metrics that can potentially affect access to nearshore fisheries resources.
- The tight linkages between land use, human population density, and the gradient of nutrient and sediment signatures in streams and nearshore reef flats directly influence spatial patterns of the benthos, the abundance of juvenile and adult herbivorous fishes, and growth and feeding rates of parrotfish and surgeonfish.
- The results of this project have direct applications to local management strategies to effectively mitigate and reduce Land-Based Sources of Pollution, and directly contributes to Goal 3 of American Samoa's Strategic Coral Reef Management Priorities to "*improve understanding of the links between land-based sources of pollution and coral reef health through focused scientific research and monitoring*".
- Our findings have potential benefits to the US Coral Reef Task Force's Strategic Goals to manage land-based pollution and improve coastal watershed quality and enhancement of coral reef ecosystem condition.

- Our integrated and collaborative ridge-to-reef research adds value to the American Samoa Community College's Marine Science Program, reef condition reporting of the National Marine Sanctuaries of American Samoa, and to the American Samoa Environmental Protection Agency's watershed monitoring programs and water quality standards.
- We provide a model scientific and management framework for other Pacific Island Countries and Territories (PICTs) through high-quality scientific outputs, training, and network-building collaborative focus. We further contribute to the baseline of ridge-to-reef knowledge for volcanic high island communities in the South Pacific.

Executive Summary

1. The tight coupling between land and sea plays an important role in the condition of shallow, nearshore reef habitats. As ridge-to-reef management moves to include the protection and management of coral reef fisheries resources, improved understanding of biological processes that help to explain observed patterns of coral reef assemblages is necessary. This is particularly relevant in easily accessible nearshore fringing reef habitats that are critically important for early growth and development of reef fishes but are directly impacted by terrestrial run-off and targeted fishing.
2. Despite the widespread adoption and application of ridge-to-reef management approaches, building a mechanistic understanding of land-based pollution impacts on fisheries requires detailed knowledge of the roles of topography, seasonal patterns, and variable land use changes in the transport, delivery and fate of nutrients and sediments to adjacent reef areas. Furthermore, poor water quality may influence populations and assemblages of fishes directly by affecting their behavior and physiology, or indirectly through changes in benthic habitats and species interactions. Thus, integrated management of land-sea systems and fisheries resources will require understanding of the processes influencing the delivery of terrestrial run-off to nearshore reef systems and the potential flow-on effects on fish-habitat associations, fish community composition, and ecosystem functioning.
3. We address the land-sea knowledge gap in American Samoa by first examining the spatio-temporal variation of nutrients and suspended particulate matter (SPM) in streams and reef flats over a 12-month period across six sites that spanned a gradient of anthropogenic impact in the adjacent watersheds (e.g., pristine, intermediate, disturbed). We also related this spatial variation in nutrients and SPM to the benthic community structure on adjacent reef flat and reef slope habitats. We then quantified patterns of recruit abundance of the major piscine herbivores (parrotfishes, and surgeonfishes), and the mechanisms driving these densities in the same six watersheds. Building on the static patterns of spatial variation of recruit abundance and benthic cover, we investigated how

growth rates of the two of the most abundant parrotfish and surgeonfish in nearshore reefs on Tutuila, (*Chlorurus spilurus* and *Ctenochaetus striatus*, respectively), were influenced by terrestrial run-off. Lastly, we assessed the impacts of terrestrial run-off on adult population structure of parrotfishes and surgeonfishes adjacent to the six watersheds. Water quality parameters exhibited spatial structure across watershed types with higher concentrations and accumulation rates in the intermediate and disturbed watersheds and a general trend of higher nutrient concentrations during the drier winter (August 2018) season, and higher sediment accumulation rates in the wet season (Nov-18, Feb-19). There were also distinct differences among watershed types in the composition of shallow water benthic communities. Generally, sites adjacent to pristine watersheds were characterized by high cover of hard coral and crustose coralline algae (CCA). In contrast, sites adjacent to intermediate and disturbed watersheds had higher cover of macroalgae, turf algae, and rubble. Parrotfishes were more abundant than surgeonfishes across reef locations and watershed types, especially on shallow reef flats. Interestingly, the highest abundances of juvenile parrotfishes and surgeonfishes were recorded on reefs adjacent to disturbed watersheds with low live coral cover (a measure of reduced reef health). Feeding rates also showed distinct spatial delineation with higher feeding rates in intermediate watersheds compared to disturbed watersheds, which may reflect differences in the quality and/or quantity of dietary resources among sites, or density-dependence. Growth rates varied among *C. spilurus* and *C. striatus* with a general trend of faster growth in individuals from intermediate watersheds compared to those from disturbed watersheds. The body condition of *C. spilurus* did not differ between disturbed and intermediate watersheds. However, *C. striatus* body condition may be more sensitive to locality-specific environmental conditions with lower condition in individuals from disturbed watersheds compared to intermediate sites. The densities of adult parrotfishes and surgeonfishes varied among the six sites with clear partitioning among watershed types. Both reef flat and reef slope habitats adjacent to the disturbed watershed, Nu'uuli, had higher parrotfish and surgeonfish densities but generally smaller body sizes. In contrast, sites adjacent to intermediate and pristine watersheds were characterised by lower abundances, but larger body sizes of parrotfishes and surgeonfishes.

4. *Synthesis and applications.* Warming ocean temperatures and declining water quality are significantly affecting coral reefs, leading to changes in reef configuration and composition, with potential impacts to the capacity of reefs to maintain provisioning of food sources. Our project expands the metrics of reef health by including critical ecological processes, benthic cover and herbivore fish recruitment, processes which support the continued replenishment and maintenance of food sources in American Samoa. Our results showed that herbivorous reef fishes were not adversely affected by intermediate levels of terrestrial run-off, and in fact, thrived in these environments; which may provide some insurance to moderate declines in water quality. Further, our project leverages results from, and the expertise of, existing integrated management initiatives in American Samoa, by investigating pathways of terrestrial run-off influence on the ecological processes of important reef habitats and fisheries resources. We add value to locally-led integrated management programs by increasing understanding of complex land-sea processes and the variability of responses of social-ecological systems within the land-sea nexus. Finally, we reaffirm our commitment to sustaining old, and, forging new partnerships, and building local technical capacity through focused training, cross-sectoral collaboration, and the co-development and co-production of knowledge products.

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Comeros-Raynal MT[1], Brodie J[1], Bainbridge Z[2], Choat JH[3], Curtis M[4], Lewis S[2], Stevens T[2], Shuler CK[5], Sudek M[6], Hoey AS[1] (2021) Catchment to sea connection: Impacts of terrestrial run-off on benthic ecosystems in American Samoa. Marine Pollution Bulletin 169:112530

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Electronic Supporting Material

LINK: https://github.com/mcomeros21/catchment-to-sea-noaacrcp

Repository of data and data analysis scripts using R markdown

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Building a research agenda that facilitates and promotes equitable, diverse, and inclusive partnerships through close collaboration and focused training with project partners

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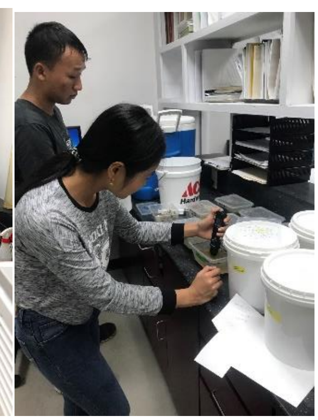
Integrated management of land-sea systems and fisheries resources will require understanding of the processes influencing the delivery of terrestrial run-off to nearshore reef systems and predictions of potential flow-on effects on fish-habitat associations, fish community composition, and ecosystem functioning









Introduction

American Samoa faces accelerated ecosystem transformations from the interacting impacts of watershed development, increasing human population density, nutrients from streams and groundwater, and fishing. Together with regional and global stressors, these cumulative impacts have the potential to diminish reef health and resilience to predicted increases in the frequency and intensity of future disturbances. In American Samoa, 100% of the population live within 5 km of the coastline, and 61% of the population live within a kilometer of the coastline (1). High human populations concentrated in the coastal plains of high islands, and the varying degree of land use and disturbance to vegetation and soil erosion means that complex reef systems such as those in American Samoa, are highly vulnerable to the negative effects of human uses in watersheds and in nearshore coral reefs (2-5). It also provides opportunity for watershed restoration actions in these areas to result in a positive recovery of valued nearshore systems.

Integrated watershed and coral reef management is an important tool in prioritizing and protecting resources that millions of people depend upon for livelihood, cultural, and aesthetic uses. For example, integrated island management approaches such as ridge-to-reef (R2R) management frameworks, which operate at multiple scales of ecological, social, and physical processes, are increasingly being implemented in Pacific Island countries and Territories (PICTs) to improve socio-ecological adaptive capacity, revitalize customary management efforts, and in setting relevant thresholds to achieve management goals (6, 7). R2R management aims to simultaneously address and manage upstream activities and downstream ecological conditions; with approaches ranging from conservation planning and prioritization (8-11), to monitoring coral reef ecosystem condition (2, 5, 12-14). Additionally, R2R management has also been applied to set relevant water quality management thresholds (15-17), and protect coral reef fisheries resources (18-22).

The impacts of poor water quality on coral reef condition and function have largely been informed by the responses of benthic communities to enriched nutrients, turbidity, and sedimentation (23-34). Understanding of the effects of declining water quality on reef fishes, however, has lagged behind those of benthic assemblages. In particular, understanding of the direct influence of declining water quality on fish behavior and physiology, or the indirect impacts on species-habitat interactions. Recent studies of the direct effects of suspended sediments have shown that for small-bodied site attached damselfishes (Pomacentridae) elevated levels of suspended sediments can damage gill tissue and/or lead to a remodeling of gill structures (35, 36), potentially compromising their capacity to uptake oxygen from the environment. Further, elevated suspended sediment levels have been shown to impair sensory (i.e., visual and olfactory) functions, inhibiting their ability to locate and hence ingest planktivorous food items (34, 37), and detect and settle to suitable benthic habitats (38). In turn, these impaired sensory functions are likely to lead to reduced growth, body condition, and survival. While these studies are informative, they focus on small and site-attached reef fishes (i.e., damselfishes), and relatively little is known about the effects of declining water quality on larger mobile fishes – important components of coral reef fisheries. In particular, the effects of declining water quality and changing quality and quantity of coral reef habitat, on habitat use, feeding behavior, and early development of larger mobile fishes is largely unknown. This knowledge gap precludes effective ridge-to-reef management of fisheries(39).

Linking fish and water quality

The nature of interactions between herbivorous fish and the benthic biota is a matter of ongoing debate. How herbivorous fish are influenced by the impacts of declining water quality and changes in climate is of critical importance as parrotfish and surgeonfish have an established and increasing food value to coastal populations. These are the dominant piscine herbivores of the Pacific and contribute to several important ecological functions including the bioerosion of reef carbonates and re-working of reef sediments (40-45). In many high islands, parrotfishes and surgeonfishes are economically and culturally important food fishes. For instance, these herbivorous fishes have been an important food source on American Samoa for ca. 3,000 years, are a major component of contemporary catches, and are the primary targets in shore-based fishing activities (through spearfishing) on the main island of Tutuila, American Samoa (46, 47). The propensity of these herbivorous fish species to settle into live coral reef habitats, together with their life history traits render them vulnerable to the effects of poor water quality and exploitation.

Despite successful coral reef conservation and management efforts in American Samoa operating at a high-level of collaboration and coordinated actions, gaps remain in our understanding of the direct and indirect impacts of pollution on the demographic rates and ecology of parrotfishes and surgeonfishes. We build on previous work led by the Department of Marine and Wildlife Resources to improve understanding on the effects of bottom-up processes on the demographic rates (i.e., recruitment and growth) and feeding ecology of herbivorous reef fishes at relevant management units. The relationship between impaired water quality, benthic, coral, and reef fish assemblages is complex; and responses to different levels of disturbances are expected to be species-specific and dependent on the underlying environmental conditions. Moreover, the magnitude, frequency, and mode of delivery of pollution to nearshore coral reef habitats are highly influenced by a suite of environmental processes such as precipitation, topography, and proportion of land use and cover. Therefore, better understanding of the processes that drive the species and population level responses of coral reef community assemblages to perturbations is crucial in planning for, and managing, coral reef resources.

Here, we investigated the effects of increased nutrients and sediment accumulation rates on demographic processes to provide a mechanistic understanding of the effects of declining water quality on parrotfish and surgeonfish assemblages in American Samoa. Our overall objective was to document process-based responses of populations and assemblages of parrotfishes and surgeonfishes to varying levels of land use, and hence nutrient concentrations and sedimentation rates. We used a multi-faceted approach (Fig.1) to address our objective: 1) assessed the spatio-temporal variation of nutrients and suspended particulate matter (SPM) across six sites in American Samoa over a 12-month period and used exploratory path analysis to relate dissolved inorganic nutrients, land use, and natural and anthropogenic drivers to benthic assemblages on adjacent shallow reefs; 2) examined the patterns of recruit abundance of the major piscine herbivores, parrotfishes and surgeonfishes, in six nearshore fringing reefs across an environmental gradient in Tutuila, and investigated the mechanisms driving these densities;

3) quantified growth and feeding rates two of the most abundant parrotfish and surgeonfish species in nearshore reefs on Tutuila, *Chlorurus spilurus* and *Ctenochaetus striatus*, respectively; and, 4) assessed the impacts of terrestrial run-off on the adult population structure of parrotfishes and surgeonfishes. Importantly, we worked closely with project partners to develop a research agenda that facilitated inclusive and equitable partnerships through focused training and co-production of knowledge products.

Overall objective to document process-based responses of populations and assemblages of parrotfishes and surgeonfishes to varying levels of nutrient concentrations, sedimentation rates, and land uses.

Ridge-to-Reef framework

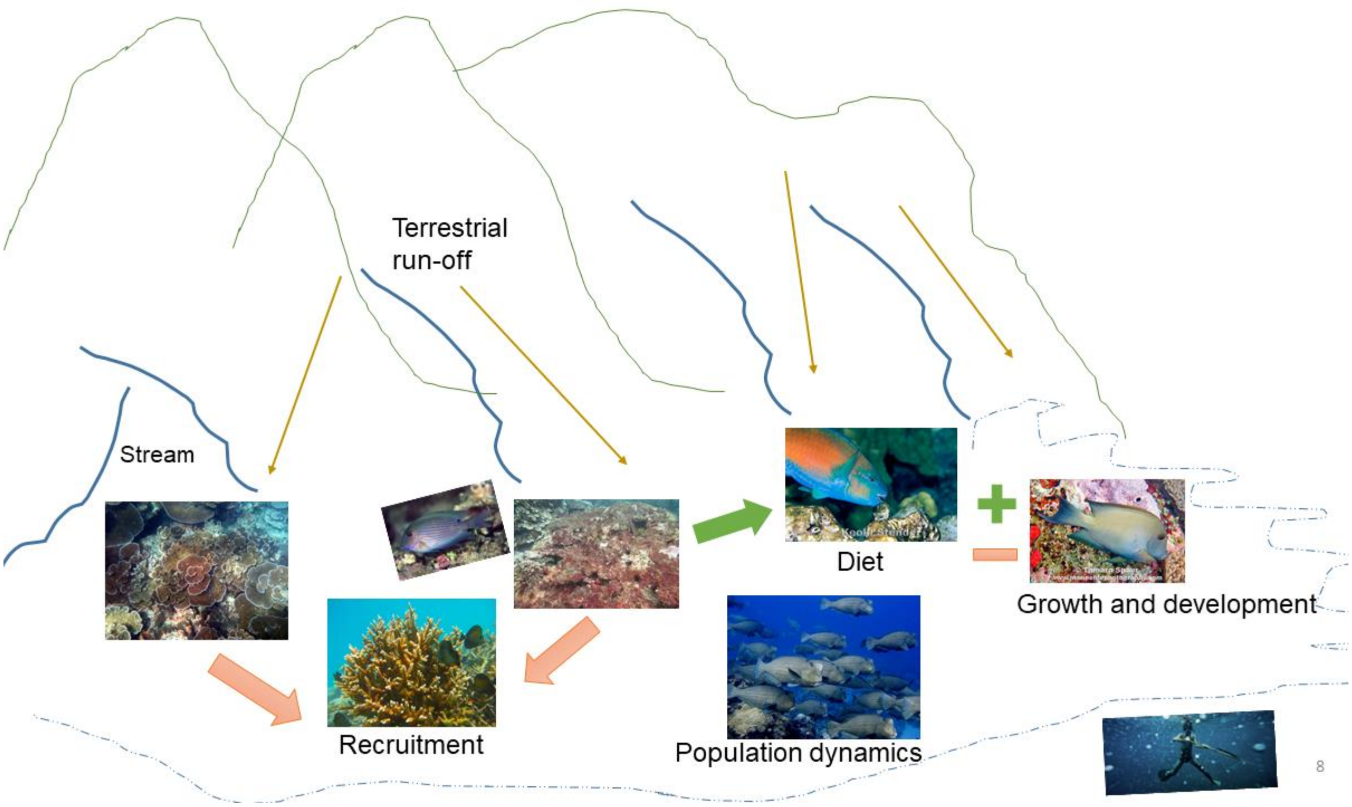


Figure 1. Diagram of ridge-to-reef project framework. The first task measured sediment and nutrient run-off from six watersheds spanning an environmental gradient on Tutuila, American Samoa, and examined the influence of terrestrial run-off on benthic assemblages in adjacent reefs. Task 2 investigated variation in habitat structure in the six sites and quantified the spatial pattern of herbivore recruit abundance and the mechanisms driving recruit densities. Task 3 examined the variation in growth and feeding rates of the most abundant parrotfish and surgeonfish species. Task 4 looked at overall population structure as a function of varying levels of terrestrial run-off, benthic habitats, recruit abundance and demographic rates. Red arrows represent negative impact, green arrows represent positive influence.

Methods:

1| Study Location

American Samoa has five main volcanic high islands (Tutuila, Aunu'u, Ofu, Olosega, and Ta'u) and two atolls (Rose and Swains), and is the southernmost U.S. Territory in the South Pacific at 14.27°S, 170.13°W. Our study was conducted on Tutuila, the largest and most populous of the main high islands in American Samoa with an area of 138 km^2 and a population of 55,000 (48). Tutuila is an extensively eroded volcanic island comprised of a central ridge of steep mountains which lead sharply to a narrow coastline (49, 50). Tutuila has a tropical climate with uniform temperatures between 26 and 28 °C and high humidity throughout the year. The mean annual rainfall on Tutuila is 3,810 mm/year with peak rainfall during the wet season from October to April and lower rainfall occurring in the dry season from May to September (51, 52). Precipitation on the islands generally increases with elevation and ranges from 2,388 mm/year at the shorelines to 6,350 mm/year at ~480 m above sea level elevation (53).

<u>Land Use and Land Cover</u>

Over 65% of Tutuila is natural forest while agriculture and development combined covers 24% of the island and is concentrated on the south-western coast (53). From 2004 to 2010, there has been a 4.8% increase in developed land and a 6.8% rise of impervious surfaces, while agriculture has increased by 17% (54).

***Method sections 1-4 adapted from: Comeros-Raynal et al. 2021. Catchment to sea connection: impacts of terrestrial run-off on benthic ecosystems in American Samoa. Marine Pollution Bulletin* 169:112530

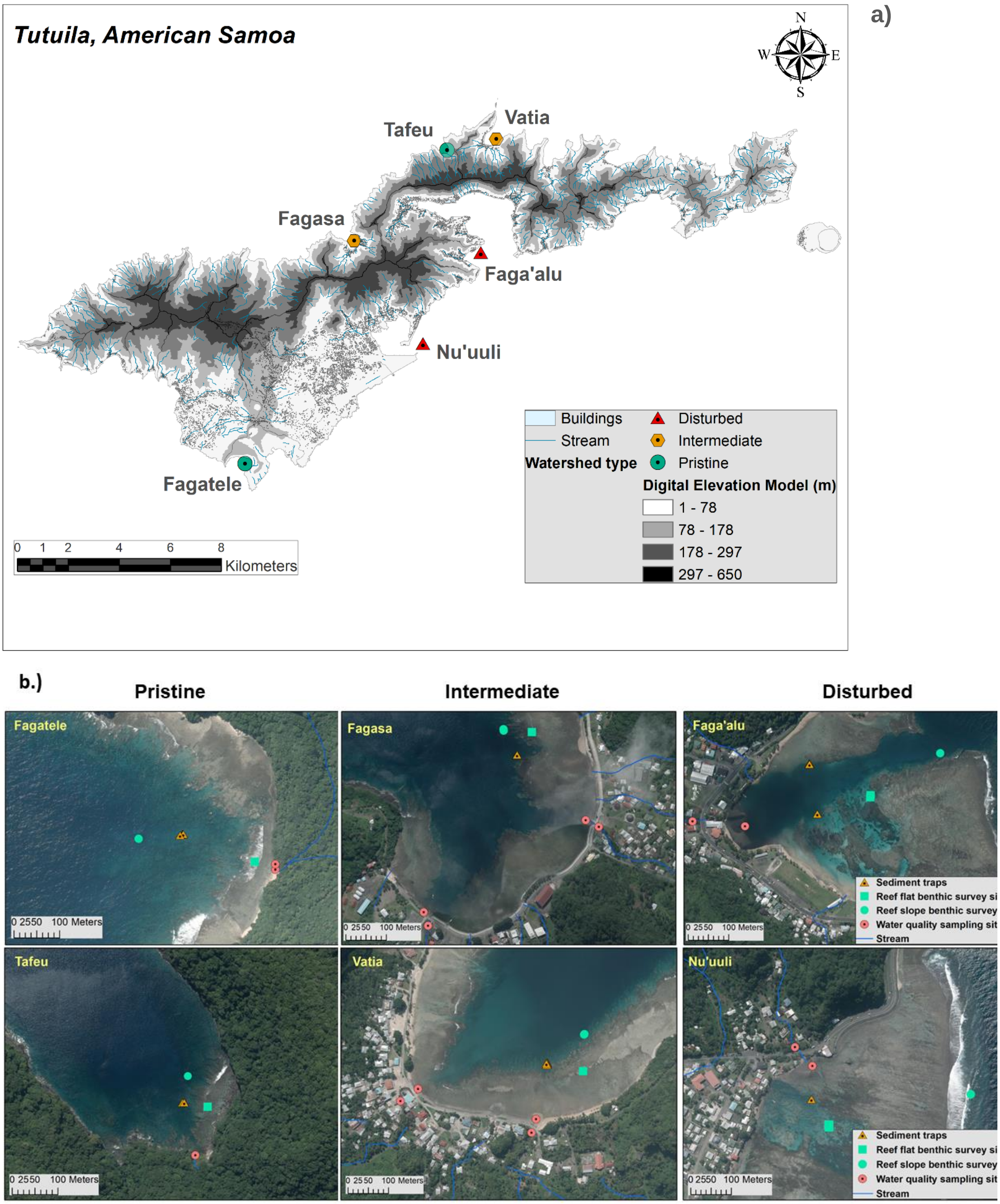

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Figure 2. Map of Tutuila, American Samoa showing the location of the six study sites. (a) Map of Tutuila showing the location of two study sites within each of three watershed types as classified by the American Samoa Environmental Protection Agency (AS-EPA). Red triangles: disturbed; orange hexagon: intermediate; green circle: pristine. (b) Satellite images of the six sites showing the approximate location of data collection sites for water quality, sediments, and benthic surveys.

2 | Study design

Water quality, benthic and fish assemblage data were collected from six sites that spanned a gradient of anthropogenic impact in the adjacent watersheds (Fig. 2). Specifically, we selected two sites within each of three watershed classifications (pristine, intermediate, and extensive) characterized by the American Samoa Environmental Protection Agency (55, 56). For clarity, the term ‘disturbed’ is used hereafter when referring to extensive watersheds, and is classified as having a human population density of >290 individuals km^{-2}. Disturbed watersheds (Nu’uuli and Faga’alu) had large (>2.33 km^2) watershed areas and were characterized by greater proportions of disturbed land (28% and 13% of the total watershed area, respectively). Similarly, intermediate watersheds (Fagasa and Vatia) had large watershed areas and low to moderate proportions of disturbed land (7% and 5%, respectively). Pristine watersheds (Fagatele and Tafeu) had the smallest (<1.55 km^2) watershed areas, and low proportions of disturbed land (5% and 0%, respectively).However, the Fagatele watershed includes the major landfill on Tutuila, and had the highest agroforest and cultivated land percent cover (Table 1).

3 | Environmental data

To evaluate the relationships of terrestrial run-off on nearshore shallow reef habitats, water quality data were collected at 3-monthly intervals for 12 months across the six watersheds to account for baseflow conditions and storm events over the two rainfall seasons in American Samoa: from June through September representing the drier winter season, and from October through May, representing the wetter summer season (51). The major streams in each watershed were sampled, with a single stream sampled in Faga’alu, Fagatele, Nu’uuli and Tafeu, and two streams in Vatia (Gaoa and Faatafe) and Fagasa (Leele and Agasii). Water samples were collected every 3 months from August 2018 – May 2019, and included two sampling periods in the wetter summer months: November 2018 and February 2019; and in the drier winter months: August 2018 and May 2019. Water samples were collected from the mouth of each stream at approximately 1 m above sea level during low tide to minimize mixing of coastal water during each sampling period. Upstream land use distance to stream mouth varied, with some point-source/cleared areas within 1 km of the sampling site. Stream mouth distance to the reef flat and reef slope sampling sites varied across sites (50-550 m) due to inherent differences in reef geomorphology and stream locations across sites. Sediment samples were collected using SediSampler® patented traps deployed on the reef slope at depths of 5-7 m.

Water samples were taken from the surface waters of the stream and reef flat by rinsing 500 ml and 60 ml polyethylene bottles three times with sample water prior to filling. Samples were placed on ice after collection and returned to the laboratory. Samples in 60 ml bottles were immediately frozen (unfiltered samples for total nitrogen and total phosphorus analysis), and the samples in 500 ml bottles were filtered using 0.7 µm GF/F Whatman filters, and the filtrate stored frozen until analysis. Filtration was conducted to remove most bacteria and other microorganisms that could affect the stability of filtered nutrient constituents.

The frozen water samples were then sent to the University of Hawaii's SOEST Laboratory for Analytical Biogeochemistry (S-LAB) for analysis of dissolved nutrients: sum of nitrate and nitrite (N+N; and ammonium (NH^{4+}), hereafter referred to as dissolved inorganic nitrogen (DIN)), ammonium (NH^{4+}), phosphate (PO_4^{3-}), silicate (SiO_4^{4-}); and Total Nitrogen (TN), and Total Phosphorus (TP) (57-60). Subsequent analysis of stable isotope of dissolved nitrate was conducted by the Biogeochemical Stable Isotope Facility at the University of Hawaii using the denitrifier method on a Thermo Finnigan MAT 252 Mass Spectrometer using a continuous flow GC-interface with a Triplus autosampler (61, 62).

Suspended particulate matter

To quantify suspended particulate matter (SPM) matter (i.e. sediments and associated particulate matter), we deployed three SediSampler® patented traps (Integral Aqua Pty Ltd) on the reef slope at each site in 5-7 m depth (Figure 1b). Each trap was attached to a steel bar driven into the substratum so that the mouth of the trap was positioned approximately 1 m above the substratum (Figure 2). The sediment traps were deployed at the time of the water sampling (i.e., November 2018, February 2019, May 2019, and September 2019) and collected after ~3 months. An additional three SediSampler® traps were placed at the mouth of the stream in Faga'alu to account for the previously reported differences in the spatial distribution of sediments at this site (4, 63, 64). After three months deployment on the reef, the 1 L sample bottles were carefully removed from the SediSampler® traps and the bottles capped underwater to avoid loss of sediments. The sample bottles were placed on ice within 10 minutes of collection and transferred to a refrigerator until sample processing.

Figure 3. Three SediSampler® patented traps (Integral Aqua Pty Ltd) on the reef slope at each site in 5-7 m depth. Each trap was attached to a steel bar driven into the substratum so that the mouth of the trap was positioned approximately 1 m above the substratum

Figure 4. PI Mia Comeros securing sediment sample bottles.

In the laboratory, each 1 L sediment trap sample bottle from each site was transferred to individual containers that had been pre-rinsed with distilled water, and the samples were well mixed for two minutes to ensure even distribution of particles prior to subsampling. Twenty-one aliquots (each 30 ml) were collected from each sampling date, three from each smaple bottle for Total Suspended Solids (TSS) analysis (65). The 21 aliquots for TSS analysis were then placed in a refrigerator and the remaining sample (~370 ml) prepared for salt removal. Similar to SPM, TSS includes both terrestrially-derived and marine-derived organic matter and mineral sediment.

Thus, we refer to TSS as SPM from here on. The remaining wet sediment samples from each sediment trap were transferred to individual 1 L plastic beakers, and left for 24-48 hours to allow sediment particles to settle. The supernatant was then decanted, taking care to ensure sediment particles were not lost. Distilled water (900 ml) was then added to each sample, agitated for two minutes to ensure mixing, allowed to settle for 24 hours and the supernatant carefully decanted as described above. This process of rinsing in distilled water, settling, and decanting was repeated until salinity was < 200 µs/cm (i.e., 3-4 rinses). The three sediment trap samples from each site were then combined in a pre-rinsed container and agitated for 2 minutes. Seven 30ml aliquots were collected from the combined sample for particle size analysis.

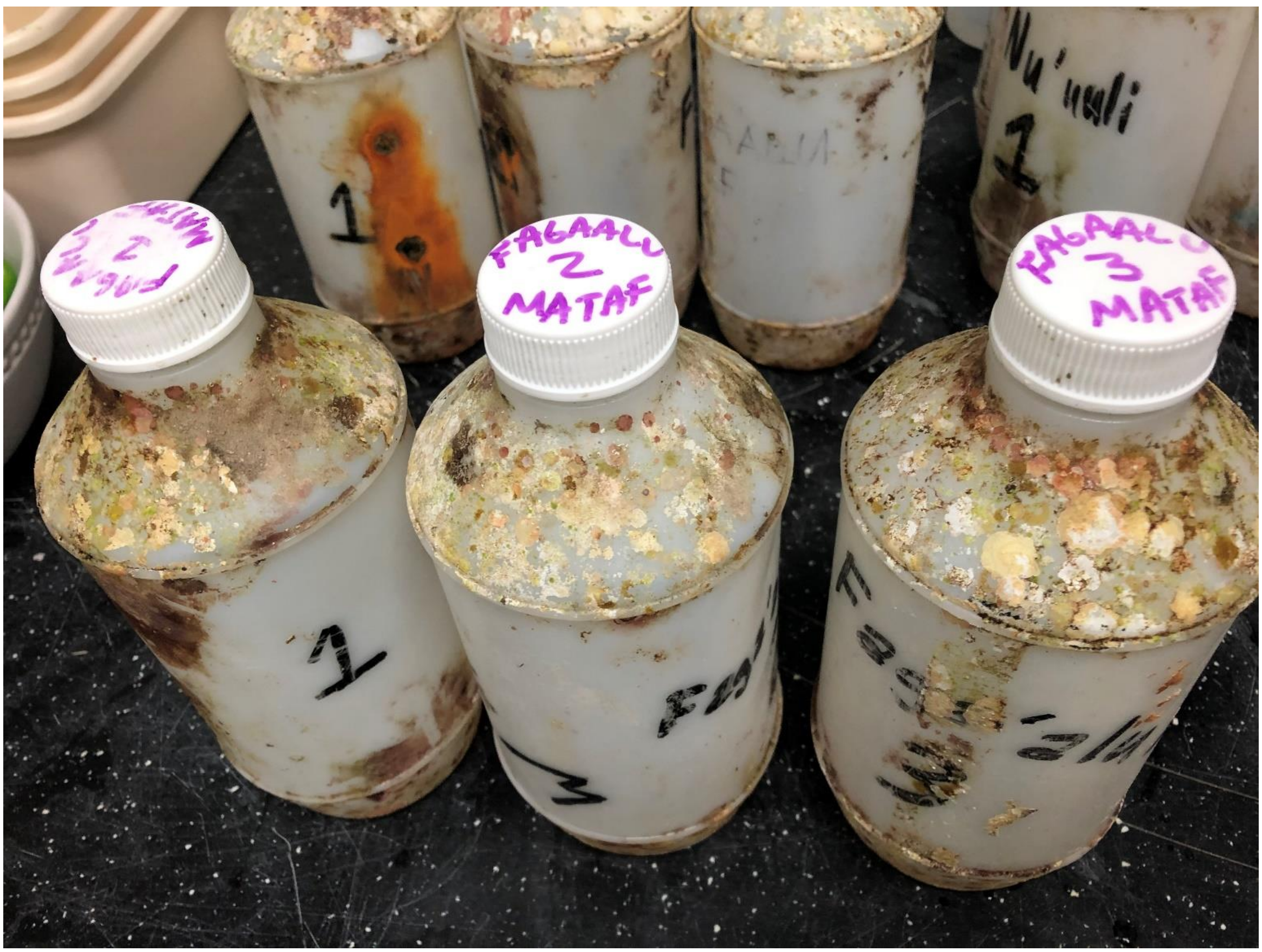


Figure 5. Sediment trap bottles collected after being deployed for 3-months at Faga’alu

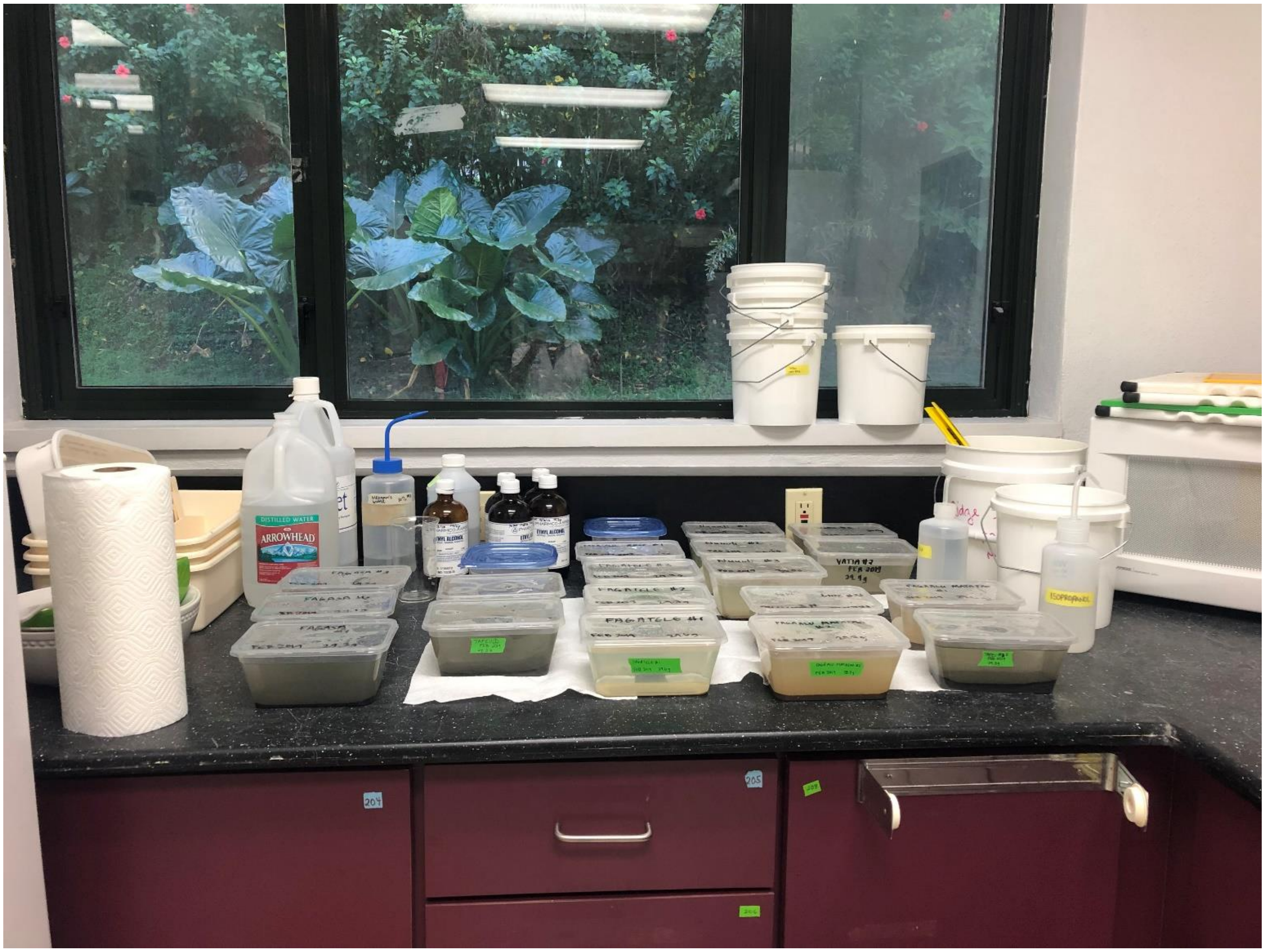


Figure 6. Sediment salt removal process. Wet sediment samples from each sediment trap were transferred to individual 1 L plastic containers, and left for 24-48 hours to allow sediment particles to settle.

The remaining bulk sample from the combined trap sediment samples were left to settle for 24 hours, decanted, and transferred to a 1 L beaker. Samples were then dried in an oven at 60°C for 24-48 hours with approximately 7 individual bags per deployment for sediment composition analysis. A total of 28 wet samples (30 ml tubes) and 7 dried samples from each deployment were then transported to Australia and analyzed at the TropWATER Laboratory, James Cook University (JCU) for Suspended Particulate Matter and Loss on Ignition (LOI), and the School of Earth and Environmental Science laboratory, JCU for particle size analysis.

The Loss on Ignition method was used to determine proportions of mineral, organic, and carbonate content by consecutively weighing the dried sediment samples after heating at suitable temperatures (66). Sample organic matter content was determined using Standard Method 2540E (65). Briefly, a crucible containing a pre-weighed quantity of each sediment trap sample (dried at 105°C to remove moisture) was ignited in a Carbolite (AAF1100) ashing furnace at 550°C for 4 to 5 hours, and reweighed. The weight lost during ignition represents the total volatile solids, an approximation of the organic matter content of each sample (weight % LOI, 550°C) (67). To determine marine sediment trap carbonate content, the sample crucibles were returned to the ashing furnace and heated at 950°C for at 2 hours, with the weight lost on ignition representing carbon dioxide released from calcium carbonate (68).

Figure 7. ASCC Marine Science students Warren Sevaaetasi and Perosi Vaofanua processing sediment samples in the Natural Resource laboratory with salt-removed sediment samples (foreground) allowed to settle for 24-48 hours prior to drying in the oven.

The TSS method was used to measure the sediment accumulation within each sediment trap (n=21 per deployment). Briefly, we pipetted 1ml of wet sample from each sediment trap with-salt aliquot, into a filtering manifold which was prefilled with 250 ml of RO water, and filtered the measured volume through a pre-weighed Whatman GF/C (1.2 µm pore size) filter paper (69). The filter paper was then dried for ~24 hours at 105°C and weighed again to measure the weight of total suspended solids (mg/L). This concentration was then converted to accumulation rate per day per cm^2 using the length of each deployment and the internal cross-sectional area (21.52 cm^2) of the sediment trap head (i.e. $mg/cm^2/day$).

Particle size distributions from salt removed wet samples were determined using the Malvern Mastersizer 3000, a laser diffraction particle-size analyzer following the parameterization method of Sperazza et al. (2004) and Bainbridge et al. (2012). This analysis was conducted on a sub-sample of collected trap material, and includes mineral, organic and carbonate components. Particle sizes were reported as percentage distributions D10, D50, and D90. For example, D50 refers to median size particles, where the diameter of a sphere at which 50% of the particles in the sample is smaller.

4 | Environmental and land use variables

We quantified land use, environmental (i.e., rainfall and wave energy), and anthropogenic (surface runoff, and Dissolved Inorganic Nitrogen (DIN) load) factors for each of the six sites. We used ArcMap 10.4 to calculate percent cover of land that was forest, agroforest, cultivated, developed and other land-use types using high resolution and LIDAR remote sensing habitat maps produced by the American Samoa Department of Marine and Wildlife Resources (53). Monthly modeled average discharge rates (rainfall and surface runoff) for each of the six watersheds were estimated using an open-source water budget model for Tutuila (70). Wave energy for each site was calculated using 10-year average wind speeds for Tutuila using the Wave Energy tool in ArcGIS (71). DIN loads exported from each watershed were taken from Shuler and Comeros-Raynal (2020).

Biological surveys: Benthic composition was quantified along four replicate 50 m point-intercept transects within each of two habitats, the reef flat (1 – 4 m depth) and reef slope (5 – 9 m depth), at each of the six sites in May 2019. The substratum directly under the transect tape was recorded at 50cm intervals along each transect (n=101 points per transect). Benthic categories were recorded as crustose coralline algae (CCA), turf algae (primarily filamentous algae <10mm in height), macroalgae (>10 mm in height), hard coral, rubble, sand, soft coral, sponge, zoanthids, etc. Hard coral and macroalgae were identified to genus. Transects were laid along the reef profile with a minimum of 10 m between adjacent transects.

Juvenile fishes (i.e., less than 10cm total length) from the three major herbivorous fish families (Acanthuridae, Labridae: Scarini, and Siganidae) were recorded on SCUBA from the reef flat (1 – 4 m depth) and reef slope (5 – 9 m depth) at each of the six sites in May 2019. Four replicate 50m x 2 m (100m^2) belt transects were conducted within each habitat at each site, with a minimum of 10m between adjacent transects. The surveyor (Co-I A. Hoey) identified all juvenile herbivorous fishes within a 2-m wide belt (i.e., 1 m on each side of the transect) to species, and recorded the total length of each individual to the nearest centimeter, while simultaneously deploying the transect tape.

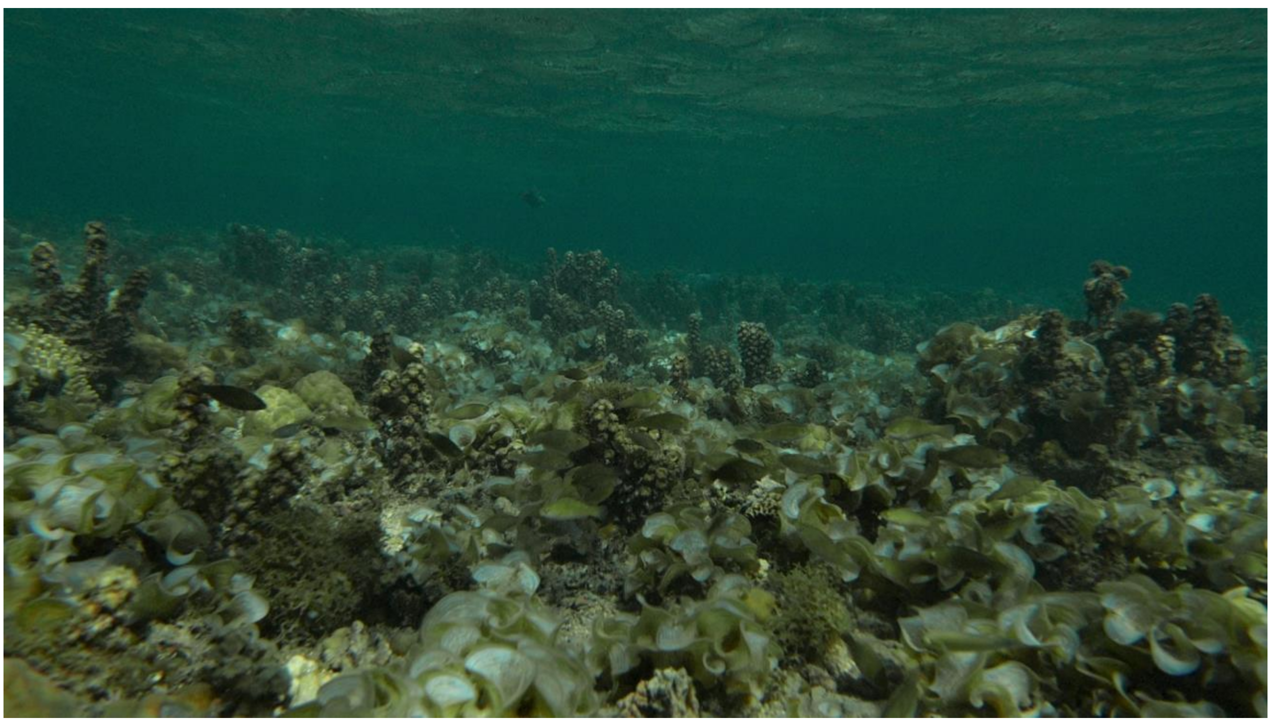


Figure 8. Parrotfish recruits in mixed algal beds (primarily *Padina* and *Turbinaria*) on the shallow reef flat in Nu'uuli

Biological surveys: fish (otolith analysis) Juvenile (<10 cm TL) and adult *C. spilurus* and *C. striatus* were collected by spearfishing from each site within pristine, intermediate and disturbed watershed types. We measured the total length (TL) of each fish and preserved specimens in > 75% ethanol after collection. Otoliths were then removed, cleaned, weighed and sent to Pacific Otolith Services for sectioning.

Individual feeding observations: We conducted short-term feeding observations on snorkel for *Chlorurus spilurus* and *Ctenochaetus striatus*, the two most abundant representatives of parrotfish and surgeonfish assemblages, respectively, on shallow nearshore reef habitats in Tutuila. We randomly selected an individual (< 20 cm TL) from one of the focal species, allowed to acclimate to the presence of the observer for 2-3 minutes, and then followed for a further ~3 minutes during which time we recorded the number of bites and feeding substrata. The body size (i.e., total length) of each fish was estimated to the nearest cm. A total of 250 individuals (30-35 individuals per species per site) were observed from reef flats adjacent to intermediate and disturbed watersheds. Feeding observations were not conducted on reef flats adjacent to pristine watersheds due to the low abundances of juvenile *C. spilurus* and *C. striatus* at these locations.

Figure 9. NOAA PIRO Fish Biologist, Michael Marsik, processing fish samples

Figure 10. ASCC Marine Science student, Ingrid Papali'i, processing juvenile fish samples (left), and Research Assistant, Jeremy Raynal (right), removing otoliths.

Biological surveys: adult parrotfish and surgeonfish assemblage (diver-operated video systems) We used a diver operated stereo-video system fitted with two GoPro Hero 5 cameras to record adult fish assemblages on SCUBA. At each site, 10 replicate timed-swims (5 m wide by 3 min long, averaging 315 m^2) were stratified at two depths: reef flat (1 – 4 m depth) and reef slope (5 – 9 m depth). From the video footage each parrotfish and surgeonfish observed within the 5-m wide belt was identified to species and life stage (initial phase and terminal phase), and their body length (Fork length, FL) estimated using the software EventMeasure (www.seagis.com.au). Fish lengths were converted to fish biomass (kg) using the formula $W=a*L^b$ where W=weight, L=length, and a and b are growth parameters obtained from regional fishery-dependent data when available (72) or from FishBase (www.fishbase.org). When growth parameters were not known for a given species, values from a closely related species were used.

Figure 11. Project partner, ASCC Marine Science Educator, Meagan Curtis (left) and PI Mia Comeros (right), setting up for diver-operated stereo-video fish survey.

Figure 12. Divers Hideyo Hattori (foreground), site liaison for the NOAA Coral Reef Conservation and Coastal Zone Management programs in American Samoa, and Meagan Curtis, ASCC Marine Science Coordinator (background) conducting underwater surveys.

Figure 13. Research assistant, Alejandro Usobiaga (left) and Professor Howard Choat (right) identifying fish species from DOV surveys

Data Analysis

Task 1: Spatial and temporal variation of nutrients and sediments

We used principal component analyses (PCA) to visualize variation in (i) benthic assemblages, (ii) nutrient concentrations, and (iii) suspended sediment characteristics among sampling locations. PCA were conducted using the “vegan” package (73) in R version 4.0.2. Analysis of benthic assemblages were based on the correlation matrix of the transect level data of each of the benthic categories, for water quality data based on quarterly collections, and for suspended sediments based on 3-month trap deployments. Separate PCA were performed for each of the two habitats (reef flat and reef slope) for benthic assemblages, and each of the two sampling locations (stream and reef flat) for water quality.

We used path analysis to explore the potential influences of both natural and anthropogenic variables relating to the catchment and wave exposure and dissolved nutrient concentrations on benthic cover in shallow coastal reef flats. Natural variables comprised of wave energy, watershed size and discharge rates, while anthropogenic variables included percent cover disturbed land area (% cover of developed, cultivated and agroforest land uses), and a proxy of human population density from DIN loads of onsite disposal systems and piggeries ($kg.day^{-1}$). Of the water quality parameters quantified, DIN and phosphate concentrations were used as explanatory variables as they are highly bioavailable and are known indicators of anthropogenic nutrient loading on shallow reef systems. Benthic cover was partitioned into two groups: turf and macroalgae, and hard coral and CCA as we expected that responses to nutrient enrichment will vary between these different benthic components. Importantly, hard corals together with CCA play key roles in CaCO3 accretion into the reef matrix, and form desirable reef health indicators from a management perspective (74, 75).

Path analysis is a multivariate technique which uses a series of structured linear regression equations to test the specified relationships between measured variables (76). The relationships are displayed in a path diagram where variables are linked by straight arrows indicating the direction of the relationship between the variables (77). In a path diagram, variables are represented as rectangles and are either exogenous or endogenous (78). The direct effects of an independent variable on a dependent variable are expressed as path coefficients. Coefficients are positive where an increase in the independent variable causes an increase in the dependent variable when other causal variables are held constant, or negative where an increase in the causal variables decreases the dependent variable. Model fit was assessed using four tests: X^2, Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA) and Standardized Root Mean Square Residual (S)RMR (78, 79). Assumptions of the linear models were validated using the *gvlma* package (80). All linear regressions performed met model assumptions.

***Task 1 Data analysis adapted from: Comeros-Raynal et al. 2021. Catchment to sea connection: impacts of terrestrial run-off on benthic ecosystems in American Samoa. Marine Pollution Bulletin* 169:112530

Task 2: Herbivore fish recruits

We fitted linear mixed models to predict cover of benthic assemblages and fish recruit abundance with watershed type using the *lme4* package in R (81). The model included watershed type (pristine, intermediate, disturbed) as a fixed effect and reef habitat (flat, slope) nested within watershed type as a random effect. Separate linear models were used for cover of CCA, hard coral, rubble, turf and recruit density (total herbivore recruit density, by family, and by species, *C. spilurus*, and *C. striatus*). For macroalgae cover, the model included watershed type and reef habitat as fixed factors and site as a random effect. We used square root transformations to satisfy assumptions of normality and heteroscedasticity. Model fit was assessed with residual plots showing approximately normal distributions and independence.

We used non-metric multidimensional scaling (nMDS) to visualize variation in fish recruit composition among sampling locations in R version 4.0.2 using the “vegan’” package (73). The nMDS of recruit assemblages were based on Bray-Curtis similarities with data standardized using Wisconsin double standardization.

Task 3: Feeding rates of *C. spilurus and C. striatus*

We fitted a linear mixed model (estimated using ML and Nelder-Mead optimizer) to predict *C. spilurus and C. striatus* feeding rates with watershed type and feeding substratum using the *lme4* package in R (81). The *C. striatus* model included total length as a random effect. For *C. spilurus* bite rates, the model included site as a random effect. We used square root transformations to satisfy assumptions of normality and heteroscedasticity. Model fit was assessed with residual plots showing approximately normal distributions and independence.

Growth

We fitted linear regression models to model growth rates by relating the age of fish to their length and weight using log transformations for recruit *Chlorurus spilurus* and *Ctenochaetus striatus* in R.

Task 4: Population dynamics

We used non-metric multidimensional scaling (nMDS) to visualize variation in adult fish composition among sampling locations in R version 4.0.2 using the “vegan’” package (73). The nMDS of adult assemblages were based on Bray-Curtis similarities with data standardized using Wisconsin double standardization.

Findings:

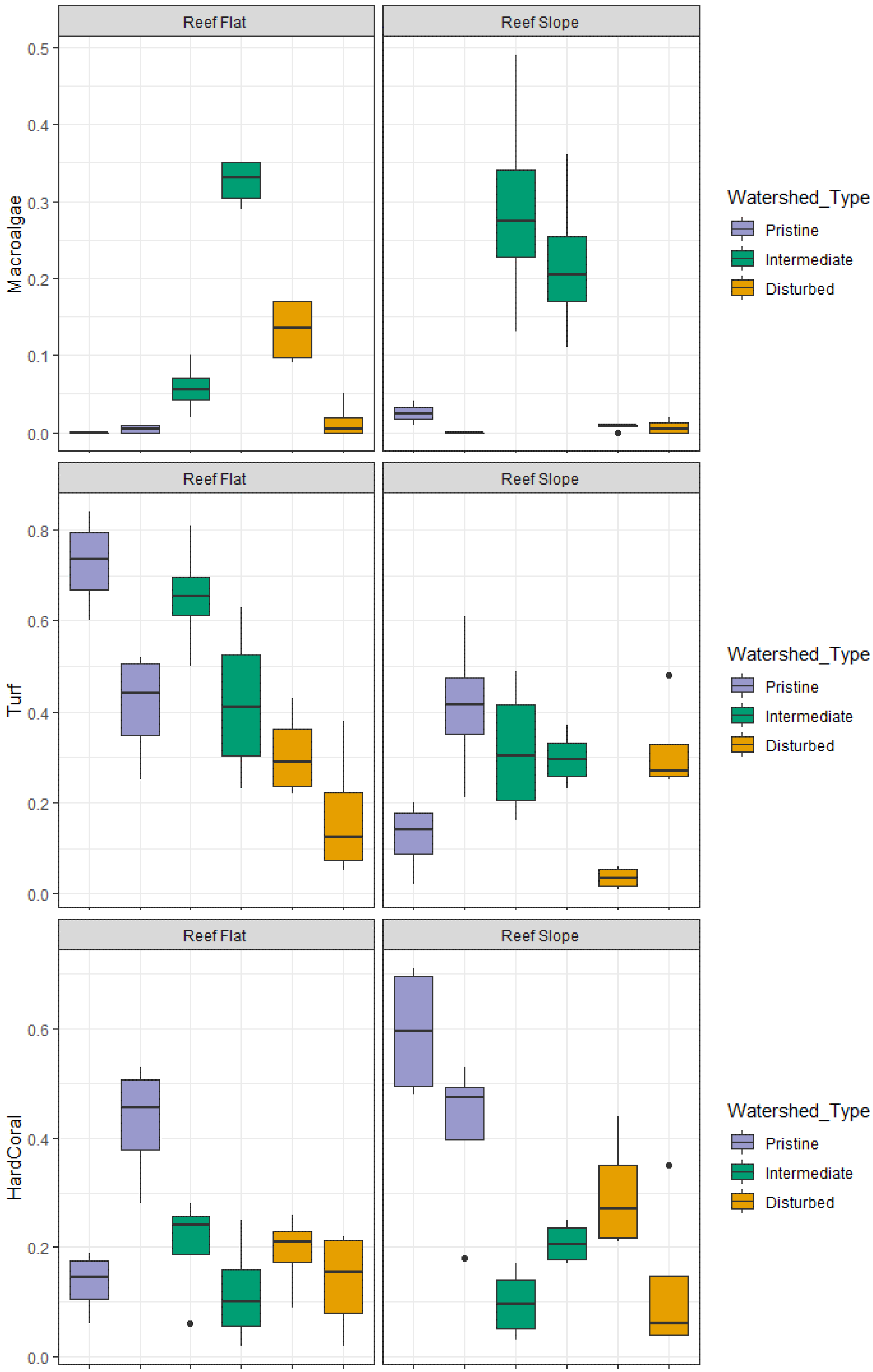
Task 1: Catchment to sea connection: impacts of terrestrial run-off on benthic ecosystems in American Samoa

*** Comeros-Raynal MT, Brodie J, Bainbridge Z, Choat JH, Curtis M, Lewis S, Stevens T, Shuler CK, Sudek M, Hoey AS (2021) Catchment to sea connection: Impacts of terrestrial run-off on benthic ecosystems in American Samoa. Marine Pollution Bulletin 169:112530*

Figure 14. Variation in benthic habitats on the reef slope: pristine watershed, Fagatele Bay (left), and disturbed watershed, Faga’alu (right)

Variation in benthic assemblages

Macroalgae, turf algae, rubble, hard coral and crustose coralline algae (CCA) exhibited clear differences among watershed types with pristine sites in both shallow and deep reef study sites characterized predominantly by hard coral and turf in the reef flat, and hard coral and CCA in the reef slope (Figure 15). In contrast, intermediate and disturbed watershed sites were generally characterized by higher percent cover of macroalgae and rubble on the reef flat, and higher relative cover of algal turfs, macoalgae and rubble on the reef slope. The first two axes of the Principal Component Analysis (PCA) for the reef flat explained 41.1% and 33.5% of the total variation, respectively, with sites within pristine watersheds being differentiated from intermediate and disturbed watersheds along PC1, and intermediate and disturbed watersheds being differentiated along PC2 (Figure 16a). For the reef slope, the first two principal components explained 58% and 18% of the total variation, respectively, with sites within pristine watersheds being represented by relatively high cover of hard coral cover and CCA and separated from intermediate watersheds that were represented by a high cover of macroalgae and rubble along PC1 (Figure 16b).


Reef Flat
Reef Slope
Macroalgae
0.0
0.1
0.2
0.3
0.4
0.5
Turf
0.0
0.2
0.4
0.6
0.8
HardCoral
0.0
0.2
0.4
0.6
Watershed_Type
Pristine
Intermediate
Disturbed

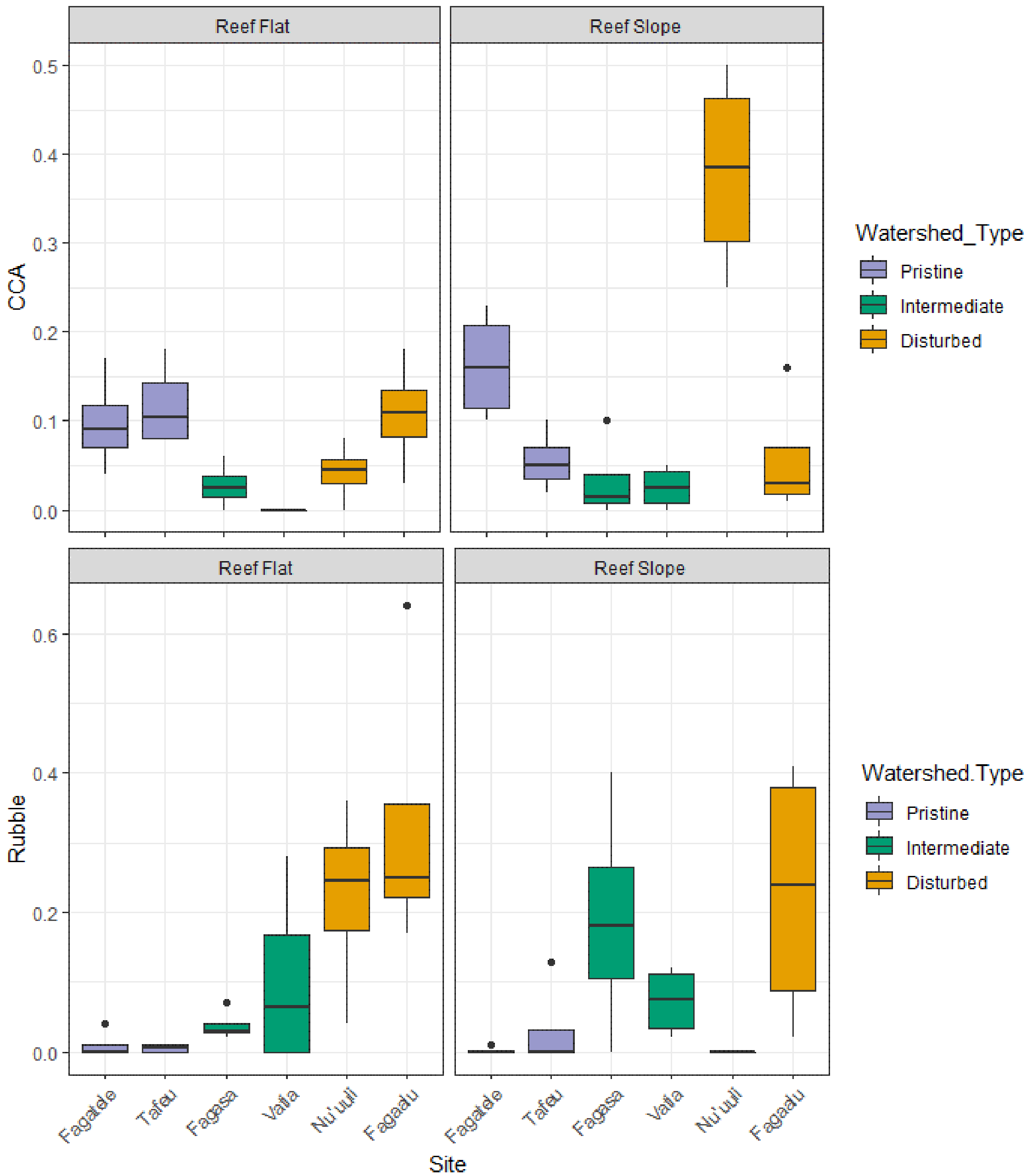


Figure 15. Percent cover of benthos across reef locations with black lines showing median values, boxes showing 25th and 75th percentile, and whisker lines above and below the box showing 5th and 95th percentile of the data.

a.) b.)

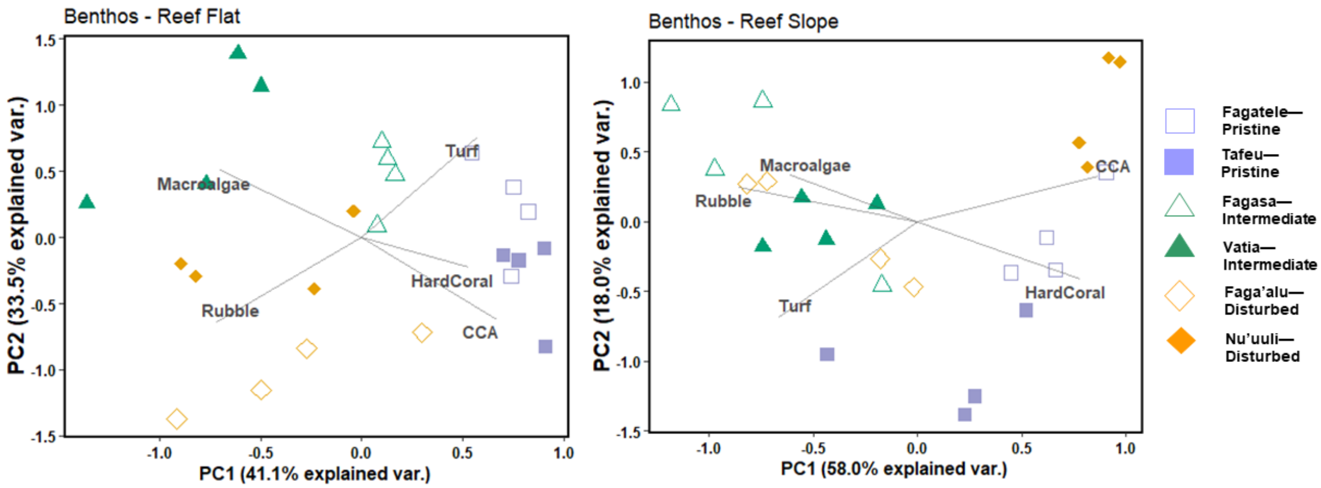
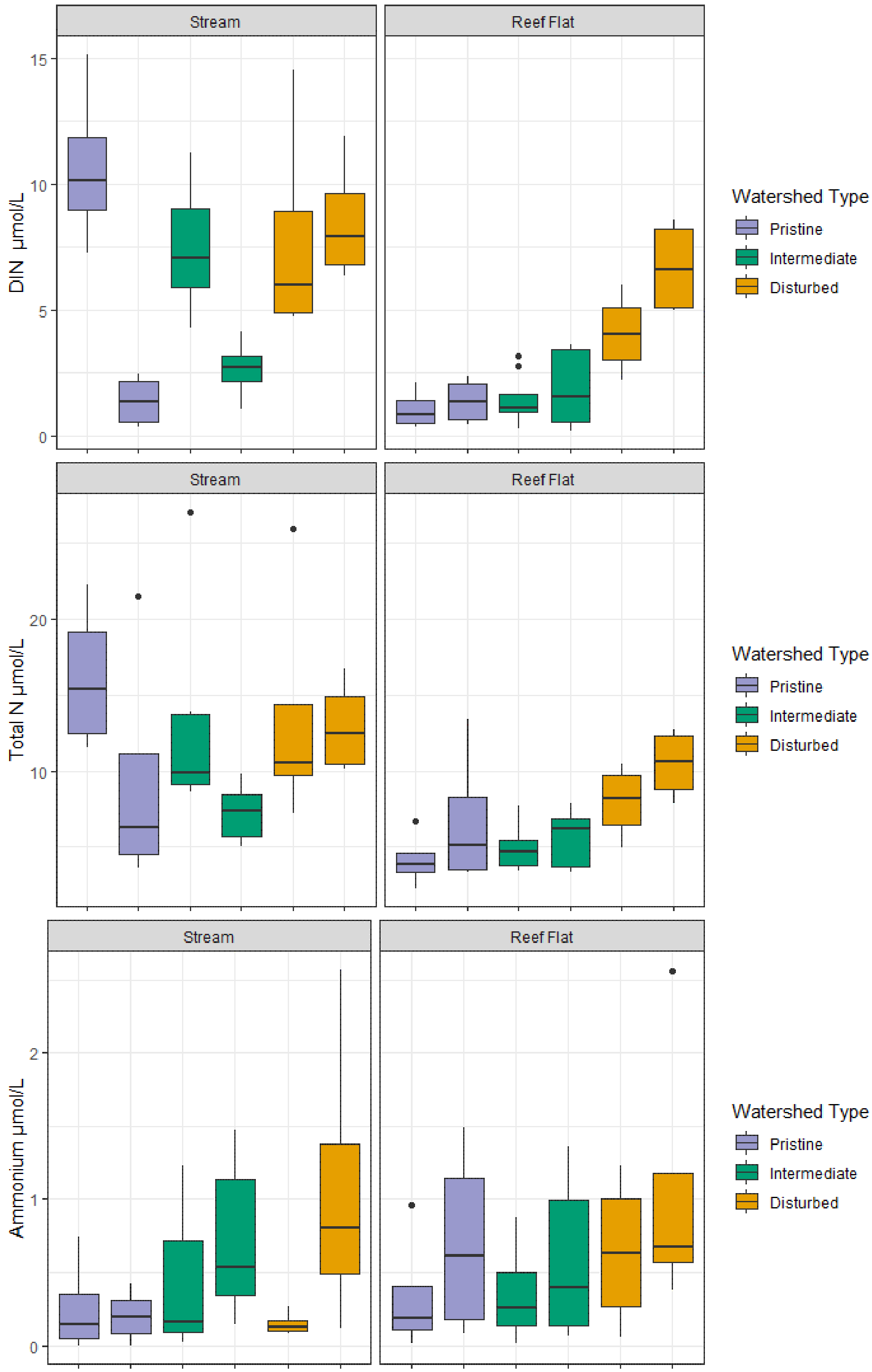


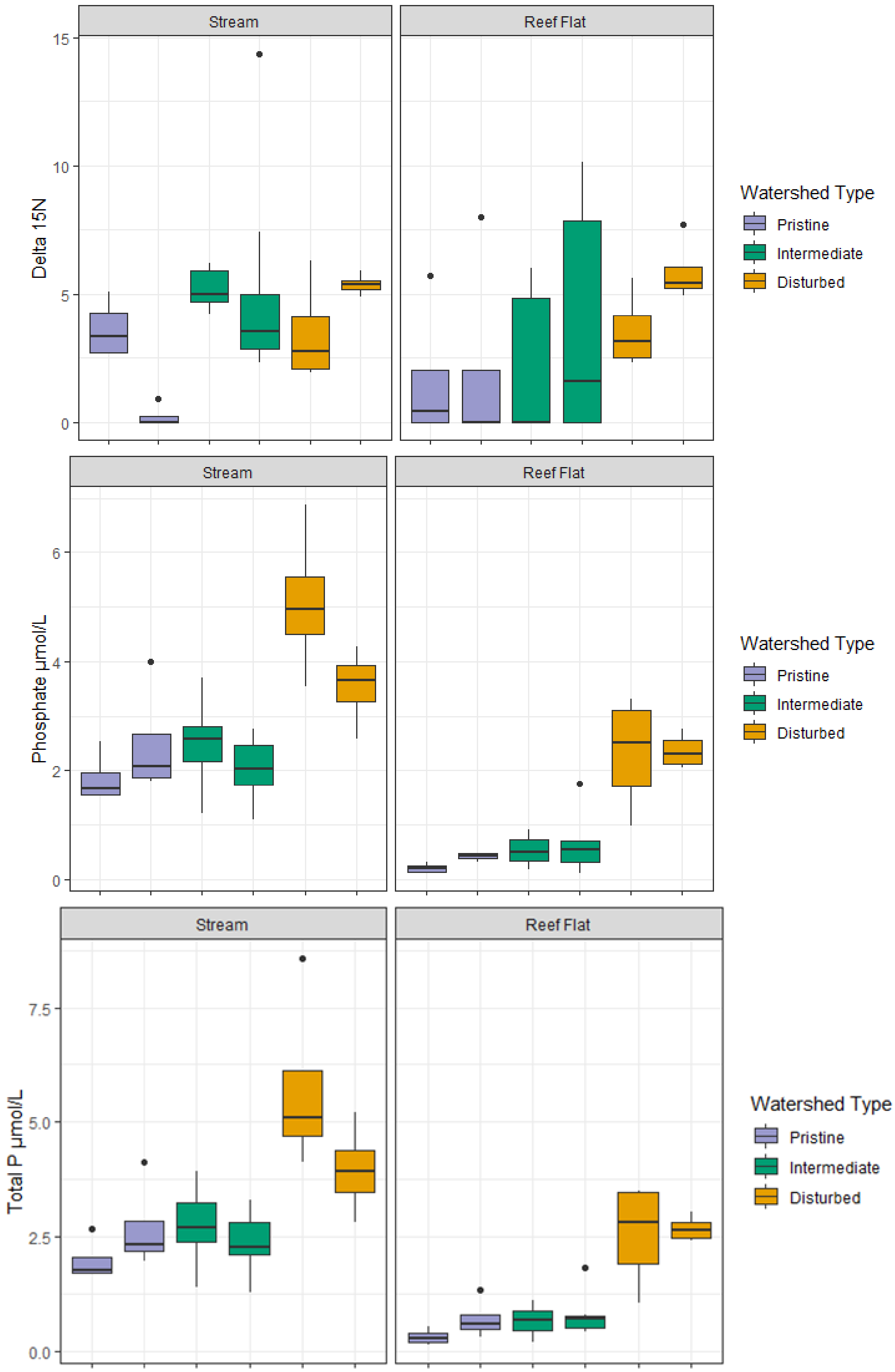
Fig 16. Principal Component Analysis (PCA) showing variation in benthic assemblages on (a) the reef flat, and (b) the reef slope among watershed types (pristine, intermediate, disturbed), and six reef sites around Tutuila, American Samoa. Analyses are based on the cover of benthic categories along four 50 m point-intercept transects within each habitat at each site. Vector lengths are proportional to correlation strengths with the primary PCA axes.

Variation in water quality: nutrients and sediments

Nutrients and sediments varied across watershed types and exhibit seasonal differences (Figures 17-19). Generally, sites adjacent to pristine watersheds had lower nutrient concentrations and sedimentation rates while intermediate and disturbed watersheds were characterized by higher nutrient concentrations and sediment accumulation. The PCA's of nutrient concentrations from stream and reef flat samples also supported the clear partitioning between pristine and intermediate watersheds (Figure 20). From the stream samples, the first two principal components explained 36.1% and 23.2% of the total variation, respectively, with samples from pristine watersheds being differentiated from intermediate watershed samples along PC2 due to lower concentrations of total DIN and, ammonium, and lower values of $\delta^{15}N$ (Figure 20a). For nutrients on reef flats, the first two principal components explained 59.9% and

12.8% of the total variation, respectively. Samples from pristine watersheds were largely differentiated from disturbed watersheds along PC1, with samples from disturbed sites characterized by higher concentrations of all nutrients than those from pristine sites (Figure 20b). Samples from intermediate watersheds were separated from pristine watersheds along PC2, and were characterized by a higher DIN load than pristine sites.


Stream
Reef Flat
DIN µmol/L
0
5
10
15
Watershed Type
Pristine
Intermediate
Disturbed
Stream
Reef Flat
Total N µmol/L
10
20
Watershed Type
Pristine
Intermediate
Disturbed
Stream
Reef Flat
Ammonium µmol/L
0
1
2
Watershed Type
Pristine
Intermediate
Disturbed



Stream
Reef Flat
Delta 15N
15
10
5
0
Watershed Type
Pristine
Intermediate
Disturbed
Stream
Reef Flat
Phosphate µmol/L
6
4
2
0
Watershed Type
Pristine
Intermediate
Disturbed
Stream
Reef Flat
Total P µmol/L
7.5
5.0
2.5
0.0
Watershed Type
Pristine
Intermediate
Disturbed

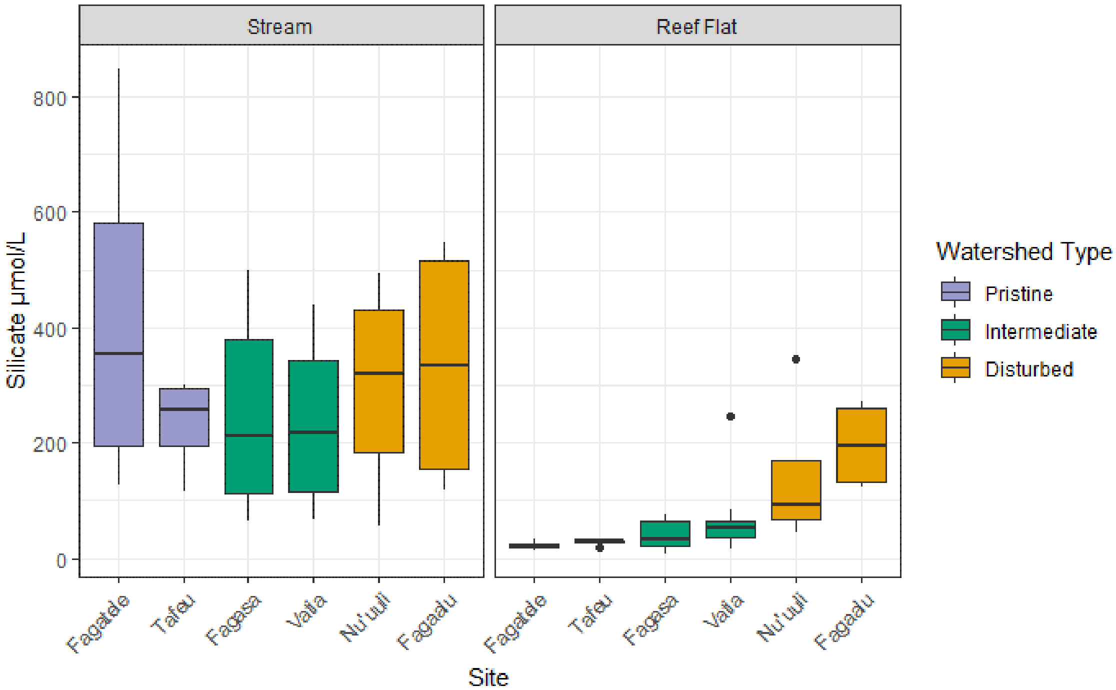
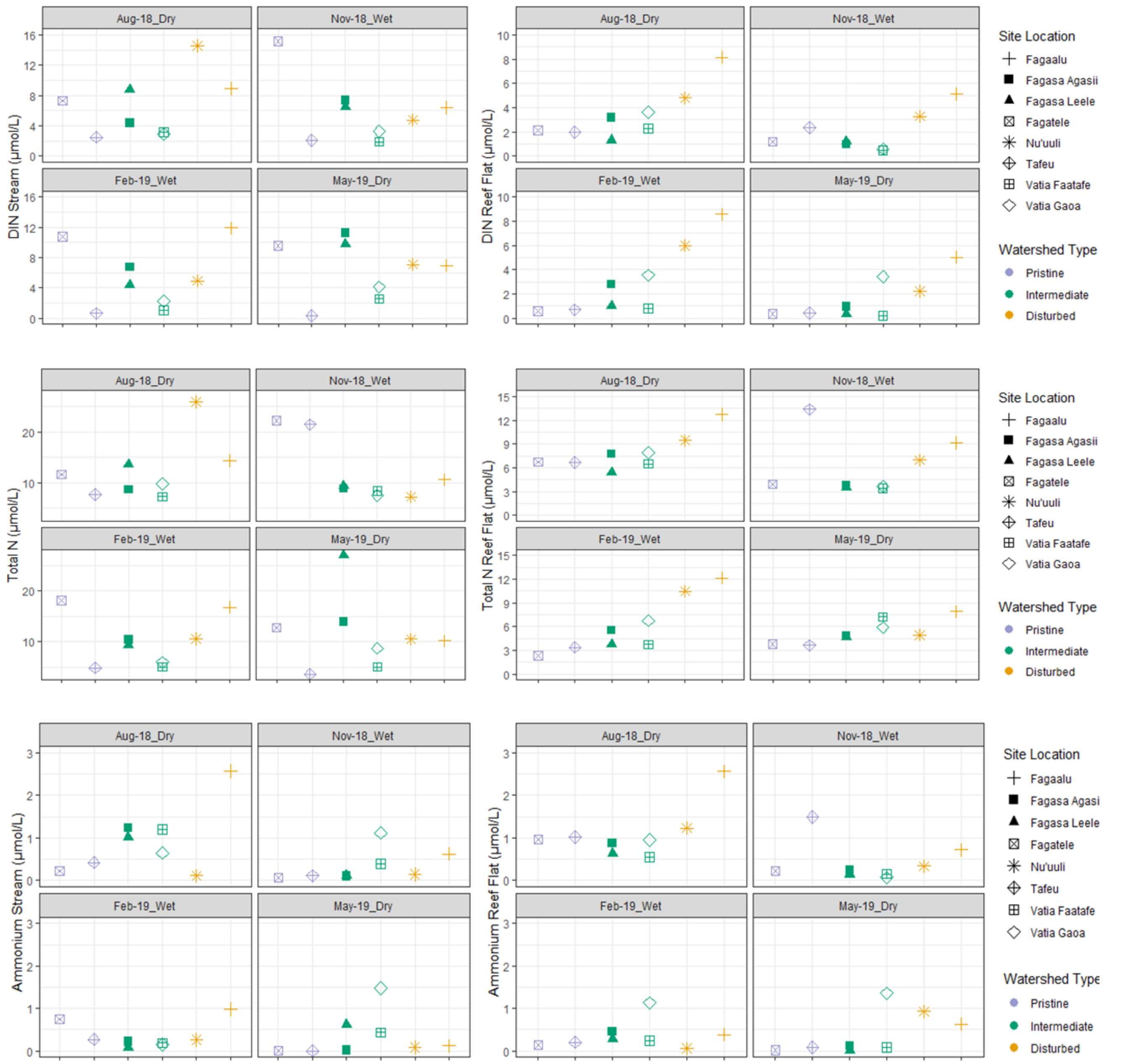
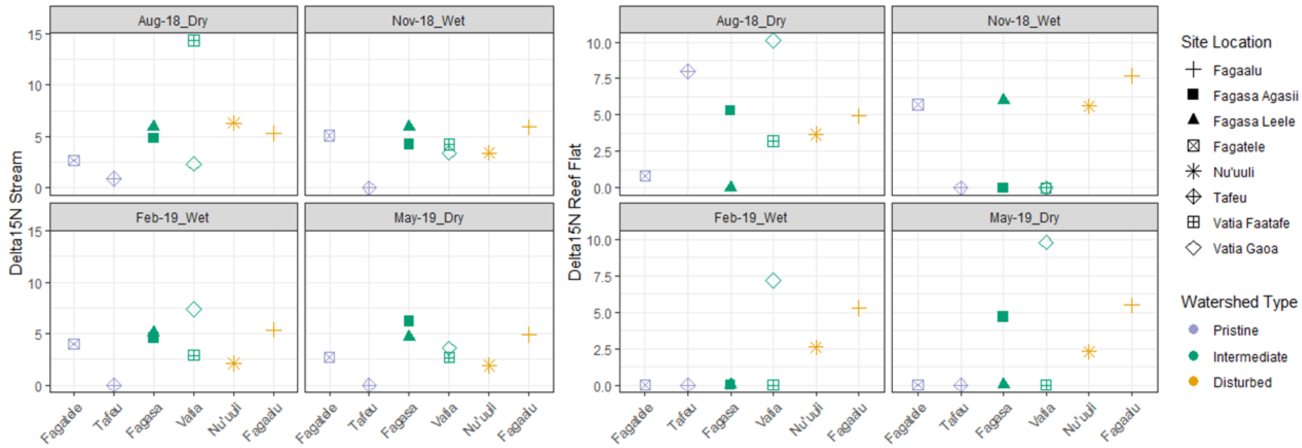
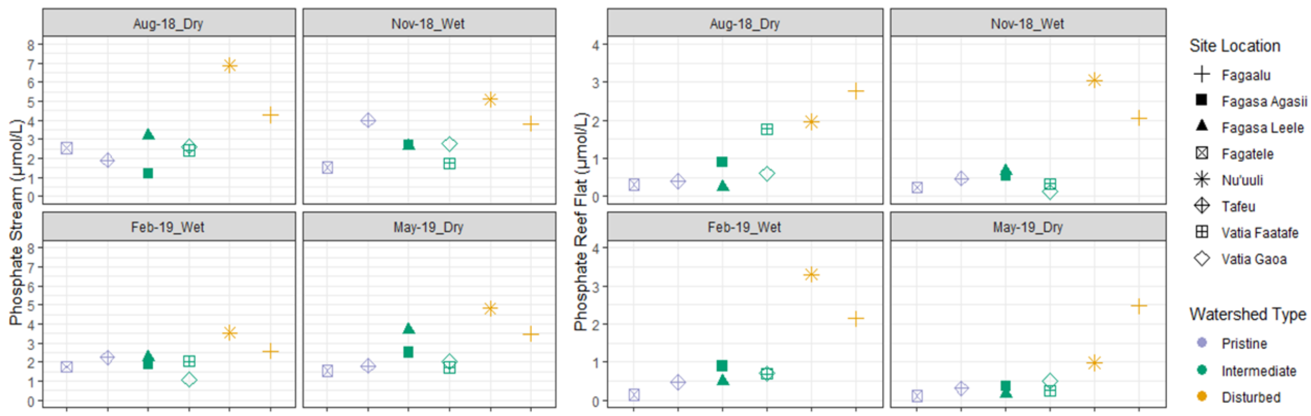
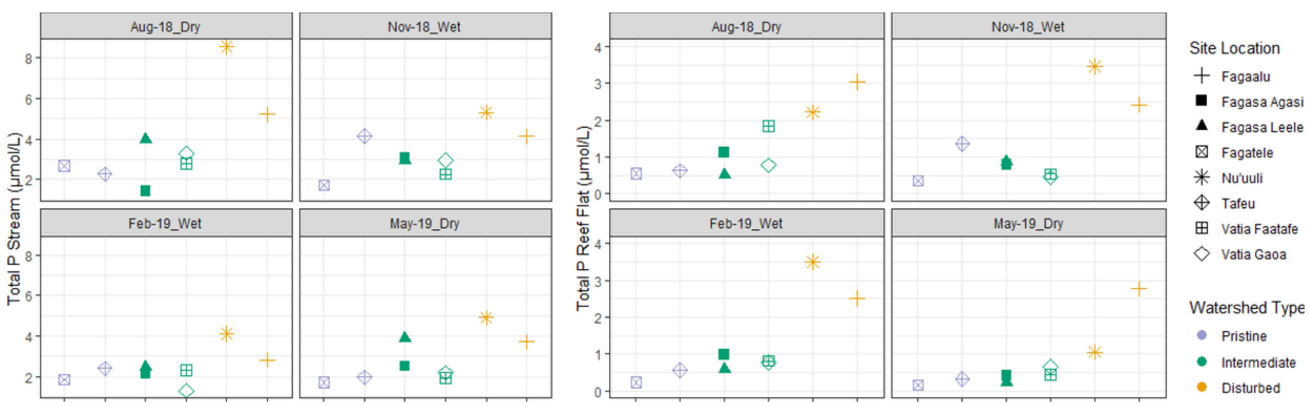


Figure 17. Nutrient concentrations (µmol/l) across all deployments by sampling location, with black lines showing median values, boxes showing 25th and 75th percentile, and whisker lines above and below the box showing 5th and 95th percentile of the data.


DIN Stream (µmol/L)
DIN Reef Flat (µmol/L)
Total N (µmol/L)
Total N Reef Flat (µmol/L)
Ammonium Stream (µmol/L)
Ammonium Reef Flat (µmol/L)
Aug-18_Dry
Nov-18_Wet
Feb-19_Wet
May-19_Dry
Site Location
Fagaalu
Fagasa Agasii
Fagasa Leele
Fagatele
Nu'uuli
Tafeu
Vatia Faatafe
Vatia Gaoa
Watershed Type
Pristine
Intermediate
Disturbed


Delta15N Stream
Delta15N Reef Flat
Aug-18_Dry
Nov-18_Wet
Feb-19_Wet
May-19_Dry
Fagatele
Tafeu
Fagasa
Vatia
Nu'uuli
Fagaalu
Site Location
Fagaalu
Fagasa Agasii
Fagasa Leele
Fagatele
Nu'uuli
Tafeu
Vatia Faatafe
Vatia Gaoa
Watershed Type
Pristine
Intermediate
Disturbed


Phosphate Stream (µmol/L)
Phosphate Reef Flat (µmol/L)
Aug-18_Dry
Nov-18_Wet
Feb-19_Wet
May-19_Dry
Site Location
Fagaalu
Fagasa Agasii
Fagasa Leele
Fagatele
Nu'uuli
Tafeu
Vatia Faatafe
Vatia Gaoa
Watershed Type
Pristine
Intermediate
Disturbed


Total P Stream (µmol/L)
Total P Reef Flat (µmol/L)
Aug-18_Dry
Nov-18_Wet
Feb-19_Wet
May-19_Dry
Site Location
Fagaalu
Fagasa Agasi
Fagasa Leele
Fagatele
Nu'uuli
Tafeu
Vatia Faatafe
Vatia Gaoa
Watershed Type
Pristine
Intermediate
Disturbed

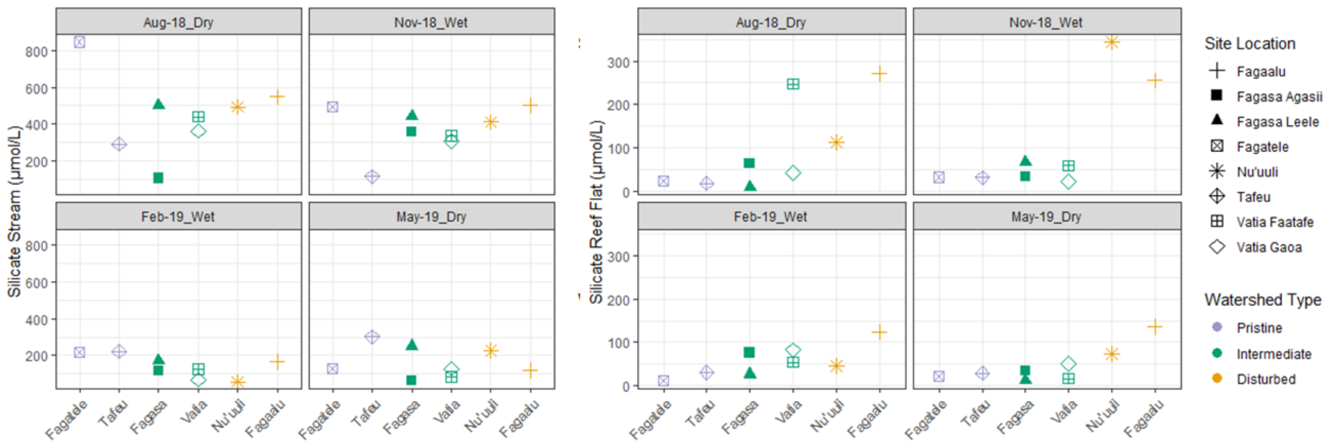


Figure 18. Nutrient concentrations for each sampling period collected from stream and reef flat locations.

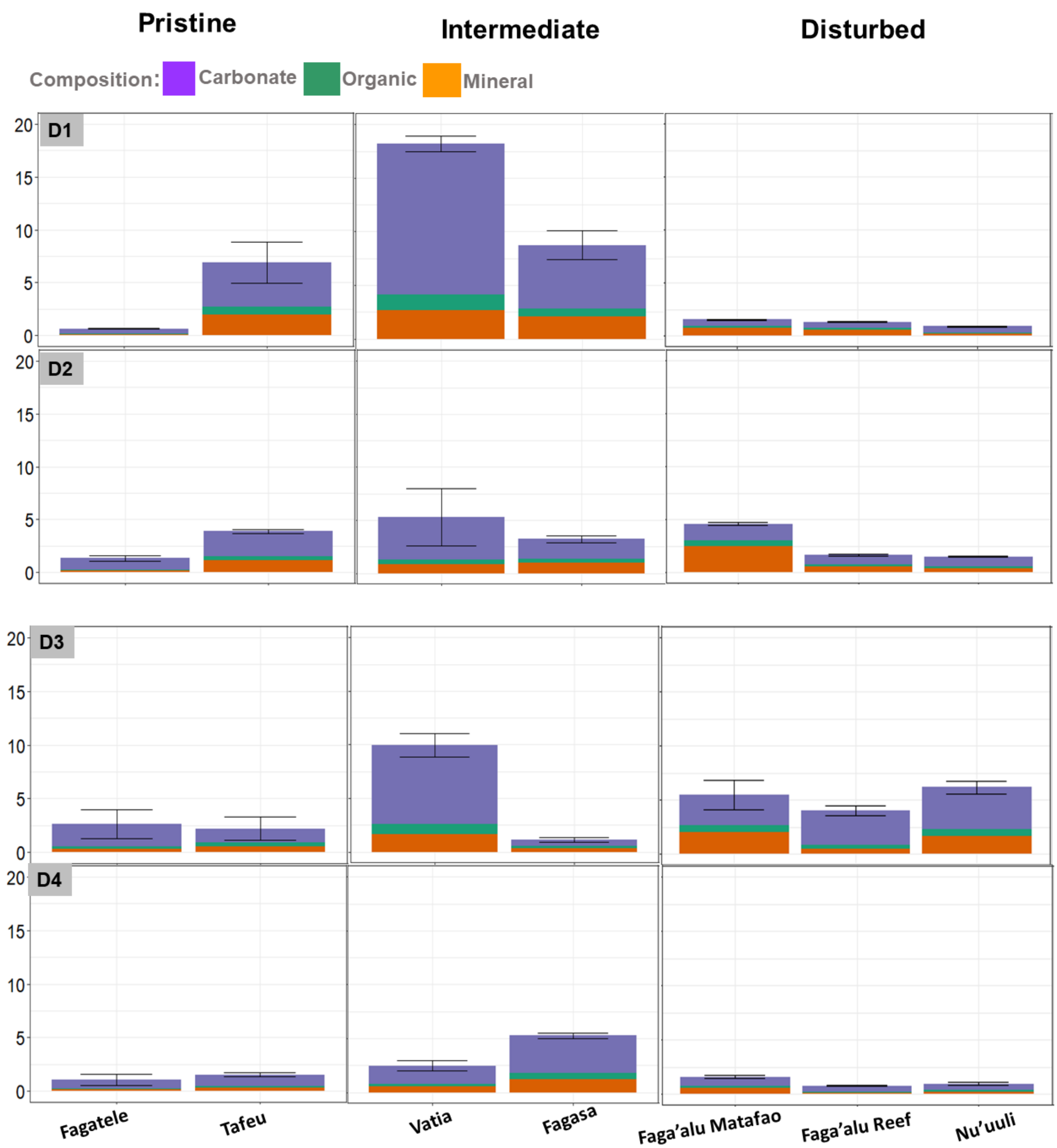


Figure 19. Pooled samples of carbonate, organic and mineral sediment composition is represented within each bar. D1 and D2 represent wet season deployments (Nov-18 – Feb-19; Feb-19 – May-19); D3 and D4 represent dry season deployments (May-19 – Sep-19; Sep-19—Nov-19).

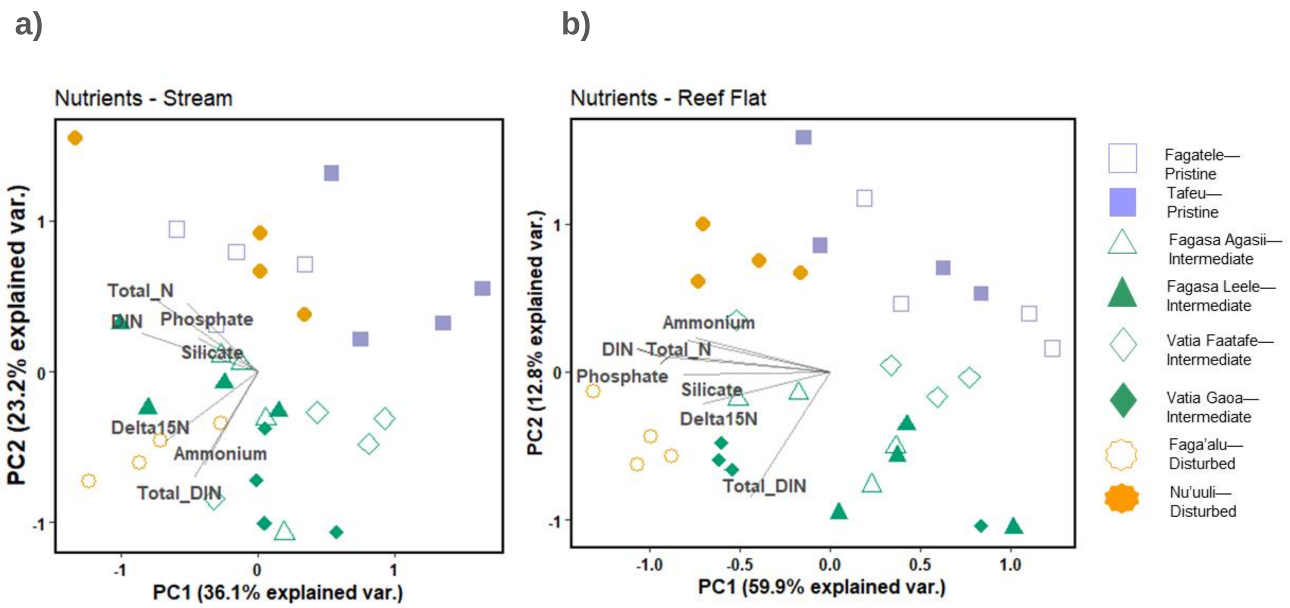


Fig 20. Principal Component Analysis (PCA) showing variation in nutrient concentration on (a) the stream, and (b) the reef flat among watershed types (pristine, intermediate, disturbed), and eight stream sites around Tutuila, American Samoa. The two major streams in Vatia (Gaoa and Faatafe) and Fagasa (Leele and Agasii) were sampled. Analyses are based on nutrient concentrations collected from quarterly sampling within each habitat at each site. Vector lengths are proportional to correlation strengths with the primary PCA axes.

Path analysis

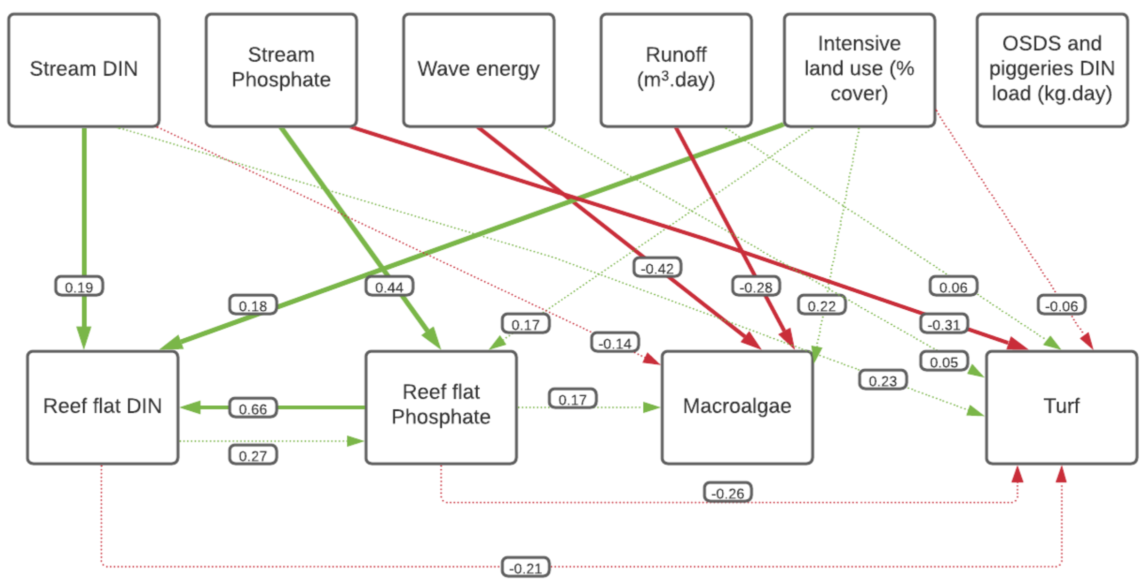
There was a direct and positive link between stream DIN and intensive land use with reef flat DIN, and positive links between stream phosphate, reef phosphate and reef DIN (Fig. 21; Table 2).

Macroalgal cover on the reef flat was best predicted by wave energy and surface runoff, both having a significant negative effect on macroalgal cover (Figure 21a), while turf algae cover was negatively influenced by stream phosphate concentrations.

Hard coral cover was positively related to surface runoff and DIN load from On-Site Disposal Systems and piggeries (path coefficient 0.28 and 0.32, respectively) (Figure 21b; Table 2). CCA cover was positively influenced mainly by surface runoff.

The lack of association between nutrient concentrations, intensive land uses, or DIN loads from piggeries and On-Site Disposal Systems and macroalgae cover was unexpected given the widely accepted positive effects of nutrient availability on macroalgal growth. Similarly, the positive relationships between surface runoff and DIN load with hard coral cover was unexpected because these drivers are generally thought to be detrimental to coral condition. These findings highlight the complexity of the processes shaping benthic assemblages because land use, tidal, wind, and wave forcing, and geomorphic controls act in concert to influence the genesis, transport, and fates of terrestrial run-off. The relationship between hard coral cover and surface run-off, especially, warrants further investigation because of the important role of discharge in driving sediments on adjacent reef habitats and the sensitivity of corals to increased sedimentation (82).

a)



b)

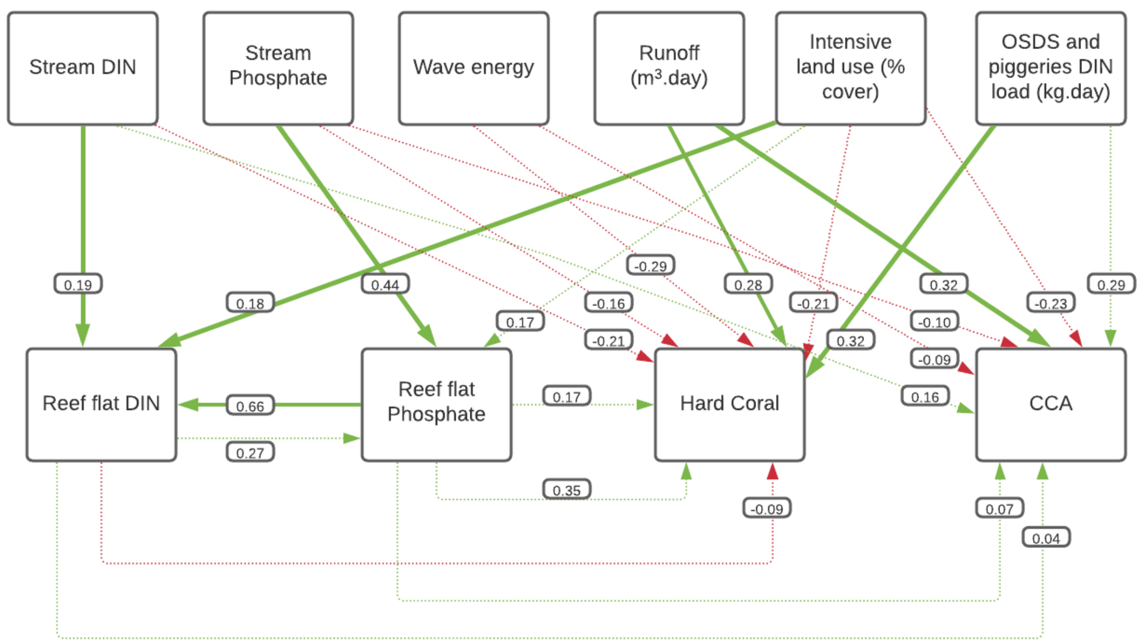


Figure 21. Path analysis showing pathways through nutrients, wave, runoff, intensive land uses and DIN loads affect (a) macroalgal and turf, and (b) hard coral and CCA cover on the reef flat. The two models represent the two groupings of benthic assemblages (see Methods for more details). Arrows in red and green with grey text boxes represent negative and positive path coefficients respectively. The bold straight line arrows represent significant (p-value < 0.05) path coefficients, broken lines represent non-significant path coefficients.

TASK 1: FINDINGS

Analyses highlight the potential complexities of the processes shaping benthic communities on coral reefs, and the need to consider multiple factors simultaneously

Task 2: Recruitment of herbivorous reef fishes

The majority of piscine herbivore recruitment has been shown to occur in shallow reef habitats (83), mostly in live coral (84), rubble (85, 86), and macroalgae including pomacentrid territories (87, 88). To examine the influence of a gradient of terrestrial run-off on recruitment habitats and its effects on herbivore fish recruitment, we quantified recruitment patterns of the two dominant families of piscine herbivores; surgeonfishes (f. Acanthuridae) and parrotfishes (f. Labridae, Tribe Scarini). A total of 1,118 herbivorous fish recruits (<10 cm TL) from 31 species were recorded across the six reef locations. 719 of the 1,118 individuals recorded were parrotfishes, with one species, *Chlorurus spilurus*, accounting for 54% of all parrotfishes recorded. Surgeonfishes (Acanthuridae) comprised 29% of the recorded recruits (324 of the 1,118 individuals), with *Acanthurus triostegus* and *Ctenochaetus striatus* both accounting for 25% of the surgeonfish recruits recorded.

Variation in recruit abundances

Overall, herbivorous fish recruit densities were higher in shallow reef flats adjacent to disturbed watersheds compared to pristine and intermediate sites (Figure 22). Total herbivorous fish recruit density was almost fourfold higher in more disturbed watersheds (3.564 ± 1.525 SE) than pristine watersheds (2.329 ± 1.078 SE; $p < 0.05$, Table 3). Taxon-specific recruitment patterns showed variation among watershed types and habitats with high parrotfish abundances in both disturbed watershed locations, primarily in shallow reef habitats (Figure 23). On reef slopes, a single intermediate watershed, Vatia, displayed high numbers of parrotfish recruits. In contrast, surgeonfishes displayed a different pattern with no clear distinctions between habitats and locations other than higher abundance at two reef slope locations, Vatia and Faga'alu. The high abundance of fish recruits in disturbed watersheds were driven by *C. spilurus* which was 3.45 times (± 1.2535 SE) more abundant in disturbed habitats compared to pristine watersheds (Table 3). In contrast, there was no detectable effect of watershed type effects on the abundance of *C. striatus* recruits ($p > 0.05$), despite higher abundance of acanthurids in disturbed watersheds.

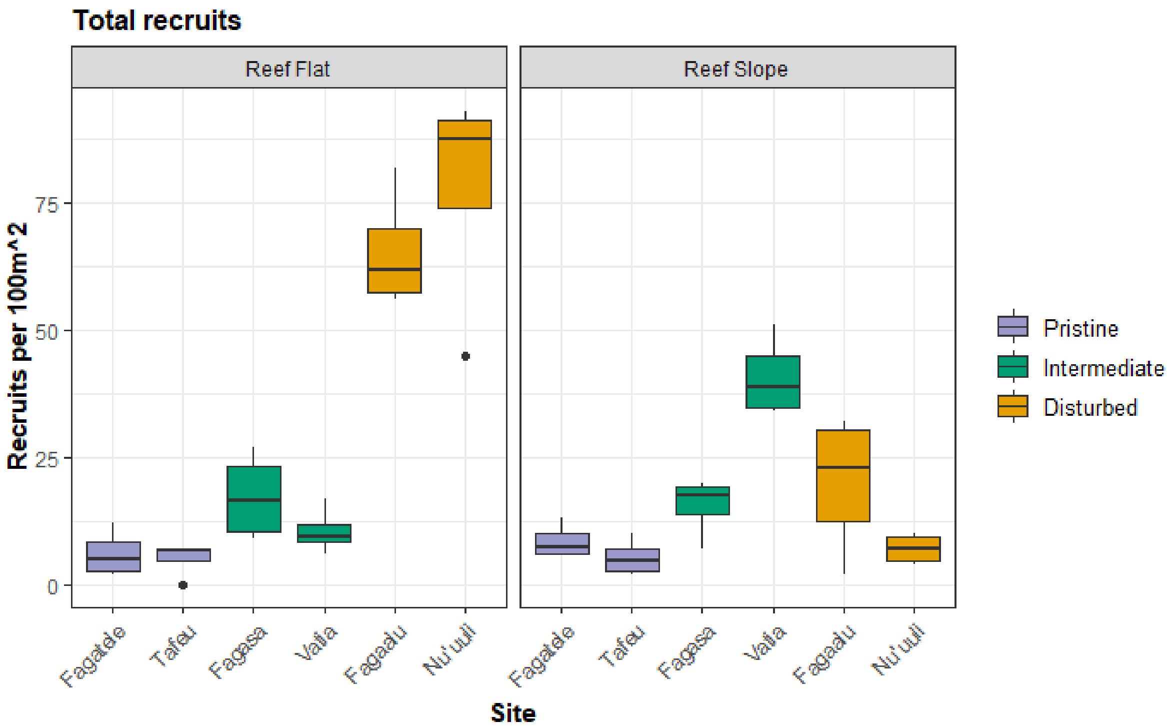
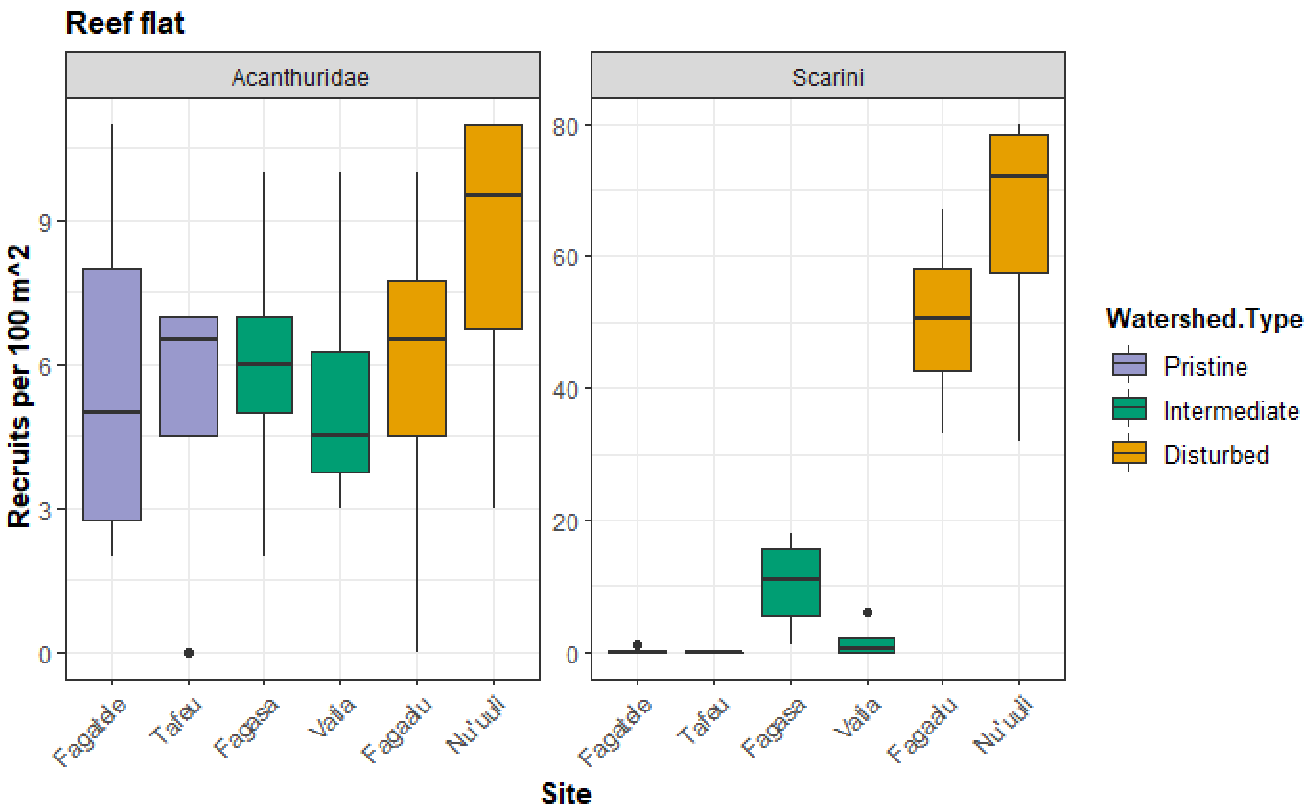


Figure 22. Recruit abundance across study sites by watershed type and reef location. (a) Overall recruit abundance across study sites by reef habitat, with black lines showing median values, boxes showing 25th and 75th percentile, and line showing 5th and 95th percentile of the data. Data points are outliers.

Recruit abundance by family



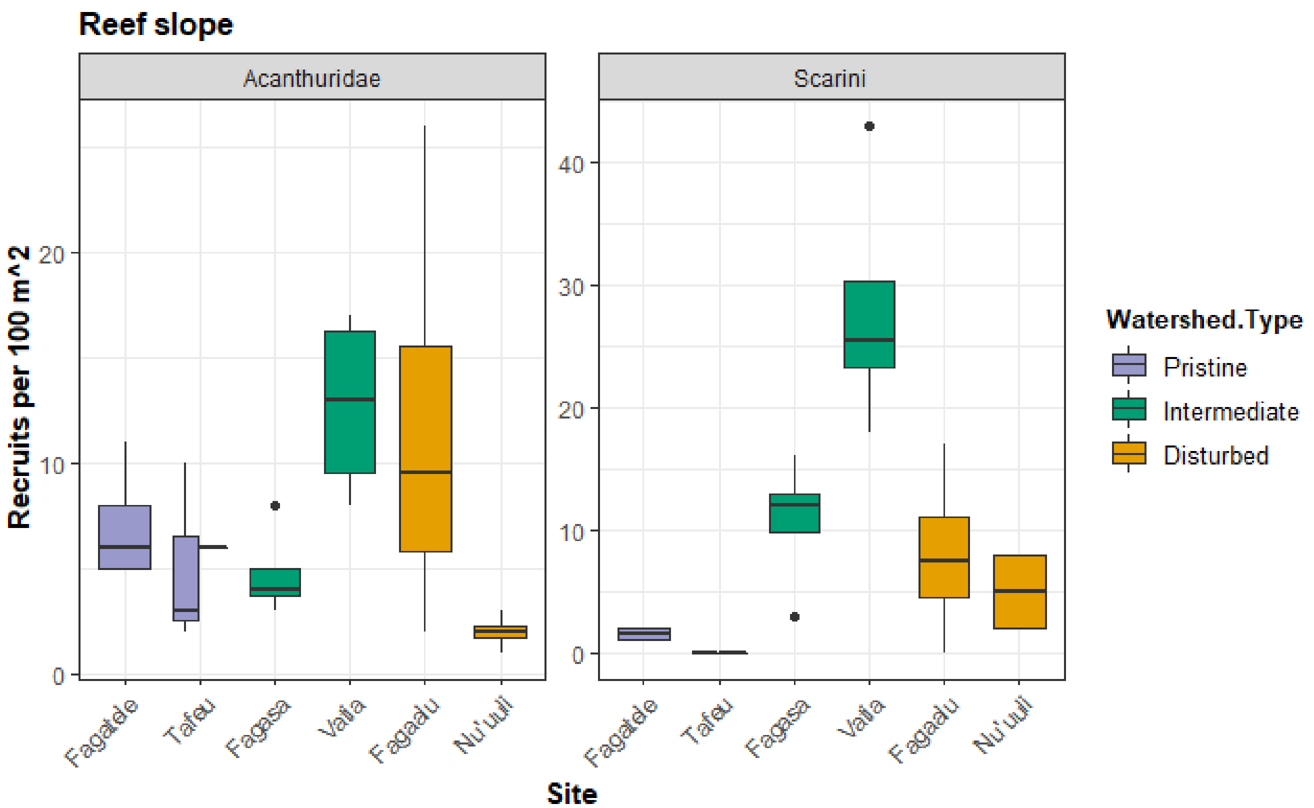


Figure 23. Recruit abundance across study sites by watershed type and reef location. Acanthurid and scarine recruit abundance with black lines showing median values, boxes showing 25th and 75th percentile, and line showing 5th and 95th percentile of the data. Data points are outliers.

Recruit assemblage structure

Herbivore fish recruits exhibited clear separation among watershed types with disturbed reef flat sites characterized by high densities of parrotfishes, *C. spilurus* and *Scarus globiceps*, and the surgeonfish, *C. striatus* (Figure 24a). Reef flat sites adjacent to pristine watersheds were characterized by higher densities of *Acanthurus nigricans* recruits, while reef slope sites adjacent to pristine watersheds were characterized by higher recruit numbers of the surgeonfishes, *Ctenochaetus cyanocheilus* and *Acanthurus nigroris* (Figure 24a). In contrast, sites adjacent to disturbed habitats had higher recruit densities of *C. spilurus*, *Scarus globiceps*, and *C. striatus*, but no differences between the reef slope and reef crest habitats (Figure 24b). Generally, recruit parrotfishes were primarily associated with disturbed and intermediate watersheds, while surgeonfishes tended to be more widely distributed among watershed types.

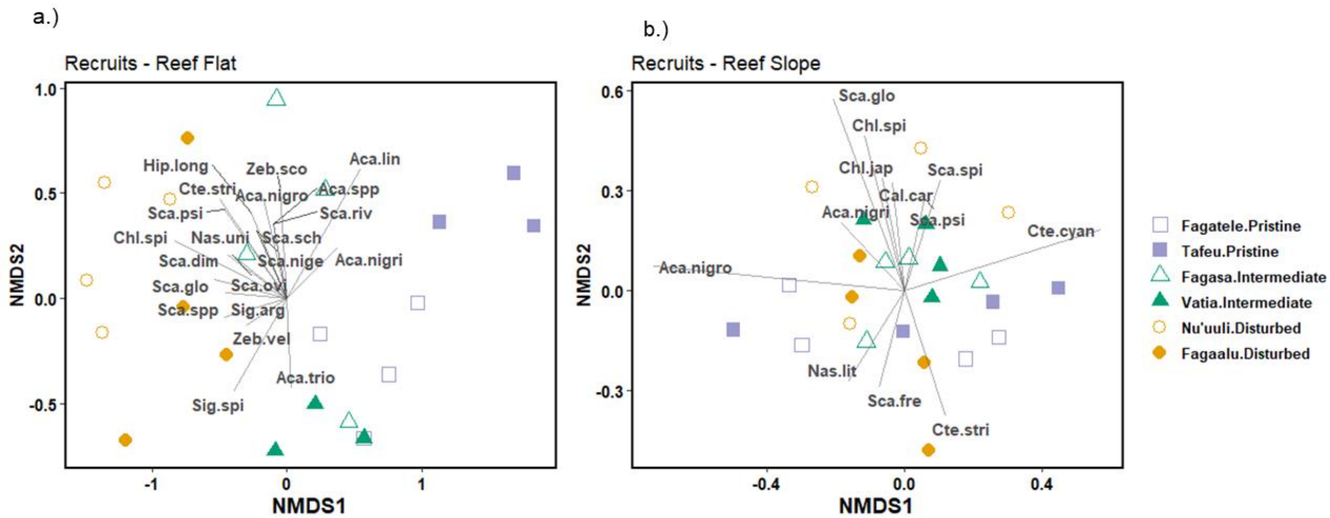


Fig. 24. Non-metric multidimensional scaling analyses showing variation in recruit abundance on (a) the reef flat, and (b) the reef slope among watershed types (pristine, intermediate, disturbed), and six reef sites at Tutuila, American Samoa. Analyses are based on transect level Wisconsin double standardization transformed data.

Task 3: Differences in feeding and growth rates

Feeding rates of both *C. spilurus* and *C. striatus* were higher in sites adjacent to intermediate watersheds compared to those adjacent to disturbed watersheds. Bite rates (bites per minute) differed for *C. spilurus* (Figure 25, Table 4), with significantly higher bite rates found in intermediate watersheds (2.45 ± 0.490 SE) compared to disturbed watersheds (2.18 ± 0.484 SE). . There were no significant differences in feeding rates across substratum types (Figure 26, Table 4). There was also variation in *C. striatus* bite rates (Figure 27, Table 4) across watershed types with higher bite rates in sites adjacent to intermediate watersheds (2.39 ± 0.281 SE) compared to disturbed sites (2.24 ± 0.273 SE) but the difference was not significant (Table 5). Rubble had a significant positive effect on *C. striatus* bite rates (4.092 ± 0.196 SE)

(Figure 28, Table 5). Watershed-specific differences in bite rates could be attributed to the quantity and quality of feeding resources at each site.

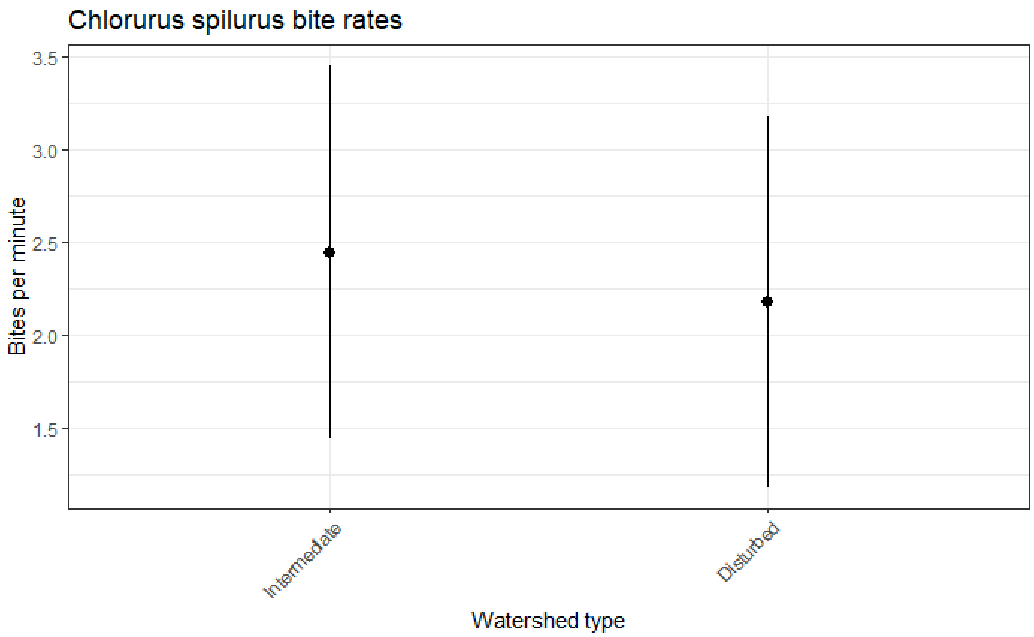


Figure 25. Modelled *C. spilurus* bite rates (± 95% confidence intervals) within each watershed type. Random effects were specified as individual species. The model included site as a random effect.

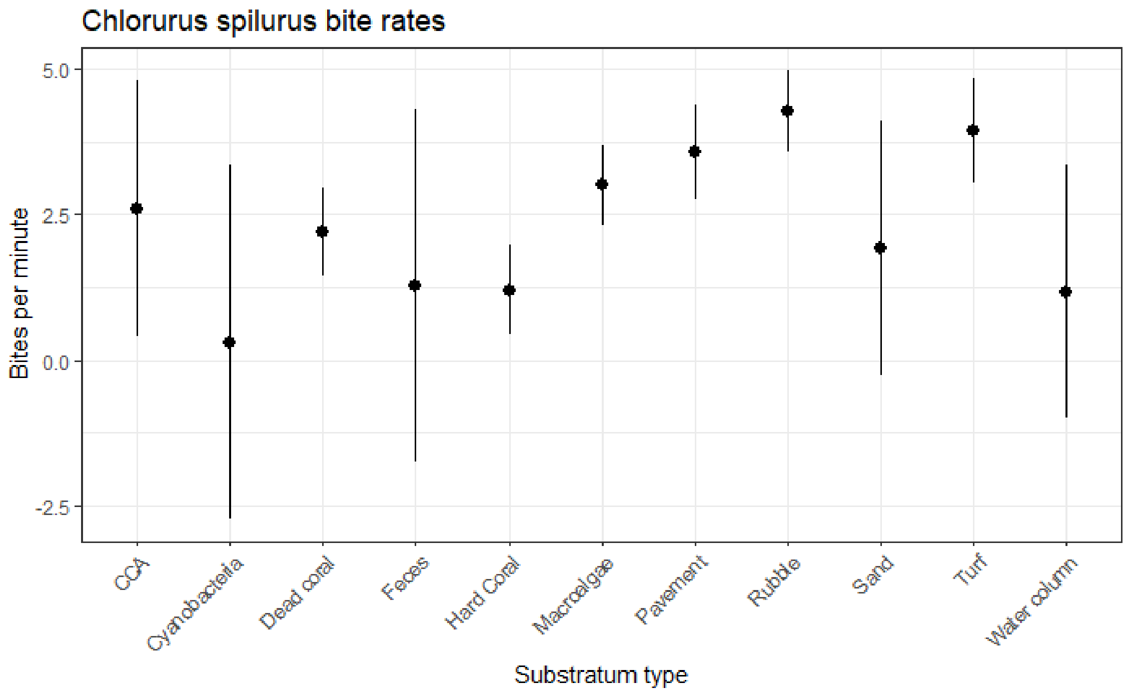


Figure 26. Modelled *C. spilurus* bite rates (± 95% confidence intervals) within each substratum type. The model included site as a random effect.

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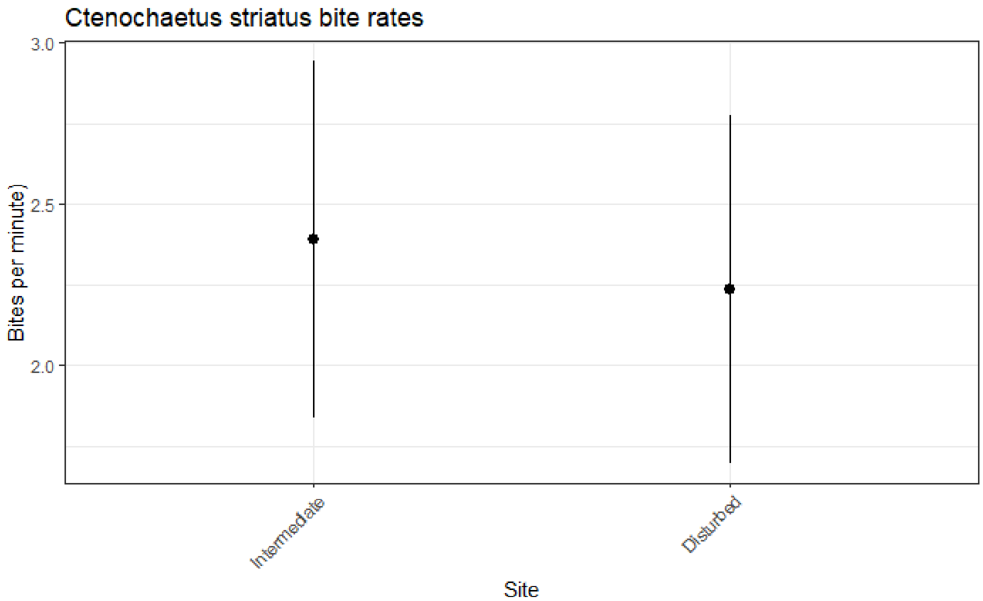


Figure 27. Modelled *C. striatus* bite rates (± 95% confidence intervals) within each watershed type. Random effects were specified as total lengths nested within observed individuals

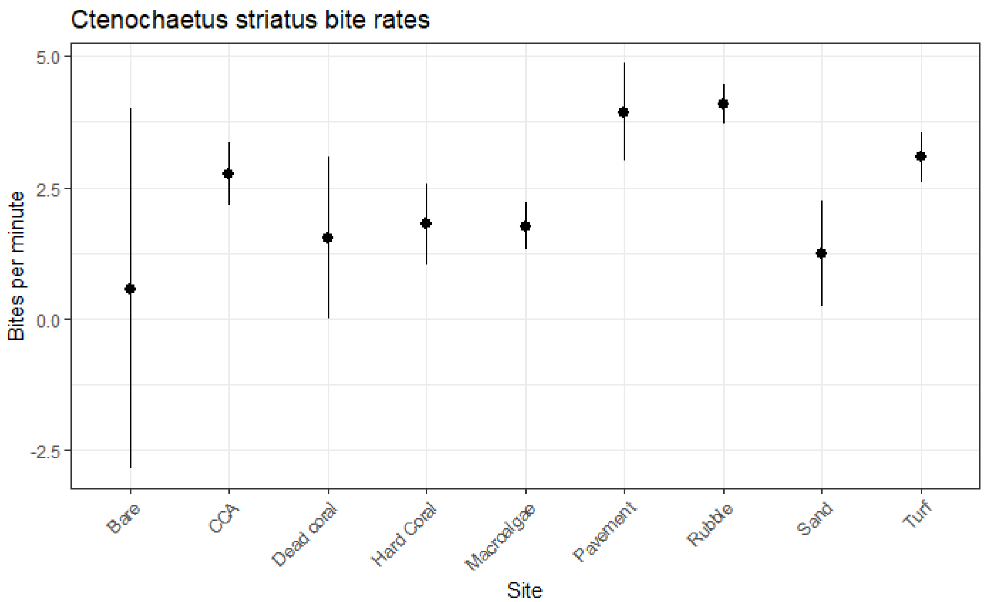
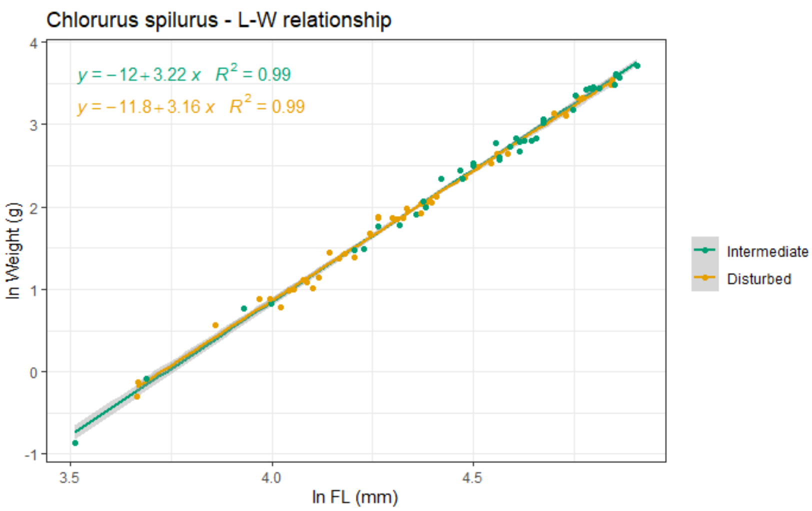


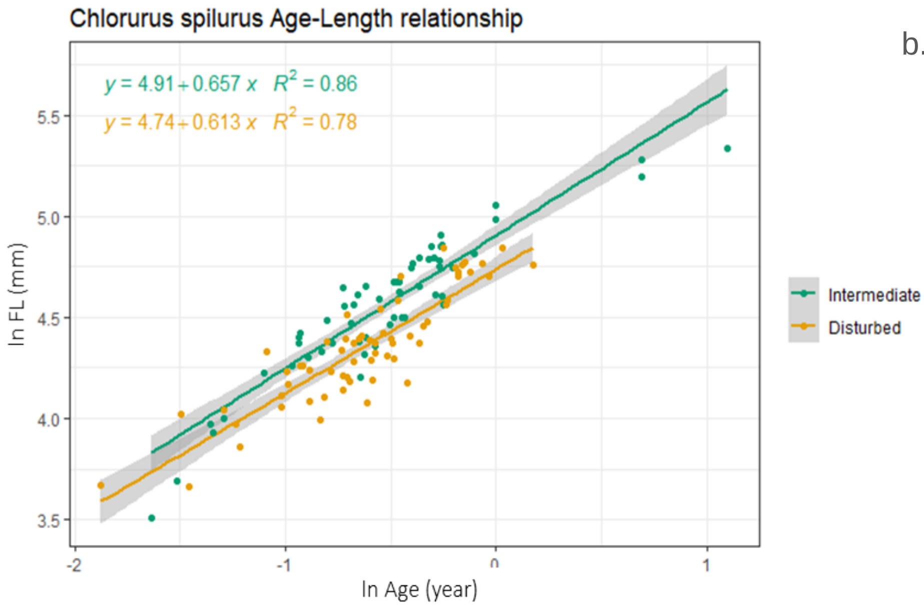
Figure 28. Modelled *C. striatus* bite rates (± 95% confidence intervals) within substratum type. Random effects were specified as total lengths nested within observed individuals

Growth rates

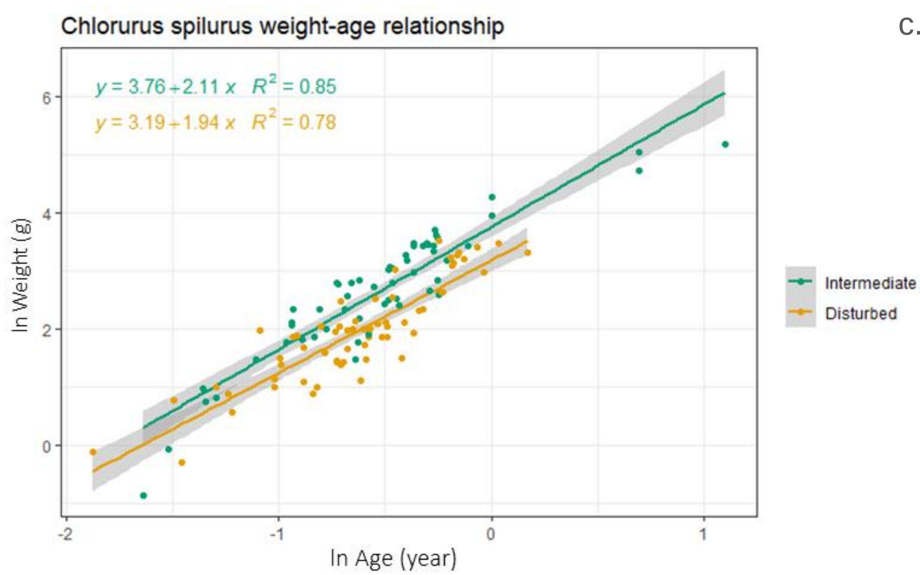
Length-weight condition plots show no difference between disturbed and intermediate watersheds for *C. spilurus* (Figure 29a). However, log-log relationships of fork length and age, and body mass and age, show that individuals of *C. spilurus* on reefs adjacent to intermediate watersheds were larger for a given age (i.e., grew faster) than those on reefs adjacent to disturbed watersheds (Figure 29b, c). For example, a *C. spilurus* at an age of 0.6 year from an intermediate watershed will be on average ~100 mm FL compared to 80 mm FL for an individual of the same age from a disturbed watershed. Individuals in intermediate watersheds were also heavier compared to those found in disturbed watersheds.



a.)



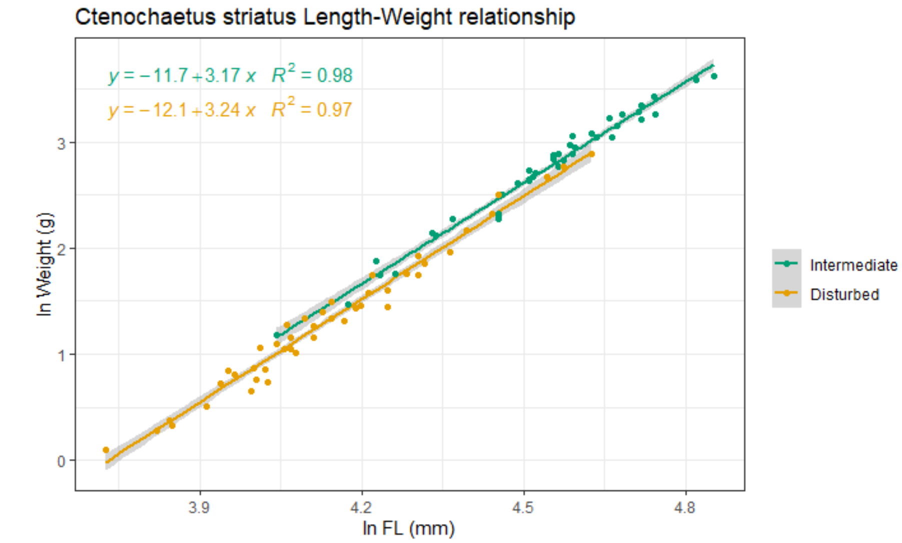
b.)

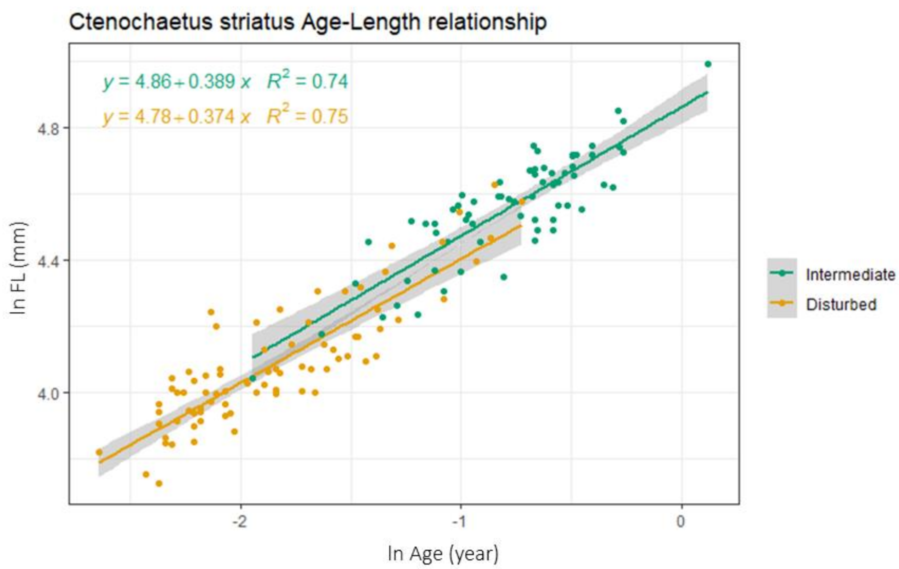


c.)

Figure 29. *Chlorurus spilurus* (a) length-weight condition plots; (b) scatterplot of log-transformed fish length and age; (c) scatterplot of log-transformed fish weight and age.

Length-weight plots suggest that *C. striatus* may be sensitive to changes in density expressed as lower condition in disturbed watersheds (Figure 30a). There is also some evidence of reduced growth in weight in individuals from disturbed watersheds (Figure 30c). The length-weight condition of *C. striatus* appears to be sensitive to locality, environmental and biotic influence. In contrast, growth rates are less sensitive to environmental or locality factors compared to *C. spilurus*.





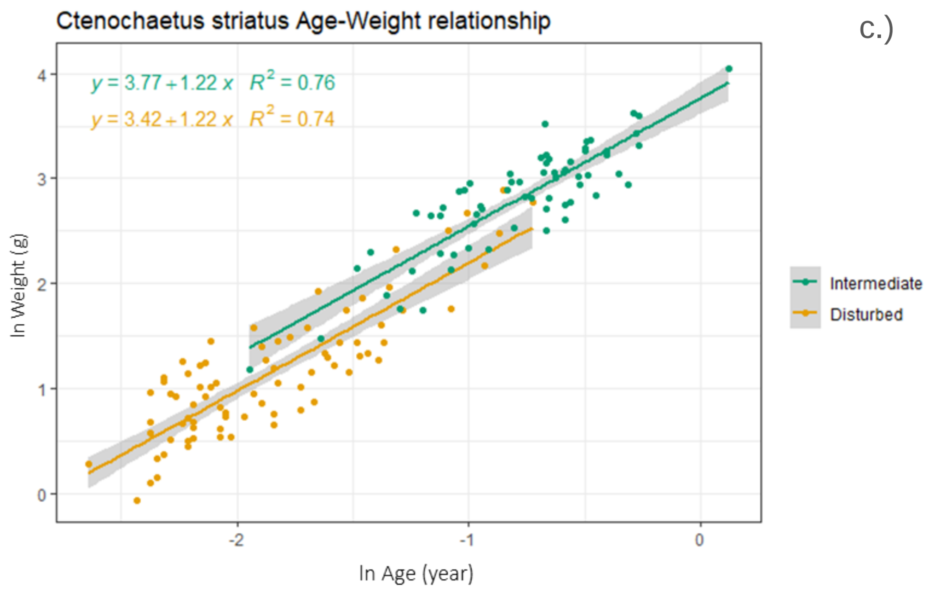


Figure 30. *Ctenochaetus striatus* (a) length-weight condition plots; (b) scatterplot of log-transformed fish length and age; (c) scatterplot of log-transformed fish weight and age.

Task 4: Population assemblage structure of adult parrotfishes and surgeonfishes

Overall, parrotfish and surgeonfish adult abundance was higher on the reef flat and reef slope in one disturbed site, Nu'uuli, compared to sites adjacent to pristine and intermediate watersheds (Figure 31). However, parrotfish body sizes differed on the reef flats and reef slopes, with sites adjacent to pristine and intermediate watersheds having higher average body sizes compared to disturbed sites (Figure 32). In contrast, there was limited variation in surgeonfish body sizes on both the reef flats and reef slopes among sites.

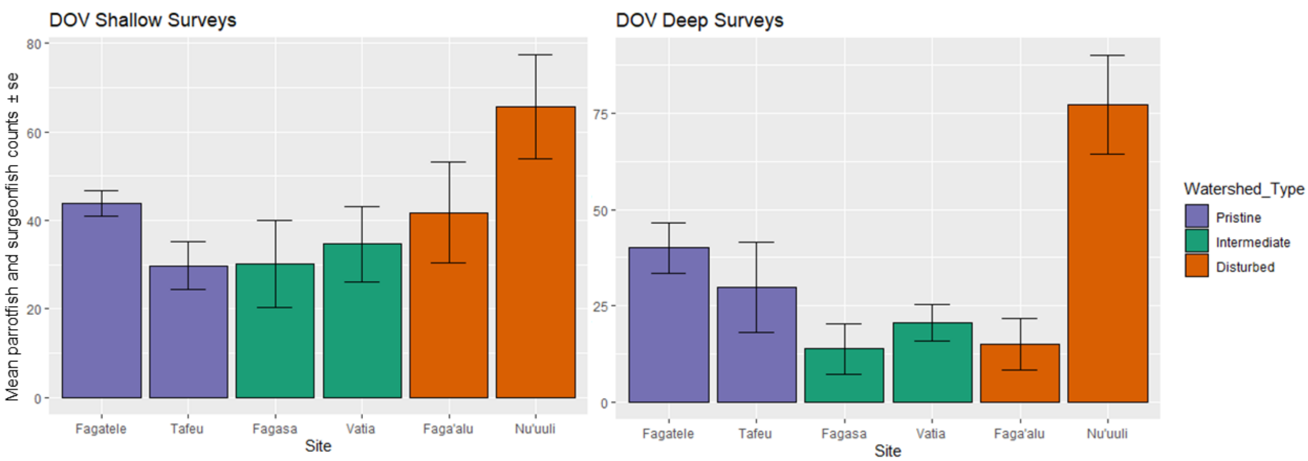


Figure 31. Mean adult parrotfish and surgeonfish distribution across study sites by watershed type and reef location (a) shallow and (b) deep reef locations (five replicate timed swims at each site, 3 min long by 5 m across, averaging 322 m^2 per reef location)

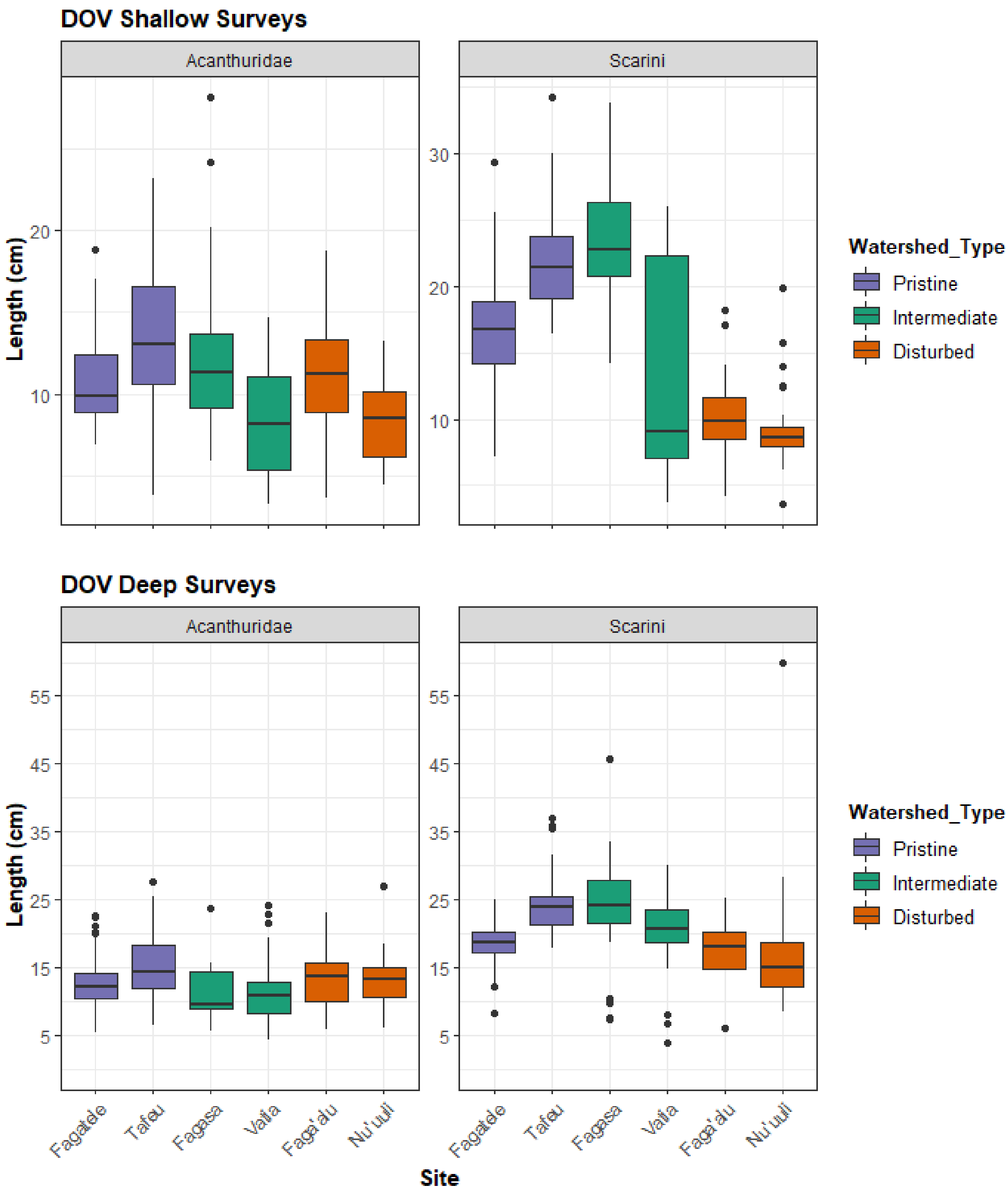


Figure 32. Adult parrotfish and surgeonfish total lengths across study sites by watershed type

and reef location (a) shallow and (b) deep reef locations with black lines showing median values, boxes showing 25^{th} and 75^{th} percentile, and line showing 5^{th} and 95^{th} percentile of the data. Data points are outliers.

Adult assemblage structure

Adult fishes exhibited clear separation among watershed types with disturbed reef flat sites characterized by high densities of parrotfishes, *C. spilurus, Hipposcarus longiceps* and Scarus *globiceps*, and the surgeonfish, *Acanthurus triostegus* (Figure 33). Reef flat sites adjacent to pristine habitats were characterized by higher densities of the surgeonfishes, *Acanthurus nigricans* and *Naso lituratus,* and the parrotfish, *Chlorurus japanensis*. There were considerable variations in shallow herbivore fish assemblages between the two intermediate sites with Fagasa characterized by high densities of *N. lituratus* and *C. japanensis*, while Vatia was characterized by higher *Acanthurus olivaceus* densities (Figure 33a). There was less distinct separation of adult assemblages among watershed types on the reef slope compared to the reef flats (Figure 33b). For instance, high densities of *C. spilurus* were recorded on the reef slopes in Fagatele (pristine) and Nu’uuli (disturbed) but only in Nu’uuli on the reef flat.

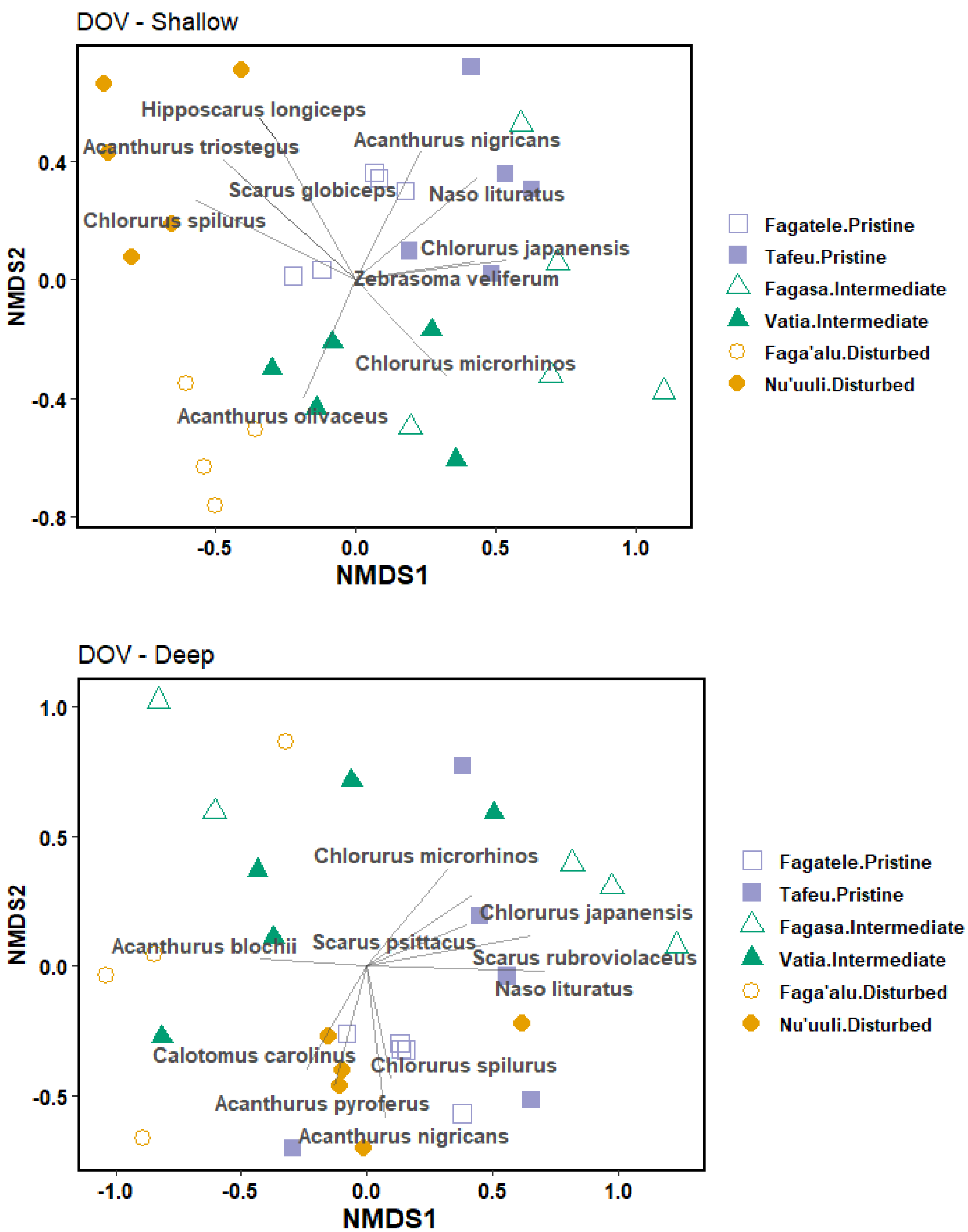


Figure 33. Non-metric multidimensional scaling analyses showing variation in adult parrotfish and surgeonfish abundance on (a) the reef flat, and (b) the reef slope among watershed types (pristine, intermediate, disturbed), and six reef sites at Tutuila, American Samoa. Analyses are based on transect level Wisconsin double standardization transformed data.

Task 5: Creating impactful research through close collaboration and coordination

We developed a research agenda grounded in partnerships to advance local technical capacity and directly address locally important issues. Throughout the project, we coordinated with project partners and advanced inter-agency collaborations by working with multiple local and federal agencies in successfully carrying out each project task. For example, CRAG, Department of Commerce Coastal Management Program, Sea Grant, NOAA PIRO, NOAA CRCP, NOAA Corps, and the National Park of American Samoa provided field and laboratory support. In addition, we received additional laboratory and analytical support from local and Australian educational institutions, the Division of Agriculture, Community, and Natural Resources of the American Samoa Community College, and the Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) at JCU, respectively.

Research agenda grounded in partnerships to advance local technical capacity and directly address locally important issues

FOCUS ON STUDENT ENGAGEMENT AND SKILLS-BUILDING

We provide value to the ASCC Marine Science Program by directly involving students in research that is highly relevant to the community and the management of local resources. The students, with the guidance of ASCC Marine Science Educator, Meagan Curtis, and PI Mia Comeros, helped collect data in the field, processed water, sediment, and fish samples, collated and queried data prior to analyses. The students contributed to essential steps of the research and were invaluable co-producers of the knowledge products of this project. In addition to developing their research skills, the students were also trained in project management and data interpretation. Importantly, we fostered an inclusive research environment that exposed students to the value of inter-agency cooperation, integrated and applied research with significant management implications, and the importance of increasing diversity and equity in addressing complex land-sea processes.

Figure 34. Presenting the project's research questions and goals to ASCC Marine Science students in August 2018.

Outreach:

We have actively engaged with partners in the co-production and transfer of knowledge products, and have maintained open communication with project partners throughout the project. For instance, we presented the project goals and objectives, and research questions with local and federal partners at the beginning of the project, reported the status of project tasks to partners at every quarterly field trip in American Samoa to evaluate the pace of progress and to receive feedback from collaborators, and pro-actively shared project findings with American Samoa resource agencies and educational institutions. We have contributed our research findings to the American Samoa EPA ridge-to-reef efforts, National Marine Sanctuary of American Samoa condition report and shared project results with the NOAA Ecosystem Sciences Division and USGS to assist with their analyses on water quality and coral reef condition in American Samoa. In addition, Co-PI Professor Andrew Hoey presented a seminar on “Rats, Seabirds, and the Productivity of Coral Reefs”, research relevant to resource agencies and ASCC, reinforcing our commitment to facilitating research exchange between ARC Centre of Excellence for Coral Reef Studies (CoE) and American Samoa government agencies and the ASCC. In addition, we have maintained communications and subsequent reviews in the coordination of the final report despite delays brought about by the covid-19 pandemic. We continued to work closely with our project collaborators to ensure that results and outputs are appropriately analyzed, summarized, and reviewed by relevant partner agencies and stakeholders. We presented our project findings in two virtual formats to American Samoa project partners, first to the American Samoa Community College where the presentation was attended by students and staff from the Marine Science and Division of Agriculture, Community, and Natural Resources; and, to local and federal partner agencies at a science brown bag session hosted by the Coral Reef Advisory Group.

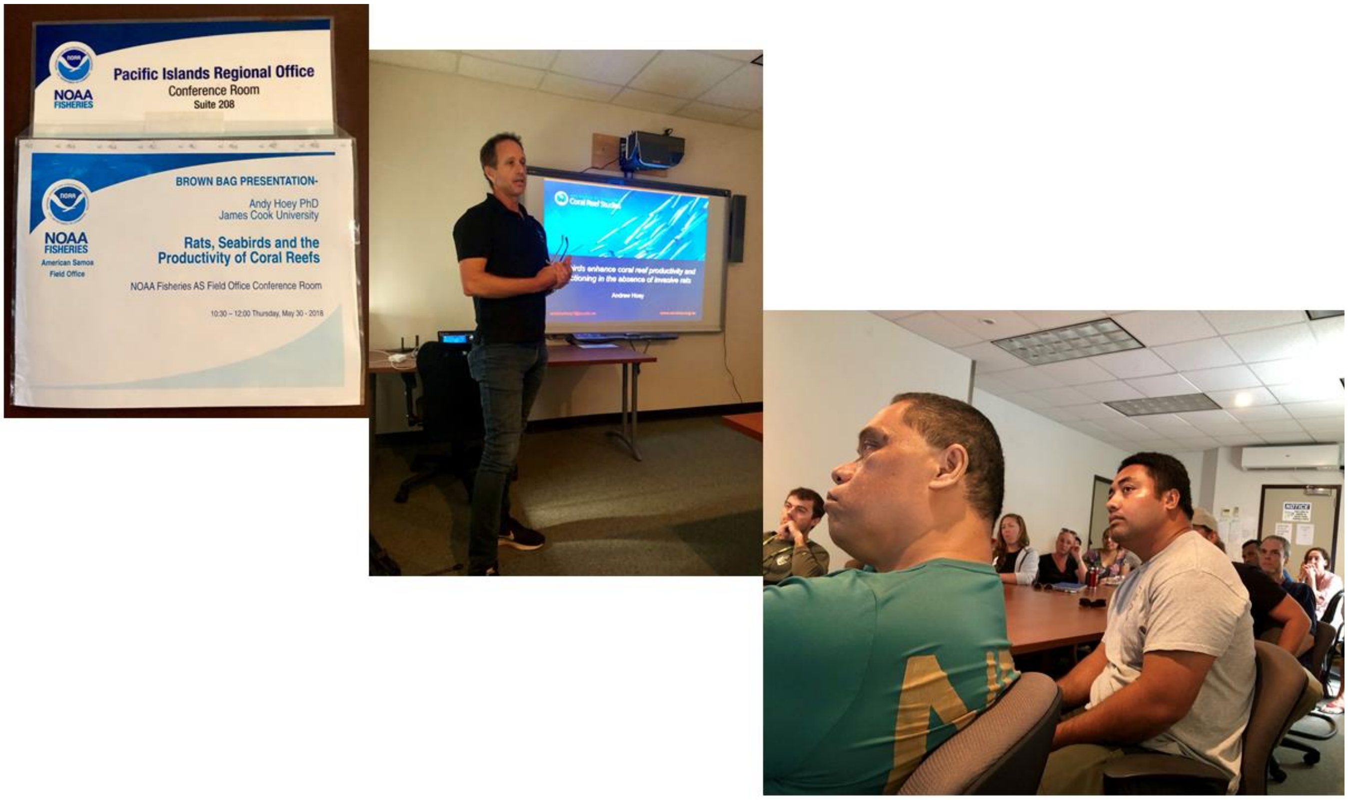


Figure 35. Professor Andrew Hoey giving a seminar to American Samoa local and federal government agencies in May 2019.

Training

We co-developed a water quality sample processing protocol with guidance from water quality experts from JCU TropWATER for the ASCC Marine Science research program (Figure 36). The laboratory protocol was modified as appropriate with feedback from marine science students, ensuring that the process was informed by on-the ground experiences and available resources. The iterative processes of updating protocols with learned experiences is necessary in our project's focus on building an inclusive research agenda.

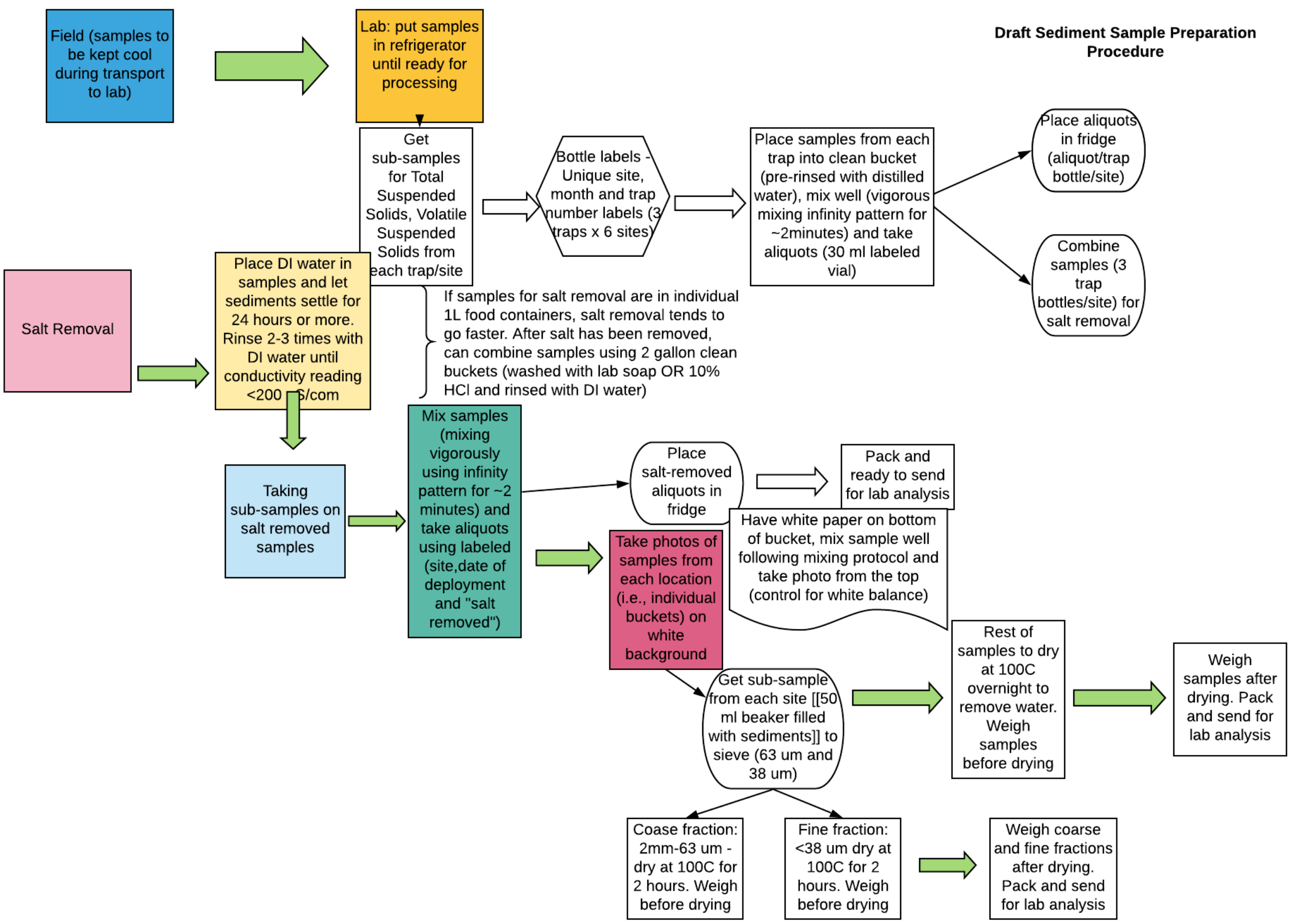
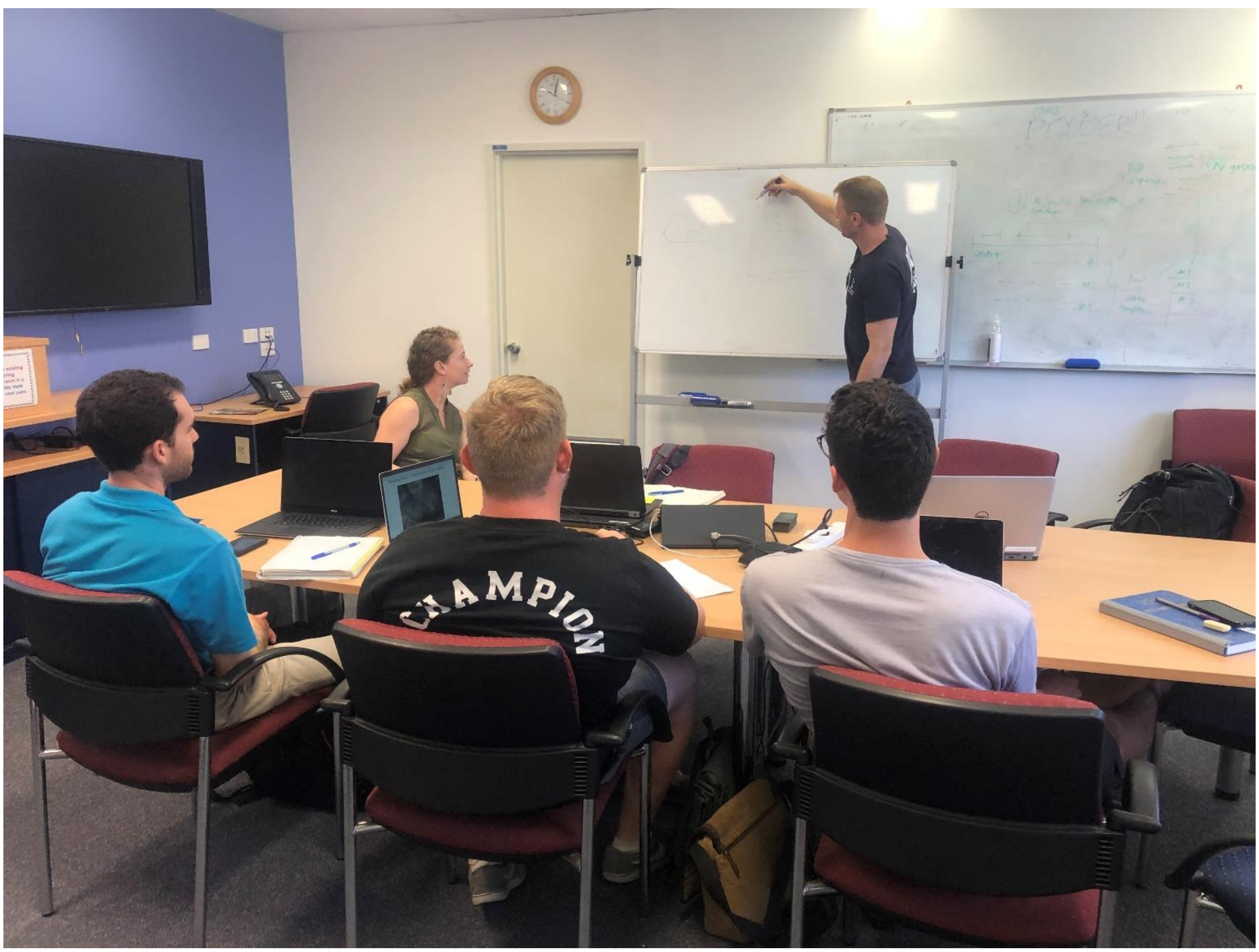
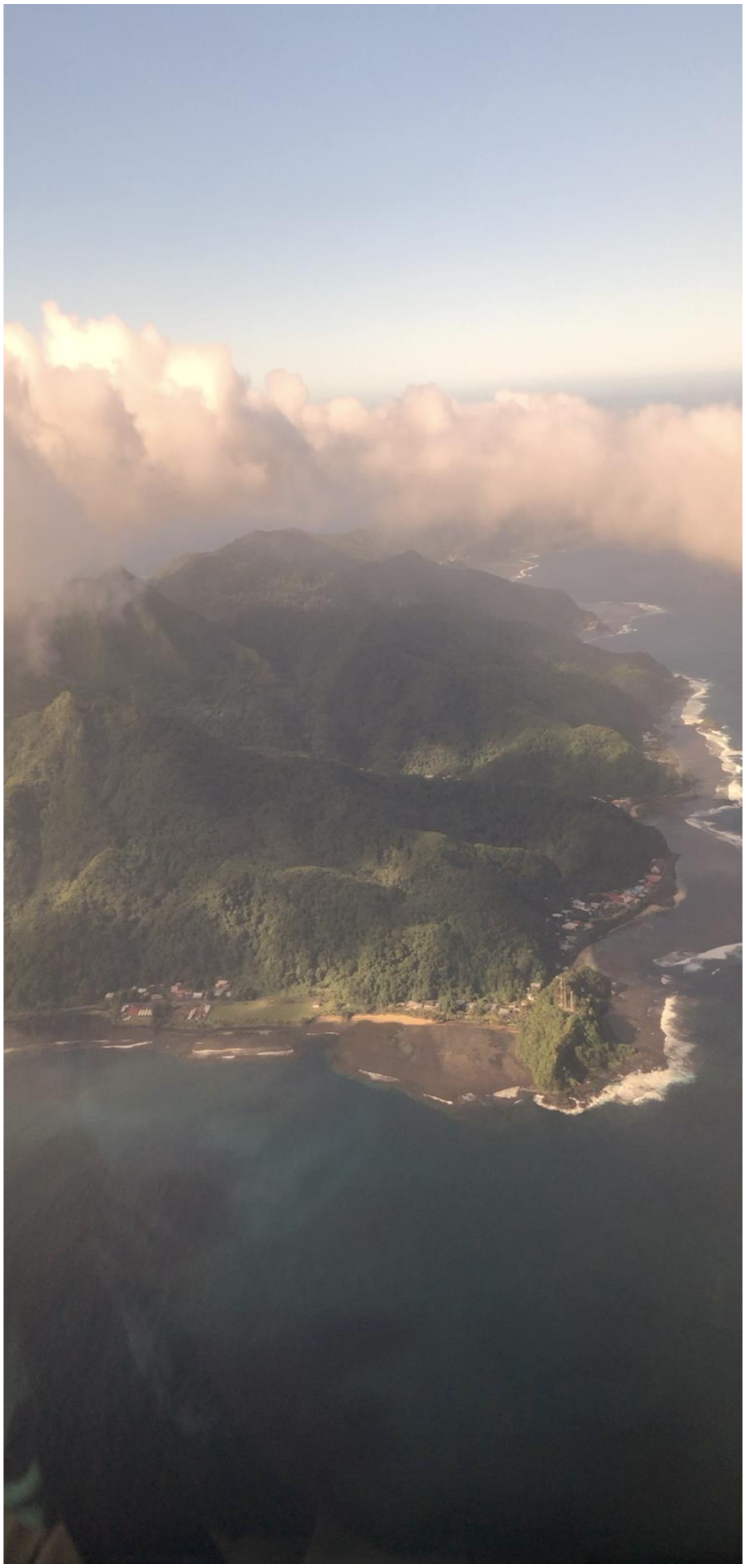


Figure 36. Flow chart of sediment sample processing (details in Methods section of Task 1).

This project also furthered training opportunities for PI Mia Comeros with training on otolith processing provided by NOAA PIRO Fish Biologist, Michael Marsik, and Dr. Jake Lowe from JCU. We also expanded training opportunities for the ARC CoE through a week-long workshop the life history and demography of herbivorous reef fishes led by Dr. Brett Taylor of the Australian Institute of Marine Science in October 2019. The workshop was critical to achieving outcomes for Activity 4: *Evaluating the effects of a pollution gradient to recruitment and population demographic parameters (age, mortality, growth) of commonly-occurring parrotfish and surgeonfish species in American Samoa*. Scientific training included the interpretation of daily and annual otolith rings of parrotfish and surgeonfishes as well as of other herbivorous fish otoliths, protocol validation and methods discussion in the analysis of life history and demographic information.



Significance and contributions



- *Project established mechanistic links between human activities, terrestrial run-off, and nearshore benthic assemblages, and the ecology of herbivorous reef fishes*
- *Evaluated reef health metrics, benthic cover and herbivore fish recruitment, critical ecological services, important to understanding the capacity of reefs to continue to deliver nutritional benefits from fish to the people of American Samoa*
- *The complexity of the genesis, transport, and fates of nutrients and sediments warrants extraordinary cautions given the complex effects of nutrients on corals and the sensitivity of corals' to increased sedimentation rates*
- *Findings directly apply to American Samoa's strategic coral reef management priorities to improve scientific understanding of the links between land-based sources of pollution and coral reef health through focused scientific research and monitoring*
- *Provided first-hand research experience, focused training, and skills advancement for ASCC Marine Science students*
- *Fostered and promoted strong inter-agency cooperation and collaboration contributing positively to reciprocal relationships and resource sustainability*

Conclusions:

Understanding the inseparable connection between land-sea is important to the provisioning of important fisheries resources, and the protection of nearshore reef environments; ecosystem services vital to the people of American Samoa. We developed a framework that examined the processes influencing the genesis, transport and, fate of nutrients and sediments, and linked terrestrial run-off to herbivorous reef fish ecological processes. The unexpected enhanced abundance of recruit and adult parrotfishes in a highly disturbed reef system highlight the complexity of the consequences of anthropogenically-generated nutrients into shallow reef systems. Thus, comprehensive and integrated sampling programs such as the framework of this project, form a foundation for investigations spanning the land-sea nexus. Our approach provides a pathway for critically examining the impacts of nutient enrichment and increased sedimentation on coral reef health through a transparent and robust scientific process. Our project, conducted in close and active collaboration with multiple agencies, resulted in an inclusive and cooperative research platform. The project's ridge-to-reef approach has resulted in models and datasets that directly align with strategic coral reef and watershed-based management priorities in the Territory. Our focus on student engagement, training, and skill-building adds value to the American Samoa Community College by providing hands-on experience to students on research that directly addresses locally important issues. Our project leverages existing watershed and coral reef management initiatives and expertise in American Samoa and contributes to a strong scientific baseline in the Territory, and increased representation of coral reef science in volcanic high lsiands in the South Pacific.

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Appendix

Table 1. Site attributes including watershed area, percent land use, number of On-Site Disposal System units and pigs.

		Natural Land Use		Intensive Land Use				
Site	**Total Watershed Area (sqm)**	**% Forest**	**% Agro-forest**	**% Cultivated**	**% Develop Land**	**% All other land use (barren land, shrub/scrub land, grass/herbaceous land)**	**Total number of Onsite Disposal System units (cesspools, septic tanks)**	**No. of pigs**
Fagatele	1,917,759	58%	10%	11%	5%	15%	0	0
Tafeu	1,669,015	90%	0%	0%	0%	10%	0	0
Fagasa	3,483,148	78%	5%	5%	7%	6%	117	118
Vatia	3,574,377	79%	4%	3%	5%	9%	100	152
Nu'uuli	17,170,233	52%	5%	8%	28%	7%	65	221
Fagaalu	2,476,211	71%	4%	2%	13%	11%	124	132

Table 2. Path analysis model outputs. We used the default estimator in the lavaan package, maximum likelihood. Standard error (SE) is based on the expected information matrix, and the z-value represents the ratio of the parameter to the standard error. P-value <0.05 represents significant parameters. Path coefficients are standardized versions of linear regression weights. R-squared values reflect the proportion of variance in the dependent variables accounted for by the equation.

	Estimate	Std.Err	z-value	P(>|z|)	Std.all	R^2
Macroalgae Reef Flat ~						40%
Wave	-0.01	0.00	-3.49	0.00	-0.42	
Reef flat DIN ~						73%
Stream DIN	0.15	0.06	2.58	0.01	0.19	
Intensive land use	0.73	0.32	2.30	0.02	0.18	
Reef flat PO_4	1.01	0.18	5.53	0.00	0.66	
Reef flat PO_4~						61%
Stream PO_4	0.55	0.14	3.90	0.00	0.44	
Intensive land use	0.44	0.26	1.71	0.09	0.17	
Reef Flat DIN	0.17	0.12	1.40	0.16	0.27	
Macroalgae Reef Flat ~						40%
Stream DIN	-0.04	0.03	-1.25	0.21	-0.14	
Reef flat PO_4	0.08	0.05	1.56	0.12	0.17	
Runoff	-0.10	0.04	-2.54	0.01	-0.28	
Intensive land use	0.29	0.15	1.90	0.06	0.22	
Turf reef Flat ~						43%
Stream DIN	0.05	0.02	1.99	0.05	0.23	
Stream PO_4	-0.16	0.06	-2.60	0.01	-0.31	
Reef Flat DIN	-0.06	0.05	-1.16	0.25	-0.21	
Reef flat PO_4	-0.11	0.08	-1.36	0.17	-0.26	

Intensive land use	-0.07	0.12	-0.55	0.58	-0.06	
Wave energy	0.00	0.00	0.46	0.64	0.05	
Runoff	0.02	0.03	0.58	0.56	0.06	
Reef Flat DIN ~						73%
Stream DIN	0.15	0.06	2.58	0.01	0.19	
Intensive land use	0.73	0.32	2.30	0.02	0.18	
Reef flat PO_4	1.01	0.18	5.53	0.00	0.66	
Reef flat PO_4~						61%
Stream PO_4	0.55	0.14	3.90	0.00	0.44	
Intensive land use	0.44	0.26	1.71	0.09	0.17	
Reef flat DIN	0.17	0.12	1.40	0.16	0.27	
Hard coral reef flat ~						20%
Stream DIN	-0.04	0.03	-1.49	0.14	-0.21	
Stream PO_4	-0.07	0.06	-1.07	0.28	-0.16	
Reef flat DIN	-0.02	0.05	-0.43	0.67	-0.09	
Reef flat PO_4	0.12	0.08	1.53	0.13	0.35	
Wave energy	0.00	0.00	-1.59	0.11	-0.29	
Runoff	0.07	0.03	2.12	0.03	0.28	
Intensive land use	-0.19	0.13	-1.52	0.13	-0.21	
DIN Load (OSDS + pigs) kg/day	0.06	0.03	2.10	0.04	0.32	
CCA Reef Flat ~						23%
Stream DIN	0.03	0.02	1.15	0.25	0.16	
Stream PO_4	-0.04	0.06	-0.69	0.49	-0.10	
Reef flat DIN	0.01	0.05	0.17	0.87	0.04	
Reef flat PO_4	0.02	0.08	0.32	0.75	0.07	
Wave energy	0.00	0.00	-0.49	0.63	-0.09	
Runoff	0.08	0.03	2.48	0.01	0.32	

Intensive land use	-0.20	0.12	-1.68	0.09	-0.23	
DIN Load (OSDS + pigs) kg/day	0.05	0.03	1.96	0.05	0.29	

Table 3. Linear mixed effects models of square root transformed parrotfish and surgeonfish recruits across watershed type.

	Model 1: All herbivores			**Model 2:** By family			**Model 3:** *Chlorurus spilurus*			**Model 4:** *Ctenochaetus striatus*		
	recruits.sqrt			**family.sqrt**			**spec.sqrt**			**spec.sqrt**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	2.33	0.22 – 4.44	**0.031**	1.13	-0.13 – 2.39	0.080	0.19	-1.55 – 1.92	0.832	0.38	-0.24 – 1.00	0.233
Watershed.Type [Intermediate]	2.04	-0.95 – 5.03	0.181	1.51	-0.22 – 3.23	0.087	1.75	-0.71 – 4.21	0.163	0.54	-0.34 – 1.43	0.226
Watershed.Type [Disturbed]	3.56	0.58 – 6.55	**0.019**	2.30	0.57 – 4.02	**0.009**	3.45	1.00 – 5.91	**0.006**	0.87	-0.01 – 1.75	0.052
Family [Scarine]				0.36	-0.29 – 1.01	0.273						
Random Effects												
σ^2	1.4058			2.6251			0.8082			0.6963		
τ_{00}	2.1492 Watershed.Type:Location			0.6109 Watershed.Type:Location			1.4703 Watershed.Type:Location			0.1152 Watershed.Type:Location		
ICC	0.6046			0.1888			0.6453			0.1420		
N	3 Watershed.Type			3 Watershed.Type			3 Watershed.Type			3 Watershed.Type		
	2 Location			2 Location			2 Location			2 Location		
Observations	48			96			48			48		
Marginal R^2 / Conditional R^2	0.380 / 0.755			0.227 / 0.373			0.471 / 0.812			0.140 / 0.262		

Table 4. Linear mixed effects models of square root transformed *C. spilurus* bite rates (bites per minute) across watershed type and feeding substratum.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	2.73	0.56 – 4.89	**0.014**
Watershed_Type[Disturbed]	-0.26	-1.09 – 0.56	0.530
Feeding_Substratum2 [Cyanobacteria]	-2.28	-5.83 – 1.26	0.206
Feeding_Substratum2 [Deadcoral]	-0.39	-2.52 – 1.74	0.720
Feeding_Substratum2 [Feces]	-1.31	-4.89 – 2.27	0.474
Feeding_Substratum2 [HardCoral]	-1.39	-3.54 – 0.75	0.203
Feeding_Substratum2 [Macroalgae]	0.41	-1.70 – 2.51	0.703
Feeding_Substratum2 [Pavement]	0.97	-1.19 – 3.14	0.379
Feeding_Substratum2 [Rubble]	1.67	-0.43 – 3.76	0.118
Feeding_Substratum2 [Sand]	-0.67	-3.60 – 2.26	0.656
Feeding_Substratum2 [Turf]	1.34	-0.84 – 3.51	0.228

Feeding_Substratum2 [Water column]	-1.42	-4.35 – 1.50	0.341
Random Effects			
σ^2	2.18		
τ_{00} Site	0.14		
ICC	0.06		
N Site	4		
Observations	299		
Marginal R^2 / Conditional R^2	0.298 / 0.339		

Table 5. Linear mixed effects models of square root transformed *C. striatus* bite rates (bites per minute) across watershed and substratum type.

Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.65	-2.67 – 3.97	0.700
Watershed_Type[Disturbed]	-0.15	-0.70 – 0.39	0.579
Feeding_Substratum2 [CCA]	2.18	-1.17 – 5.54	0.202
Feeding_Substratum2 [Deadcoral]	0.97	-2.69 – 4.64	0.603
Feeding_Substratum2 [HardCoral]	1.23	-2.19 – 4.64	0.481
Feeding_Substratum2 [Macroalgae]	1.19	-2.17 – 4.56	0.487
Feeding_Substratum2 [Pavement]	3.36	-0.12 – 6.83	0.058
Feeding_Substratum2 [Rubble]	3.52	0.15 – 6.88	**0.041**
Feeding_Substratum2 [Sand]	0.67	-2.81 – 4.16	0.704
Feeding_Substratum2 [Turf]	2.50	-0.84 – 5.84	0.142
σ^2	2.72		

τ_{00} Individual:Length	0.21
ICC	0.07
N Individual	34
N Length	15
Observations	334
Marginal R^2 / Conditional R^2	0.242 / 0.297