Rapid Report Markdown

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NSF Rapid Report. Markdown of major findings.

1 Black Sea Urchin Abundance Modeling

1.1 Assessing urchin abundance pre/post die-off

Our study determined that the number and size of *D. antillarum* clusters across our study site significantly declined after the die-off, with questionable signs of recovery. The number of clusters between the baseline period (~9 months before die-off) and the periods during and after the die-off dropped considerably (Table 1, Figure 1). There was an overall relative difference of -98.8% (± 0.206) between the baseline and post-die-off cluster counts at Maguey after 6 months, and -95.8% (± 0.370) after 12 months. Similarly, at Tampico, the relative differences were -98.9% (± 0.147) after 6 months and -91.9% (± 0.374) after 12 months. The difference in cluster counts of the post-die-off periods was not significant (Table 2, Figure 1.1), showing no evidence of a recovery of the *D. antillarum* population in Culebra reefs one year after this mortality event. Still, our model predicts an increase in counts 6 months after the period with the lowest counts (i.e., comparison between 6 month and 12-month periods), indicating a potential for slow recovery.

Table 1: ANOVA of GLMM Results for our negative binomial model with a log ling used for urchin cluster counts (Counts ~ Period + (1|Site)). Multiple models were tested with additional factors but the most parsimonious model included only period as a term.

Term	Chisq	df	p-value
Period	102.894	3.000	0.000

Pairwise differences within model show a significant differences between Baseline and all other periods.

Table 2: Pairwise comparisons of GLMM model (Counts ~ Period + (1|Site)).

Periods	estimate	SE	z ratio	p-value
Baseline vs. Die-Off	3.1781	0.4521	7.0293	0.0000
Baseline vs. After 4 Months	4.4789	0.6721	6.6642	0.0000
Baseline vs. After 12 Months	2.8114	0.4036	6.9653	0.0000
Die-Off vs. After 4 Months	1.3008	0.7439	1.7487	0.2985

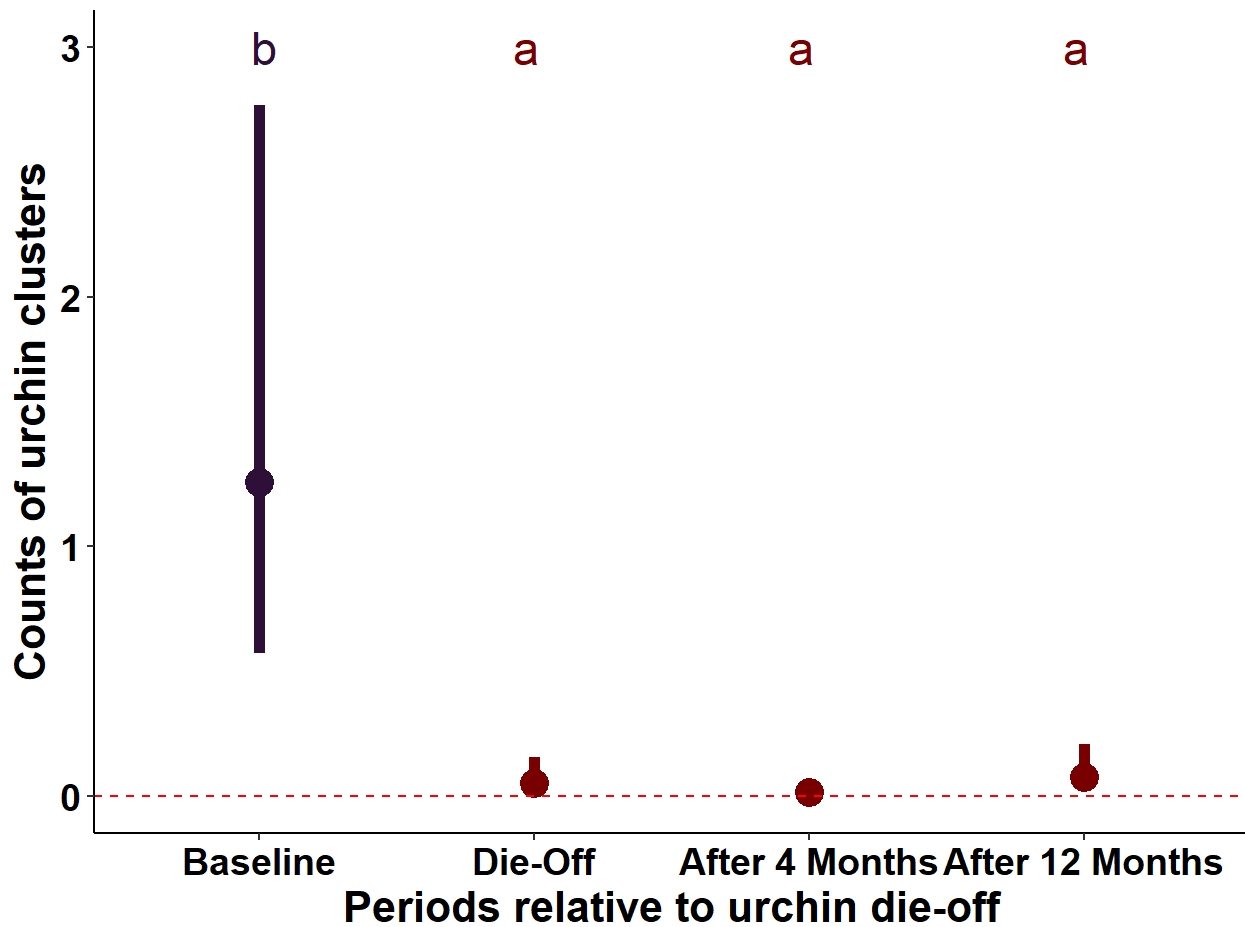| Periods	estimate	SE	z ratio	p-value
Die-Off vs. After 12 Months	−0.3667	0.5291	−0.6930	0.8998
After 4 Months vs. After 12 Months	−1.6675	0.7245	−2.3017	0.0976

Figure 1: Predicted counts of urchin clusters from GLMM model. Letters portray significance. A sharp drop is observed between baseline and all other periods. However, a slight increase is observed between the 4 month and 12 month periods, indicating at the possibility of recovery.

1.2 Evaluating Urchin Cluster sizes

Cluster sizes of *D. antillarum* across our study site decreased significantly following the die-off event. Due to the random distribution of clusters throughout our study sites, we focused only on cluster size when clusters were present and therefore only considered period as a predictor variable in our model.

Pairwise comparisons reveal a significant reduction (p < 0.05) in the size of urchin clusters between the baseline, the die-off 12 month periods after the die-off. Cluster size was not significantly different (p = 0.0755) between the baseline and 4 month period, but we suspect this is likely an effect of the analysis approach and not a biological trend since a further reduction is observed at the 12 month mark.

Table 3: GLMM results for urchin cluster size. We used a gaussian model with log link: Cluster size ~ Period + (1|Site)

Term	Chisq	df	p-value
Period	37.139	3.000	0.000

Table 4: Pairwise comparison of our gaussian model for Cluster Size

| Periods	estimate	SE	df	Z ratio	p-value
Baseline vs. Die-Off	0.3490	0.1005	52.0000	3.4730	0.0056
Baseline vs. After 4 Months	0.4955	0.2000	52.0000	2.4771	0.0755
Baseline vs. After 12 Months	0.3742	0.0751	52.0000	4.9816	0.0000
Die-Off vs. After 4 Months	0.1465	0.2176	52.0000	0.6731	0.9068
Die-Off vs. After 12 Months	0.0251	0.1195	52.0000	0.2103	0.9967
After 4 Months vs. After 12 Months	−0.1214	0.2065	52.0000	−0.5876	0.9354
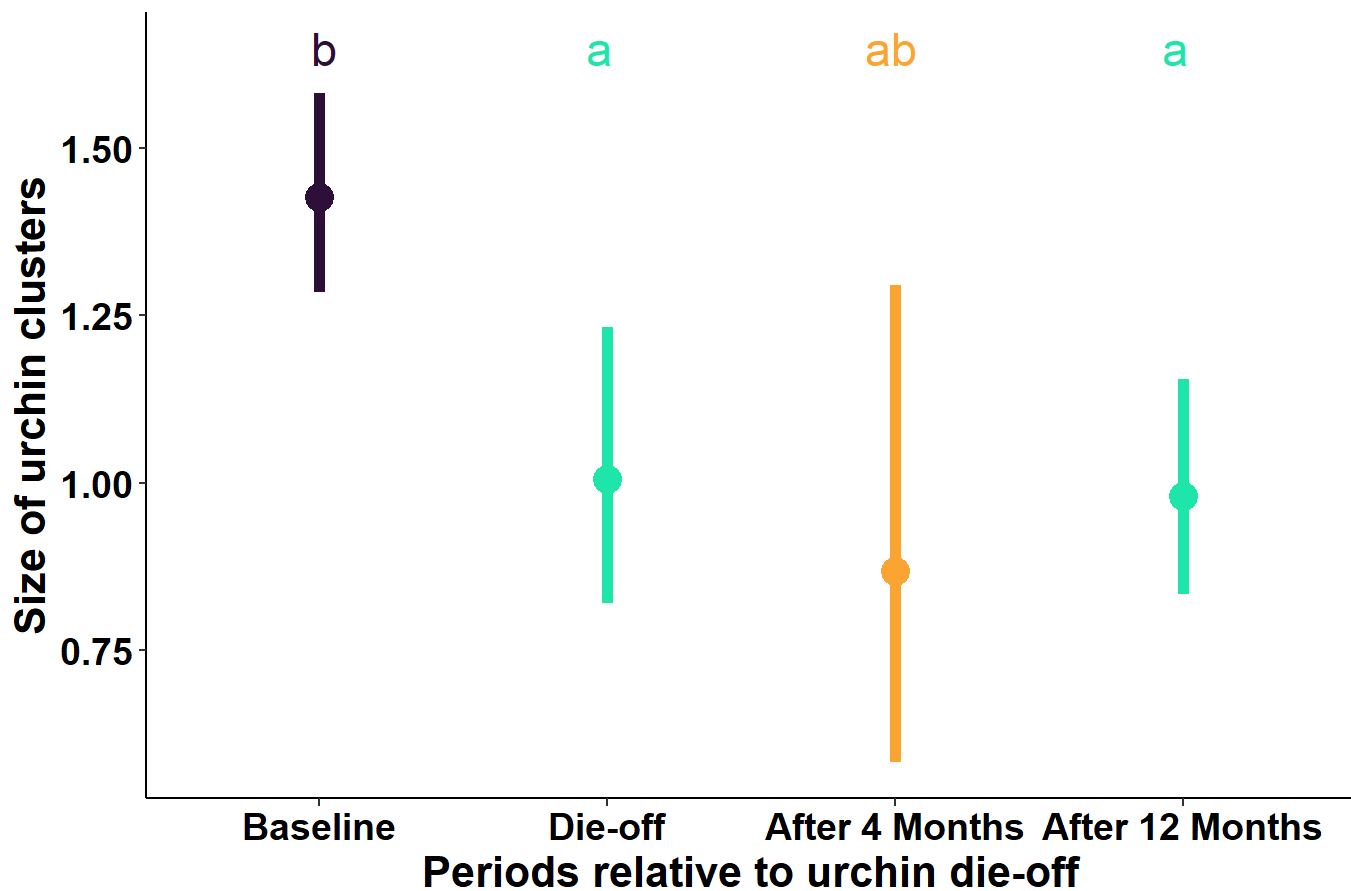
Figure 2. Predicted cluster size differenes from our gaussian model.

2 Assessing the role of black sea urchin structuring benthic communities

2.1 A multivariate approach

In order to address objective 2, we used a multivariate statistical approach which consisted of Non-metric Multidimensional Scaling (NMDS) to visualize changes in community structure across periods and locations following the *D. antillarum* die-off event. Figure 3A shows the overall community structure across different time periods. Some overlap in points across periods is visible but a general trend is observed where baseline samples appear more dispersed while later periods form tighter clusters suggesting homogenization in community structure over time after the die-off event. When comparing locations, Maguey and Tampico show distinct community structures, with Tampico having a more dispersed community structure in the baseline period compared to Maguey Figure 3B and 3C show the NMDS plots for each location separately.

In Maguey, all periods overlap, suggesting that temporal changes in community structure were less distinct. Tampico shows clearer separation among periods, specifically between the baseline and post-die-off periods indicating a more pronounced shift in community structure over time.

Figure 3: NMDS of community data. Points represent community structure across periods and locations. Overlapping points suggest similar community structure, while distinct clusters indicate differences.

We preformed a PERMANOVA to assess the observed differences in our NMDS and changes in community structure across location and period. Community structure was evaluated at the functional group level with 5 distinct categories: Corals, Macroalgae, Sponges, Gorgonians and Seagreass. We found that the community structure across sites in Culebra was significantly influenced by both location and period, with a notable interaction between these factors ($p < 0.05$). The effect of location on community structure was substantial, with communities showing clear spatial differences (Table 6, Figure 3). Specifically, location accounted for 15.44% of the variation in community structure (F = 73.11, p = 0.001), suggesting that

distinct environmental or ecological conditions (such as the *D. antillarum* mortality event) across locations significantly shape the observed community patterns.

Period also had a significant impact, explaining 8.01% of the variance (F = 12.64, p = 0.001), which indicates that community structure changed meaningfully over time. There is also a significant interaction between location and period (1.36% of variance; F = 2.15, p = 0.002), suggesting that the temporal changes in community structure were somewhat location-dependent.

Table 5: PERMANOVA results of community structure in relation to Location (Tampico & Maguey) and Periods.

Term	Df	Sum of Squares	R-squared	F-value	p-value
Location	1	3.6996	0.1544	73.1126	0.0010
Period	3	1.9180	0.0801	12.6351	0.0010
Location:Period	3	0.3262	0.0136	2.1490	0.0030
Residual	356	18.0139	0.7519		
Total	363	23.9578	1.0000		

Pairwise PERMANOVA comparisons further explained the patterns between locations and periods. Temporal comparisons revealed a significant difference in community structure between the baseline and subsequent periods. The observed die-off caused a major shift in community structure with no signs of recovery to date. There was no significant difference between the 4-month and 12-month post-die-off periods (p = 0.219), suggesting that community structure may have stabilized rather than showing signs of recovery.

Table 6: Combined pairwise PERMANOVA results for Location and Period

			Statistics			
Group	Terms	Degrees of Freedom	Sum of Squares	R^2	F-Statistic	P-Value
Location	Maguey vs Tampico	1	3.700	0.154	66.109	0.001
	Residual	362	20.258	0.846		
	Total	363	23.958	1.000		
Period	Baseline vs Die-off	1	1.310	0.107	21.260	0.001
	Residual	178	10.964	0.893		
	Total	179	12.274	1.000		

Group	Terms	Degrees of Freedom	Statistics			
			Sum of Squares	R²	F-Statistic	P-Value
Period	Baseline vs After 6mo	1	1.160	0.085	16.444	0.001
	Residual	177	12.482	0.915		
	Total	178	13.642	1.000		
Period	Baseline vs After 12mo	1	0.832	0.062	12.063	0.001
	Residual	183	12.620	0.938		
	Total	184	13.452	1.000		
Period	Die-off vs After 6mo	1	0.098	0.010	1.831	0.136
	Residual	177	9.494	0.990		
	Total	178	9.592	1.000		
Period	Die-off vs After 12mo	1	0.220	0.022	4.174	0.022
	Residual	183	9.632	0.978		
	Total	184	9.851	1.000		
Period	After 6mo vs After 12mo	1	0.086	0.008	1.401	0.219
	Residual	182	11.149	0.992		
	Total	183	11.235	1.000		

2.2 Univariate analysis of benthic functional group responses

2.3 Functional Group Responses

To assess the drivers of the observed community structures across periods in our multivariate analysis we investigated how distinct functional groups changed across periods. Our results suggest unique taxonomic responses to the *D. antillarum* die-off. The **coral** cover of the reef communities was significantly higher (Wilcoxon rank-sum test, $p < 0.05$) in Tampico than in Maguey with mean values of 1.83% (± 1.00%) in Tampico and 0.90% ± (0.49%) in Maguey (Table X, Figure 4A). The percent cover of **macroalgae** was significantly higher in Tampico than in Maguey (Wilcoxon rank-sum test, $p < 0.05$), with mean values of

30.80% (± 13.28%) in Tampico and 22.36% (± 13.77%) in Maguey (Table X, Figure 4B). The percent cover of **sponges** was significantly higher in Tampico than in Maguey (5.52% ± 6.63% VS 4.11% ± 2.90%, Wilcoxon rank-sum test, $p < 0.05$) (Figure 4C).

Across both locations coral and macroalgae cover varied similarly across the periods (Figure 4A,B, Appendix Table X). In both locations, the percent cover increased by an average of 22.92% and 10.78% for corals and macroalgae, respectively, between the baseline period and 12-month post die-off periods; however, the differences in coral cover may be influenced by sampling bias, as the point count method used to calculate percent cover may underestimate true values when cover is low (Rivas et al., in prep). Sponge abundance appears to be the greatest driver of the observed community structure shifts. Sponges experienced a sharp decrease after the die-off, with a 61% reduction in cover between the baseline and die-off periods. This reduction was followed by a slight increase in sponge cover between the die-off and 4-month post die-off periods, suggesting a potential recovery trend. However, similarly to corals, sponge recovery may be over/under-estimated due to the point count methodology used.
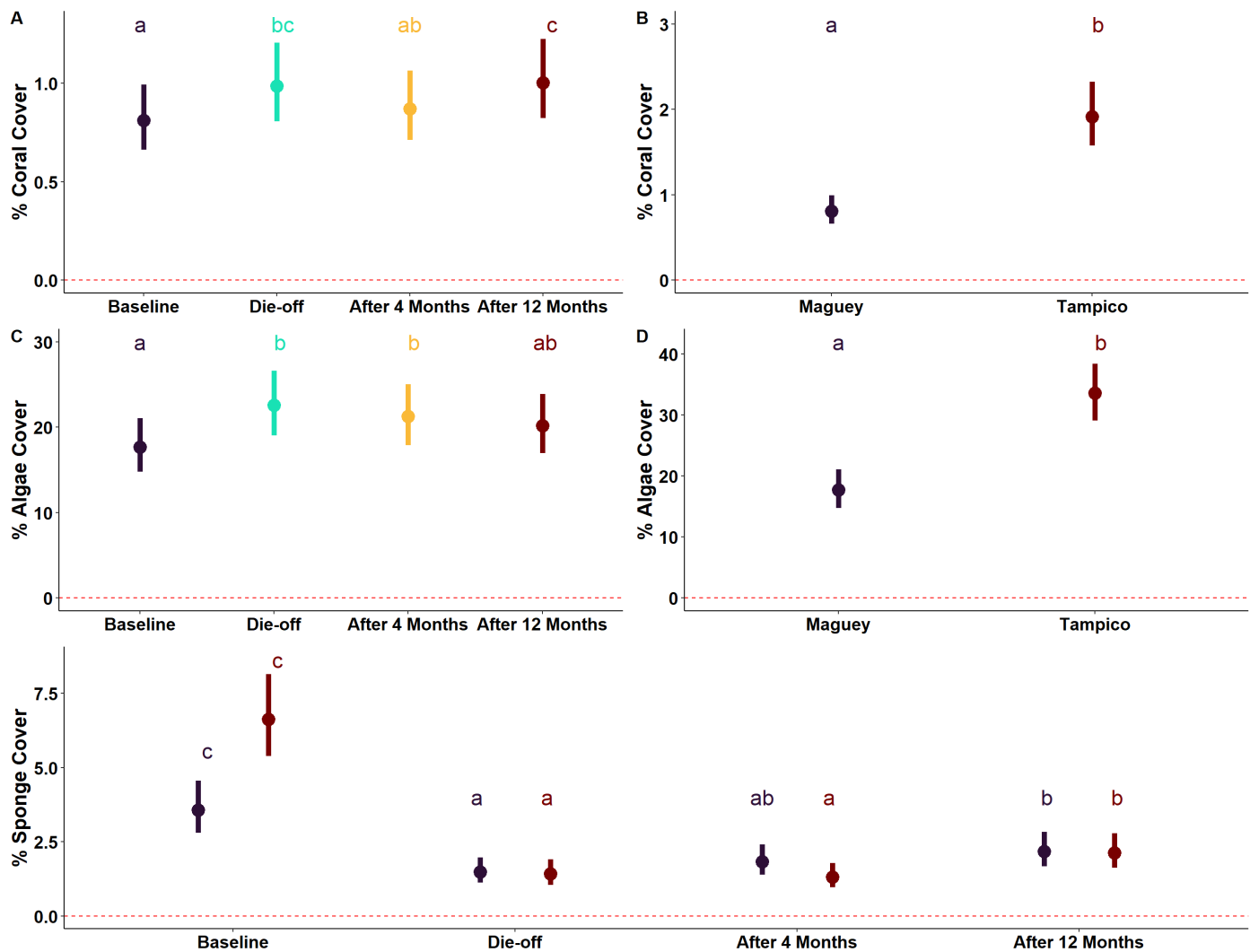
Figure 4. A) Estimated mean coral cover values (± standard error) for four time points: baseline, after 6 months, after 12 months, and following the die-off event. Error bars represent 95% confidence intervals for each estimate. Groups with different letters (a, ab, bc, c) indicate significant differences based on pairwise comparisons. B) Estimated mean cover values (± standard error) for two locations: Maguey and Tampico. Error bars represent 95% confidence intervals for each estimate. Significant differences were observed between the two locations, with Maguey (group a) having a lower mean cover compared to Tampico (group b). C) Estimated mean algae cover values (± standard error) across four time periods. Significant differences are indicated by group letters (a, ab, b), with baseline showing the lowest mean cover (group a) and die-off showing the highest (group b). D) Estimated mean algae cover values (± standard error) for two locations: Maguey and Tampico. Error bars represent 95% confidence intervals for each estimate. Significant differences were observed between locations, with Maguey (group a) having a lower mean cover compared to Tampico (group b). E) Estimated mean sponge cover values (± standard error) for two locations (Maguey and Tampico) across four time periods: baseline, after 6 months, after 12 months, and following the die-off event. Error bars represent 95% confidence intervals for each estimate. Significant differences are denoted by group letters (a, ab, b, c). Baseline values for both locations (group c) were higher compared to subsequent periods. After 6 months and following the die-off, Maguey and Tampico exhibited lower mean cover (groups a and ab), while after 12 months, both locations showed intermediate values (group b).

2.4 Correlation analysis

To further understand how algae influences community structure we performed a series of correlation tests between different functional groups (Sponge, Coral, Algae, and Gorgonian) that revealed distinct relationships within each location, Maguey and Tampico.

In Maguey, a weak relationship between algae and coral cover was observed ($r = 0.13$, $p = 0.066$). We once again attribute this to a sampling effect due to the critically low cover of coral already present in Maguey. Algae and sponge cover showed a small negative correlation ($r = -0.23$, $p < 0.01$), suggesting an inverse relationship where increases in algae cover might correspond to decreases in sponge cover.

In Tampico, the relationship between algae and coral cover is moderate (r = 0.26) and highly significant (p < 0.001), suggesting that increases in algae cover are moderately associated with increases in coral cover. As mentioned above, this correlation should be taken lightly. The algae and sponge relationship at this location is negative (r = -0.35, p < 0.001), showing a stronger inverse relationship compared to Maguey. This suggests that increases in algae cover are associated with decreases in sponge cover. The relationship between algae and gorgonian cover is moderate (r = 0.22, p = 0.003), suggesting that the two groups can impact one another.

Finally, we also assessed the relationship between algae cover and *Diadema* across locations. A negative and significant correlation was observed in Tampico for *D. antillarum* counts and mean cluster sizes (r = -0.29 & r = -0.27 p < 0.01), suggesting that increases in algae cover are associated with decreases in *Diadema* cover. In Maguey, the relationship was also negative but not statistically significant (p > 0.05).
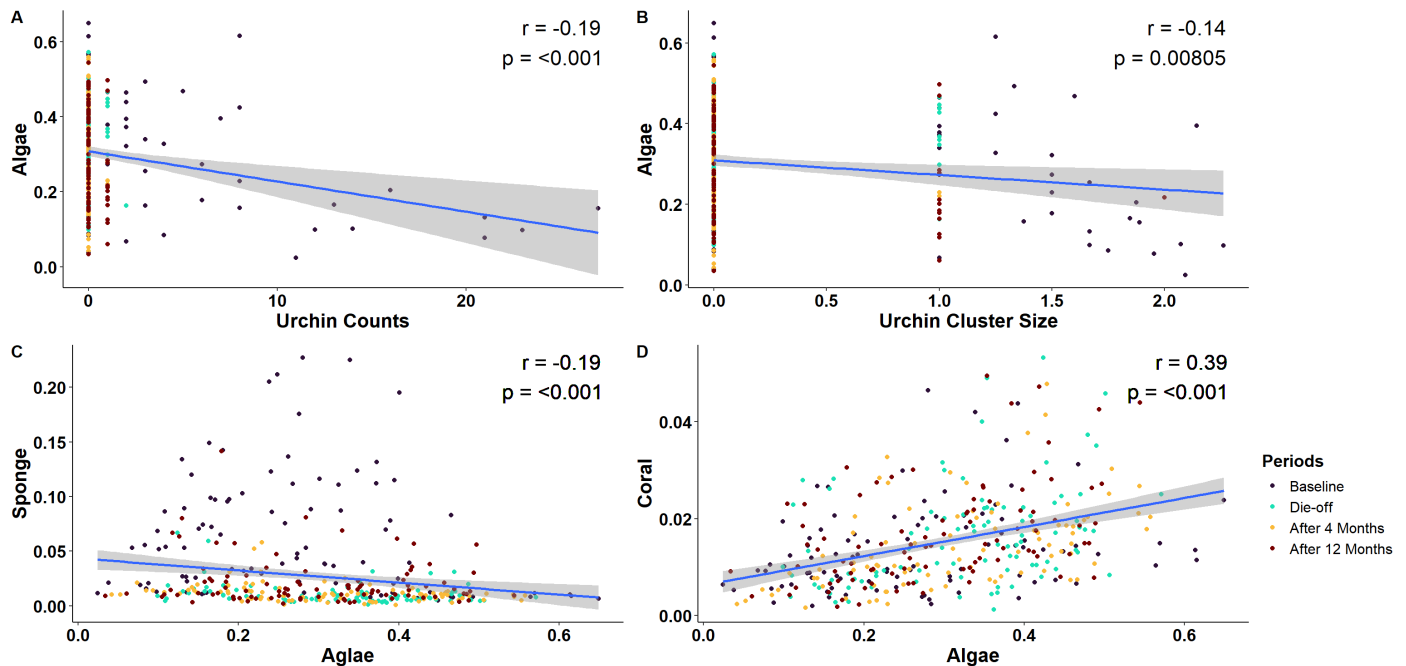

Figure 5. A) Correlation between algae and urchin counts, showing a negative relationship with a correlation coefficient of -0.29. The relationship is statistically significant (p-value < 0.05), indicating an inverse association between algae and urchin counts.
B) Correlation between algae and urchin cluster size, showing a negative relationship with a correlation coefficient of -0.27.
The relationship is statistically significant (p-value < 0.05), indicating an inverse association between algae and urchin cluster size.
C) Correlation between algae and sponge cover, showing a negative relationship with a correlation coefficient of -0.19.
The relationship is statistically significant (p-value = 0.0003), indicating a weak but meaningful inverse association between algae and sponge cover in the analyzed dataset.
D) Correlation between algae and coral cover, showing a positive relationship with a correlation coefficient of 0.39.
The relationship is statistically significant (p-value < 0.05), indicating an association between the two variables.
However, due to the incredibly low percentage of coral cover in Maguey, this relationship should be interpreted with caution.

3 The role of black sea urchins on demographic performance of coral

To evaluate the impact of *D. antillarum* decline and macroalgal increase on coral demographic performance, we analyzed the vital rates of *Porites astreoides* and *Diploria labyrinthiformis*, representing weedy and stress-tolerant functional groups, respectively. Leslie matrices were constructed for two intervals: baseline-to-die-off and die-off-to-12 months post-event.

Our results show that the vital rates of *P. astreoides* declined significantly after the die-off, with a λ (population growth rate) decreasing from 1.1 before the event to 0.988 after, indicating a potential population collapse under sustained macroalgal disturbance. Similarly, D. labyrinthiformis exhibited lower growth rates following the die-off (decrease in λ from 0.908 to 0.855), suggesting a rapid population decline.

We projected population trajectories for both species over 100 years. In a stochastic scenario where the disturbance matrix (the matrix calculated for after the die-off period) was applied 10% of the time, P. astreoides maintained resilience, with λ only slightly reduced (1.1 to 1.08). However, this model assumes a single disturbance type and does not account for additional stressors or rapid macroalgal proliferation. Under a frequent disturbance scenario (90% disturbance), *P. astreoides'* λ dropped below 1, signaling inevitable population collapse.

The sensitivity matrix shows how changes in transition probabilities (e.g., survival or reproduction) affect population growth rate (λ). Adult-to-Juvenile transitions have the highest sensitivity (2.7176), emphasizing the critical role of adult contributions to juvenile recruitment. The elasticity matrix highlights the proportional contribution of each transition to population λ. Juvenile-to-Juvenile transitions (0.5127) have the highest elasticity, emphasizing the importance of juvenile survival for population stability.

D. labyrinthiformis had much weaker population vital rates, to which λ was already negative (~0.94) before the *D. antillarum* die-off and decreased more post event (~0.82). We plotted a 100 year projection with only a 10% chance of disturbance and found that λ remained negative, indicating a quick population decline. Extinction probablity was calculated and found to reach extinction after just 20 years. The sensitivity matrix shows that population growth is most influenced by transitions within and from the intermediate stage, with intermediate-to-intermediate (0.5723) being the highest. These values suggest that survival intermediate stage are critical for the population dynamics of *Diploria labyrinthiformis*. The intermediate-to-intermediate transition also had the highest elasticity (0.4239), reflecting its central role in maintaining population stability.
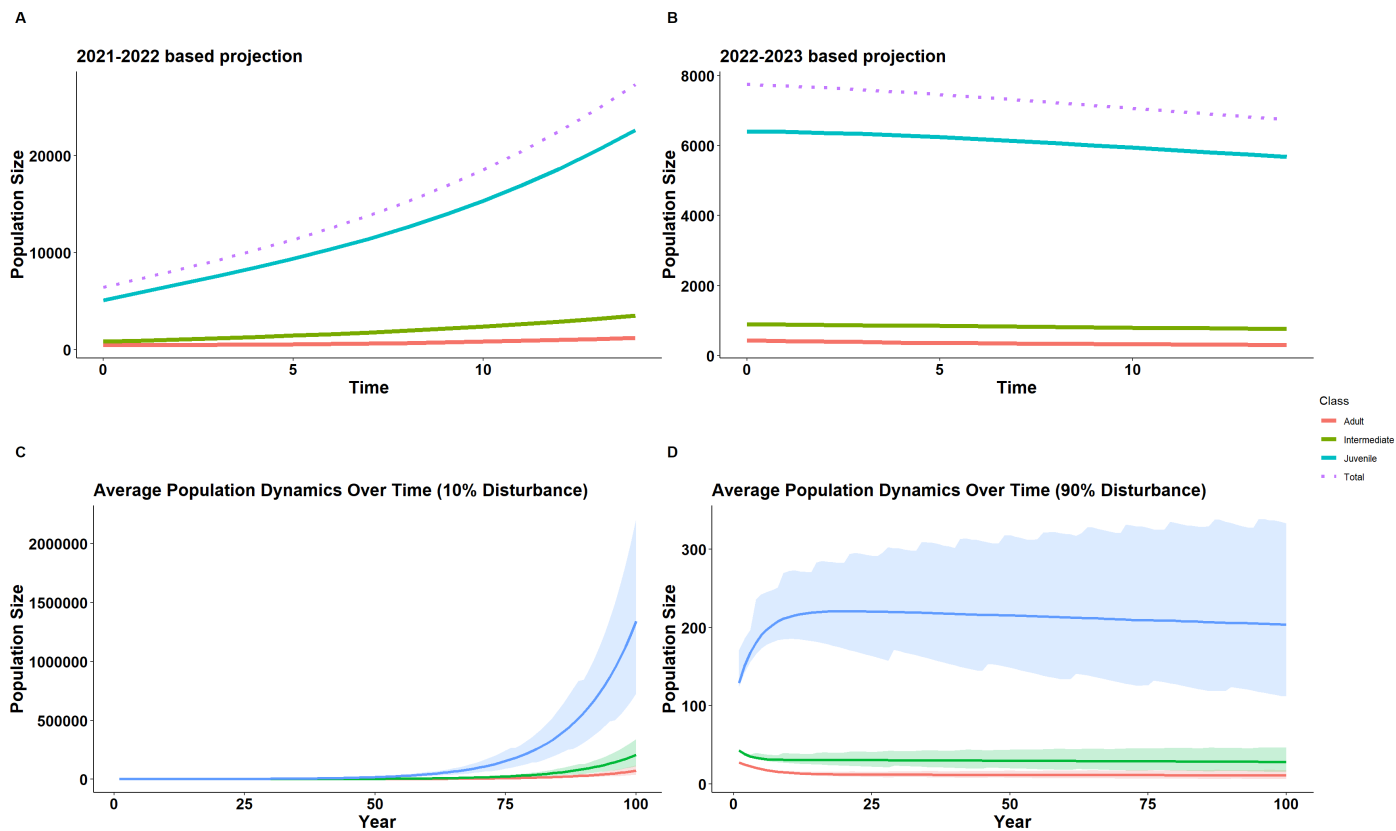
Figure 6. A) Population dynamics of *Porites astreoides* from 2021 to 2022, showing a steady increase in population size. B) Population dynamics of *Porites astreoides* from 2022 to 2023, showing a significant decrease in population growth rate from 1.1 to 0.988. C) The stochastic model with 10% disturbance shows a steady increase in population size. D) The stochastic model with 90% disturbance shows a decrease in λ to below 1, leading to an eventual population collapse.
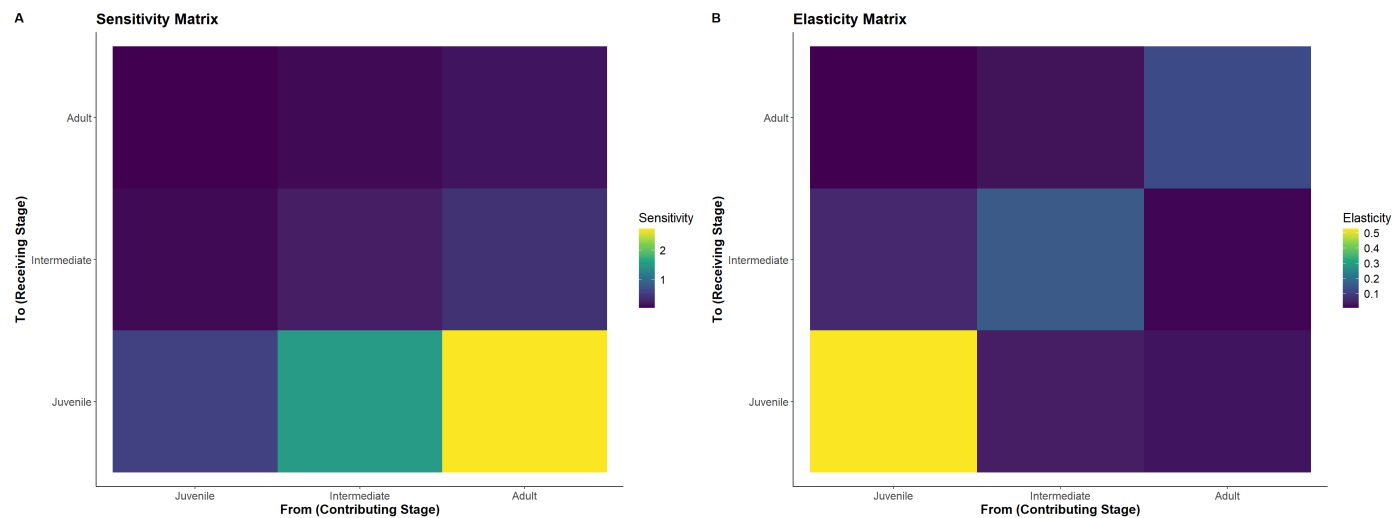

Figure 7. For *Porites astreoides* A) Sensitivity analysis of stochasitc model with equal probabilities of disturbance. B) Elasticity analysis of stochasitc model with equal probabilities of disturbance.
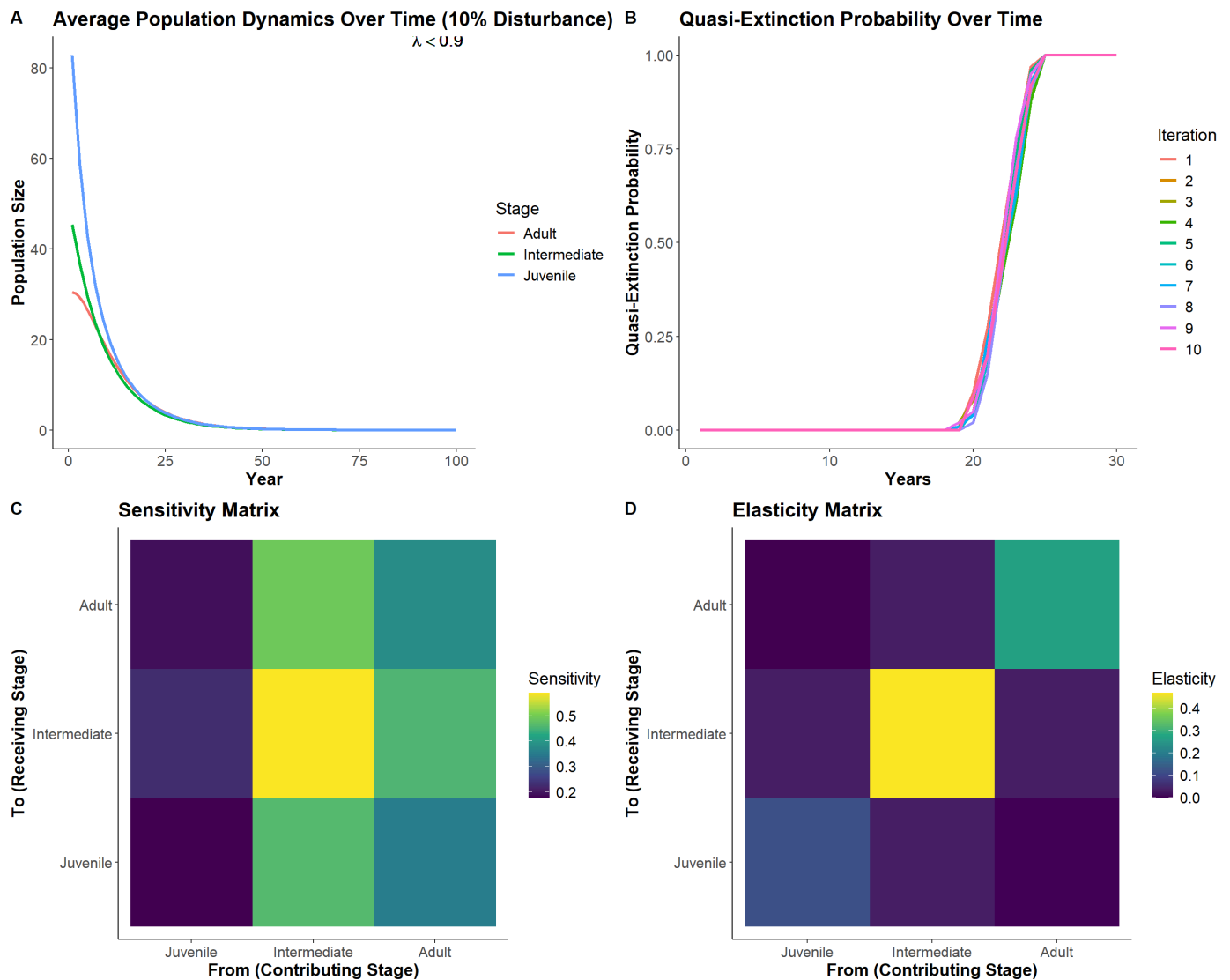
Figure 8. A) Population dynamics of DLAB from stocastic model with a 10% chance of a macroalgal bloom occuring, showing a rapid decline in population size. B) Multiple model predictors of time until population collapse (less than 20 individuals). C) Sensitivity analysis of stochastic model with equal chance of disturbance. D) Elasticity analysis of stochastic model with equal chance of disturbance

4 Macroalgae distribution before and after die-off

To assess the impact of *Diadema antillarum* on macroalgal expansion in the reef benthos, various landscape (or seascape) metrics were analyzed. Clumpiness and average gyration of macroalgal clusters remained consistent across all four periods (figure 9). However, patch density showed significant temporal variation, with a marked increase in macroalgal patch density observed four months after the die-off, followed by a decline—indicating potential stabilization or recovery of the reef (figure 9). Percent cover of macroalgae exhibited a similar pattern. These findings suggest a delayed expansion of macroalgae across the benthic floor, with a subsequent decrease observed a year after the die-off event.
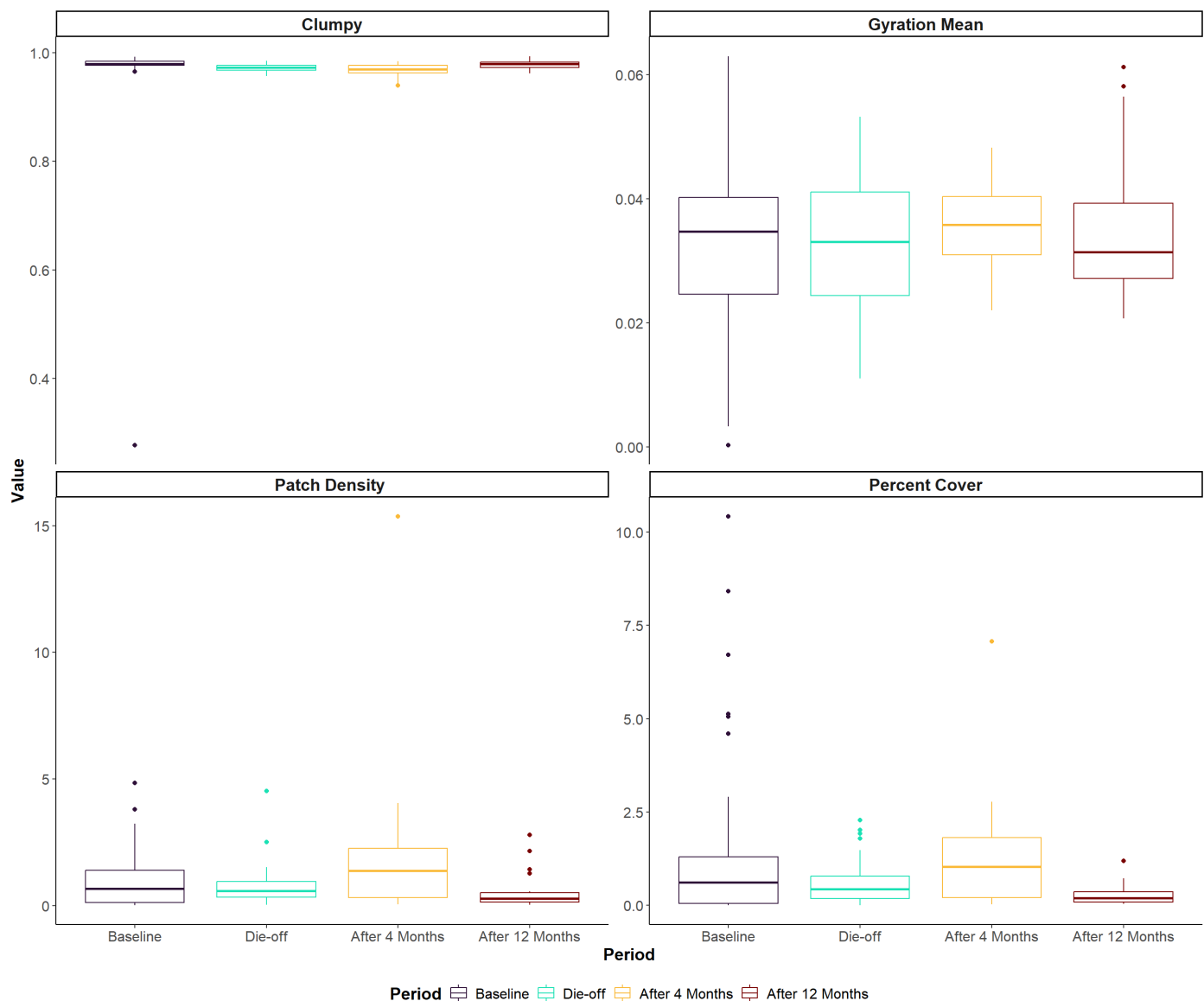
Figure 9: Multiple `seascape` metrics used to asses the expansion of macroalgae across periods.

5 Assessing trophic dynamics as a response to black sea urchin collapse

Stable isotope analysis revealed shifts in the reef food web. Before the die-off, pelagic organic matter (POM) was a primary energy source for many species. Over time, reliance on seagrass increased for omnivorous fish, while predator fish showed greater use of algae. The analysis also highlighted niche partitioning, where different species reduced overlap in their resource use, reflecting adjustments to changing environmental conditions.

Figure 9. A) Trophic niche width across the collection dates. Community is the total community niche width and multi species is the total niche width that w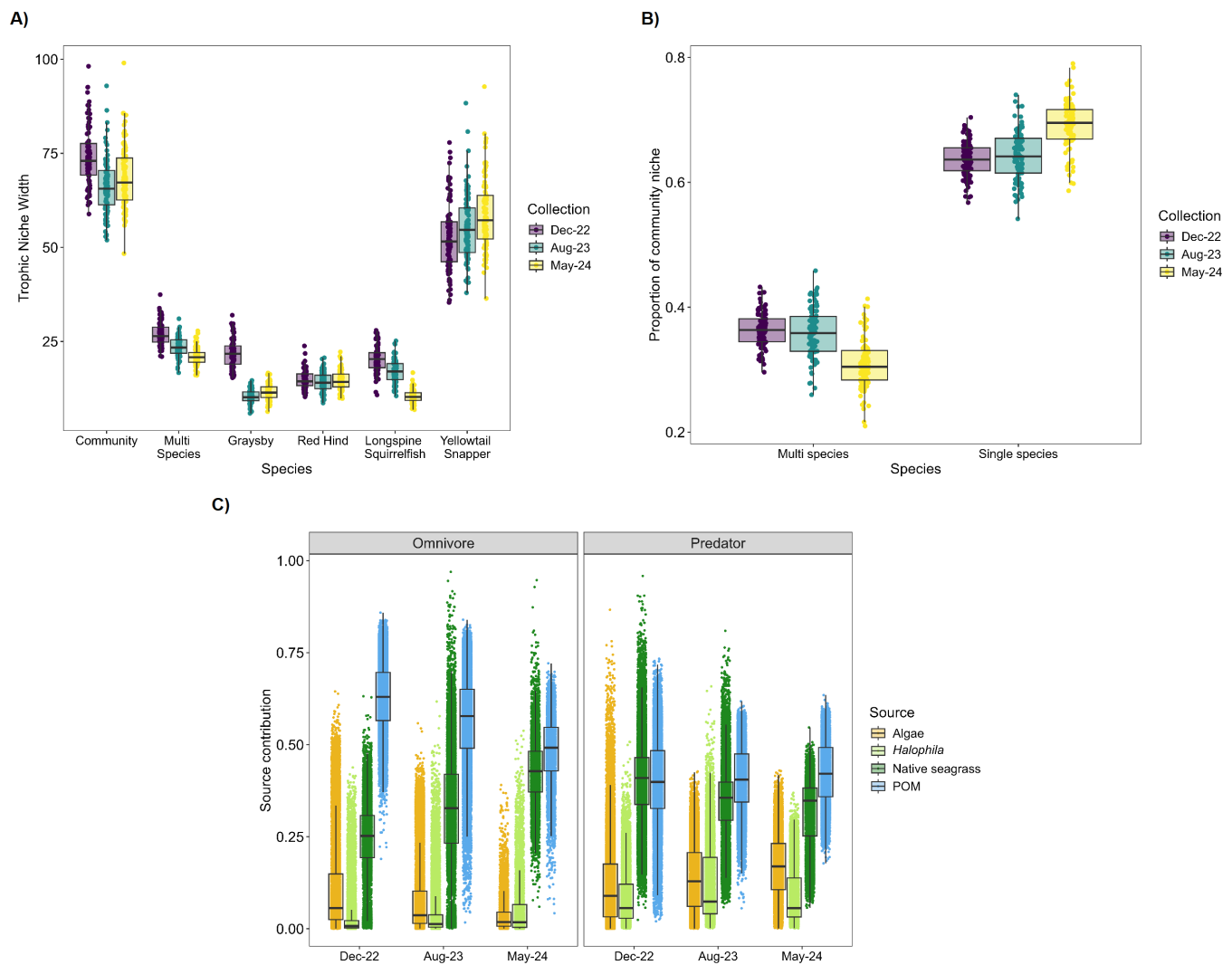as occupied by more than one species. Each point represents a bootstrap iteration. B) Proportion of the community trophic niche width that was either occupied by multiple or single species across the time periods. Each point represents a bootstrap iteration. C) Combined mixing model results for the three collection dates for omnivorous and predatory fish. Each point represents an estimate from a single run of the mixing model for the estimated resource use of a given species.

6 Supplementary

GLMM Results for Coral, Macroalgae and Sponges

Percent Cover ~ Period + (1|Site)

Group	effect	Term	Estimate	Std. Error	Z-value	p-value
Coral	fixed	Intercept	−4.808	0.104	−46.060	0.000
Coral	fixed	Location: Tampico	0.871	0.086	10.099	0.000
Coral	fixed	Period: Die-off	0.198	0.058	3.405	0.001

GLMM Results for Coral, Macroalgae and Sponges

Percent Cover ~ Period + (1|Site)

Group	effect	Term	Estimate	Std. Error	Z-value	p-value
Coral	fixed	after 4 months	0.072	0.060	1.201	0.230
Coral	fixed	Period: After 12 Months	0.216	0.057	3.777	0.000
Coral	ran_pars	sd__(Intercept)	0.413			
Macroalgae	fixed	Intercept	−1.537	0.110	−13.913	0.000
Macroalgae	fixed	Location: Tampico	0.856	0.094	9.089	0.000
Macroalgae	fixed	Period: Die-off	0.305	0.064	4.761	0.000
Macroalgae	fixed	after 4 months	0.227	0.064	3.534	0.000
Macroalgae	fixed	Period: After 12 Months	0.163	0.064	2.561	0.010
Macroalgae	ran_pars	sd__(Intercept)	0.432			
Sponge	fixed	Intercept	−3.296	0.129	−25.561	0.000
Sponge	fixed	Location: Tampico	0.651	0.154	4.214	0.000
Sponge	fixed	Period: Die-off	−0.895	0.142	−6.297	0.000
Sponge	fixed	after 4 months	−0.687	0.139	−4.957	0.000
Sponge	fixed	Period: After 12 Months	−0.509	0.132	−3.848	0.000
Sponge	fixed	Location: Tampico*Period: Die-off	−0.703	0.203	−3.465	0.001
Sponge	fixed	Location: Tampico*Period: Die-off	−0.993	0.203	−4.891	0.000
Sponge	fixed	Location: Tampico*Period: After 4 Months	−0.674	0.186	−3.615	0.000
Sponge	ran_pars	sd__(Intercept)	0.368			

Pairwise comparisons for Corals, Macroalgae and Sponges

Percent Cover ~ Period + (1|Site)

Group	location	term	Contrast	estimate	std.error	statistic	p-value
Coral	Maguey	period	Baseline - Die-off	−0.1982	0.0582	−3.4047	0.0037
Coral	Maguey	period	Baseline - After 4 months	−0.0717	0.0597	−1.2013	0.6260
Coral	Maguey	period	Baseline - After 12 months	−0.2156	0.0571	−3.7772	0.0009
Coral	Maguey	period	Die-off - After 4 months	0.1265	0.0547	2.3147	0.0947
Coral	Maguey	period	Die-off - After 12 months	−0.0174	0.0519	−0.3348	0.9871
Coral	Maguey	period	After 4 months - After 12 months	−0.1439	0.0536	−2.6840	0.0366
Coral	Tampico	period	Baseline - Die-off	−0.1982	0.0582	−3.4047	0.0037
Coral	Tampico	period	Baseline - After 4 months	−0.0717	0.0597	−1.2013	0.6260
Coral	Tampico	period	Baseline - After 12 months	−0.2156	0.0571	−3.7772	0.0009
Coral	Tampico	period	Die-off - After 4 months	0.1265	0.0547	2.3147	0.0947
Coral	Tampico	period	Die-off - After 12 months	−0.0174	0.0519	−0.3348	0.9871
Coral	Tampico	period	After 4 months - After 12 months	−0.1439	0.0536	−2.6840	0.0366
Macroalgae	Maguey	period	Baseline - Die-off	−0.3054	0.0641	−4.7606	0.0000
Macroalgae	Maguey	period	Baseline - After 4 months	−0.2265	0.0641	−3.5339	0.0023
Macroalgae	Maguey	period	Baseline - After 12 months	−0.1628	0.0635	−2.5613	0.0510
Macroalgae	Maguey	period	Die-off - After 4 months	0.0788	0.0601	1.3108	0.5560
Macroalgae	Maguey	period	Die-off - After 12 months	0.1426	0.0596	2.3934	0.0783
Macroalgae	Maguey	period	After 4 months - After 12 months	0.0638	0.0596	1.0694	0.7082
Macroalgae	Tampico	period	Baseline - Die-off	−0.3054	0.0641	−4.7606	0.0000
Macroalgae	Tampico	period	Baseline - After 4 months	−0.2265	0.0641	−3.5339	0.0023

Pairwise comparisons for Corals, Macroalgae and Sponges

Percent Cover ~ Period + (1|Site)

Group	location	term	Contrast	estimate	std.error	statistic	p-value
Macroalgae	Tampico	period	Baseline - After 12 months	−0.1628	0.0635	−2.5613	0.0510
Macroalgae	Tampico	period	Die-off - After 4 months	0.0788	0.0601	1.3108	0.5560
Macroalgae	Tampico	period	Die-off - After 12 months	0.1426	0.0596	2.3934	0.0783
Macroalgae	Tampico	period	After 4 months - After 12 months	0.0638	0.0596	1.0694	0.7082
Sponge	Maguey	period	Baseline - Die-off	0.8955	0.1422	6.2966	0.0000
Sponge	Maguey	period	Baseline - After 4 months	0.6867	0.1385	4.9575	0.0000
Sponge	Maguey	period	Baseline - After 12 months	0.5092	0.1323	3.8484	0.0007
Sponge	Maguey	period	Die-off - After 4 months	−0.2088	0.1508	−1.3841	0.5093
Sponge	Maguey	period	Die-off - After 12 months	−0.3863	0.1459	−2.6480	0.0404
Sponge	Maguey	period	After 4 months - After 12 months	−0.1775	0.1422	−1.2480	0.5962
Sponge	Tampico	period	Baseline - Die-off	1.5986	0.1469	10.8833	0.0000
Sponge	Tampico	period	Baseline - After 4 months	1.6801	0.1493	11.2558	0.0000
Sponge	Tampico	period	Baseline - After 12 months	1.1829	0.1311	9.0205	0.0000
Sponge	Tampico	period	Die-off - After 4 months	0.0815	0.1734	0.4701	0.9656
Sponge	Tampico	period	Die-off - After 12 months	−0.4157	0.1605	−2.5907	0.0472
Sponge	Tampico	period	After 4 months - After 12 months	−0.4972	0.1597	−3.1142	0.0100