METHODS

Sampling method

Three morphologically distinct species of intertidal gastropods, namely *Nerita atramentosa, Austrolittorina unifasciata,* and *Nodilittorina pyramidalis* ("Nerita", "Austrolittorina", and "Nodilittorina", respectively), were sampled in two defined zones along the shore of the southern end of the sandy beach at South Maroubra Beach, NSW, Australia. Replicated samples within each zone were taken by counting animals in a small quadrat. Separate samples were taken for the 3 species randomly and independently considering potential interaction between individuals in proximity.

Defining zones and quadrats

Zones

The accessible part of the shore was split into halves. We defined **zones** based on their relative height on the shore with visually distinct levels of humidity. Two levels of zones were characterised in this experiment: The "**Mid**" zone is that close to the water's edge and was wetter, and the "**High**" zone is that far from the water's edge and was drier. The same zones were used throughout the experiment.

Quadrats

The quadrats were square pieces of plastic mesh about 60 cm x 60 cm with a grid size of about 5 cm. These were placed around the shore randomly, and animals within 5 x 5 of the small squares within each quadrat were counted. That gives a sampling area for each quadrat of about 25 cm x 25 cm. Abundance was expressed as individual counts of one species per quadrat.

Randomising sampling sites

Originally, a stricter approach was designed using a random numbers table to generate random coordinates as quadrat locations over a defined Cartesian plane at the sampling site. Due to the large swell at Maroubra beach this year, the quadrat locations were determined via haphazard sampling instead. This approach produced random samples effectively as the quadrat location did not in any way associate with the numbers of animals within it, and the location was blinded to sampler for each measurement.

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Data recording

For each quadrat, the following data were recorded:

- the **zone** ("High" or "Mid") in which the sample was drawn; and
- the **number** of the selected snail per quadrat measured ("Abundance").

Additionally, data from previous classes in 2016-2020 were provided to contrast the species composition in the intertidal community living in rock pools ("rockpools") to neighbouring rocky habitats that were emergent at low tide ("emergent"). The organisms featured were recorded as:

- ***Algae*** (characterised morphologically as "Brown", "Red", and "Green"). Abundance was measured by *percentage coverage* on a quadrat; and
- ***Nerita, Cellana, Austrocochlea, Bembicium, Morula, Patiriella, Pyura, Actinia, and Barnicles***. Abundance was measured by *counts* in a quadrat.

Data assessment and treatment

Gastropod abundance data. It was noted that a few replicates in the Maroubra data were removed prior to analyses such that the resulting dataset is balanced. This produced 70 replicates for each of the three species per zone. This prevents any misinterpretation due to the default use of Type I sum of squares in multifactor analysis of variance (ANOVA) by R. In addition, the abundance data were assessed for normality and homogeneity of variance (HOV) as required by ANOVA using the following approaches:

Normality. The histogram of residuals was used to detect any significant departure from normality.

HOV. Residuals diagnostic plots of the ANOVA model were used to detect any data heteroskedasticity. Specifically, plots of residual versus means, and normal quantile-quantile were used. No additional transformations were applied.

Multiple species composition data. Multiple species abundance measurements (count per quadrat for gastropods, and percentage coverage for algae) were standardised using the *decostand* function with *method=max*, which divides each entry with its marginal maximum. The function is included in the vegan package in R. The Bray-Curtis dissimilarity index was used as the similarity coefficients for MDS ordination, while Bray-Curtis distance is used for multivariate dispersion using the vegan function *vegdist*.

Statistical analyses and data visualisation

The abundance of gastropods was contrasted among species and different heights on the shore using **2-factor ANOVA** with species and zone as fixed factors. The differences in multiple species composition of the intertidal community between habitats, and among sampling years, were visualised using MDS ordination, and analysed using permutational multivariate analysis of variance (PERMANOVA). MDS ordination plots were created using the Bray-Curtis dissimilarity index. Additionally, multivariate dispersion with respect to group medians was visualised as a measure of beta diversity among the two habitats and sampling years, respectively, using the *betadisper* function included in the vegan package (See also https://cran.rproject.org/web/packages/vegan/vegan.pdf, Anderson 2006). The dispersion plots were modified using the ggplot2 function *stat_ellipse* to produce confidence ellipses of the Bray-Curtis distances and to supplementally establish cleaner aesthetics and contrasts among groups.

RESULTS

Does mean abundance of gastropods vary across zones and species at Maroubra bay?

The null hypotheses on which our research question was based are rejected (Table 1, 2-ANOVA, $p < 0.001$ for each fixed factor). There is very strong evidence that gastropod species ($F = 153.50$, df = 2, $p < 2e\text{-}16$) and height on the shore ($F = 12.60$, df = 1, $p < 0.001$) are both significant factors on abundance (count per quadrat) observed at Maroubra bay. Moreover, a considerable interaction was detected between species and zone (Fig. 2, Table 1, $F = 17.56$, df = 2, $p < 0.001$). It suggests that the gastropod species observed at Maroubra bay depend on the height on the shore where they inhabit.

In addition, Tukey's post hoc test suggests that on average, *Austrolittorina unifasciata* is significantly more abundant than the other two species, regardless of height on the shore (Table 2a, Diff = -27.35 for *Nerita-Austrolittorina*, $p < 0.001$; Diff = -26.38 for *Nodilittorina-Austrolittorina*, $p < 0.001$). In addition, only *Nodilittorina pyramidalis* exhibits a trend of higher abundance with increasing height (Fig. 1, Fig. 2, Table 2b), while lower abundance is observed for *Austrolittorina* and *Nerita atramentosa* in the higher zone compared to the mid zone.

Due to significant interaction between species and height on the shore, we conclude that the underlying trends of gastropod abundance with respect to species and height on the Maroubra shore characterised here require further investigation and are subject to deeper analyses.

How does gastropods species composition distribute in rockpool vs emergent habitats, and among sampling years?

Species composition with respect to different habitats. Multiple species composition evidently differs between rock pools and neighbouring emergent habitats based on MDS ordination (Fig. 3a, stress = 0.23). We consider this difference a moderate evidence for varying species composition among habitats due to the high stress value (stress > 0.2). The composition of intertidal communities was strongly dependent on the habitat according to PERMANOVA (Table 3a, df = 1, pseudo-F = 35.98, $p < 0.001$). Additionally, multivariate dispersion plots of species composition dissimilarities (using Bray-Curtis distance from *max*-standardised abundances with respect to group medians) showed characteristic distinction of sample dissimilarity dispersion between two habitats when contrasting with respective standard deviations (Fig. 4a, 4c).[1] In summary, the multiple species abundance data collected showed a strong connection between intertidal species composition and different living environments.

Species composition with respect to sampling years. No evident contrast in intertidal species composition among sampling years was seen from MDS ordination (Fig. 3b; stress = 0.23). However, PERMANOVA suggests that sampling year is a significant factor on varying species composition (Table 3b, df = 1, pseudo-F = 15.02, $p < 0.001$). Interestingly, MDS ordination shows a specifically lower sample variation in 2016 (Fig. 3b). It was suspected if there exists growing diversity of intertidal species — if measured by higher variability of dissimilarity between species composition samples — from 2016 to 2020. Multivariate dispersion of sample dissimilarity (using the Bray-Curtis distance with respect to group medians), however, did not show distinguishing evolutionary pattern of sample dissimilarity dispersion across sampling years (Fig. 4b, 4d).[2] In summary, the multiple species composition data collected from previous classes shows moderate connection between intertidal species composition and sampling year.

[1] Considerable distinction in sample dissimilarity dispersion between the two habitats is also shown using respective 95% confidence ellipses (Fig. 5a). There is evident distinction between the confidence ellipses, while their dispersion boundaries (represented by convex hulls) largely overlap.

[2] Exceptionally, a significantly ***lower*** variation of species composition was detected in the 2016 data when compared with other sampling years using confidence ellipses of Bray-Curtis distance (Fig. 5b). This finding is consistent with the higher compactness seen for the 2016 data points in Fig. 3b, as well as the smaller convex bound for the 2016 data in Fig. 4b.

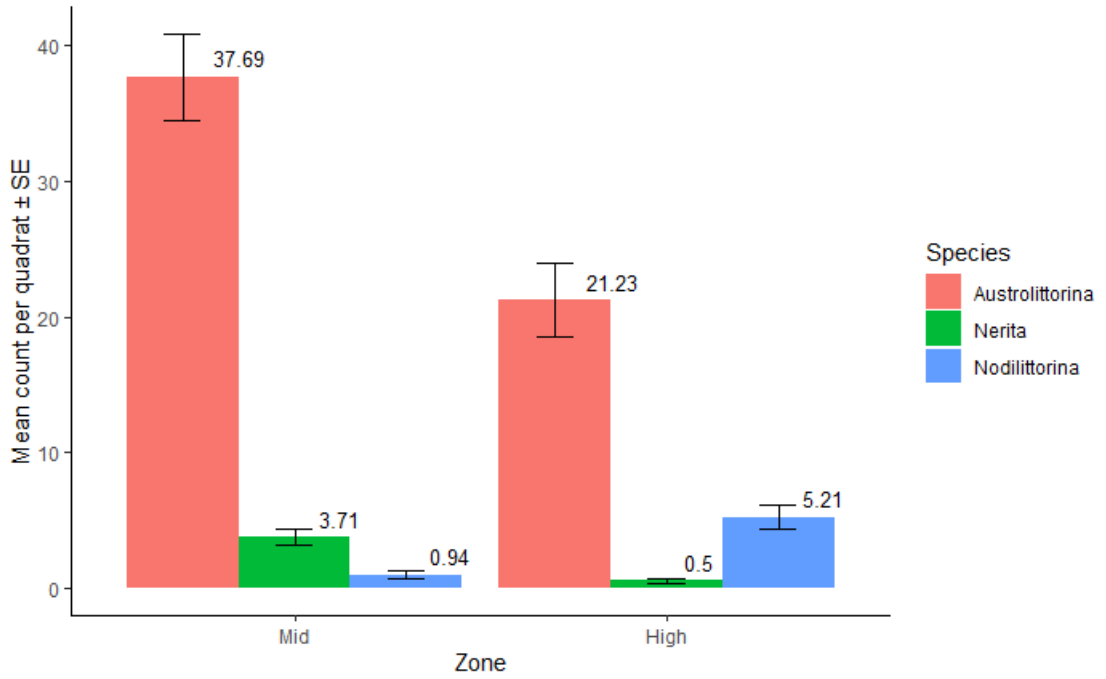

Fig. 1. Effect of height on the shore on the abundance of three species of gastropods inhabiting Maroubra bay, NSW, Australia. Data are mean counts per quadrat (±SE) of each species in one zone, reported to two decimal places and displayed above the bars. Species measured are *Austrolittorina unifasciata, Nerita atramentosa, and Nodilittorina pyramidalis*. Each replication measured one unique species independently. Zones are defined by their relative height on shore and exhibits distinction in humidity. Note that the mean abundance of *Austrolittorina unifasciata* differs significantly from other species in both zones, as confirmed by Tukey's post hoc test. (n = 70 per species per zone)

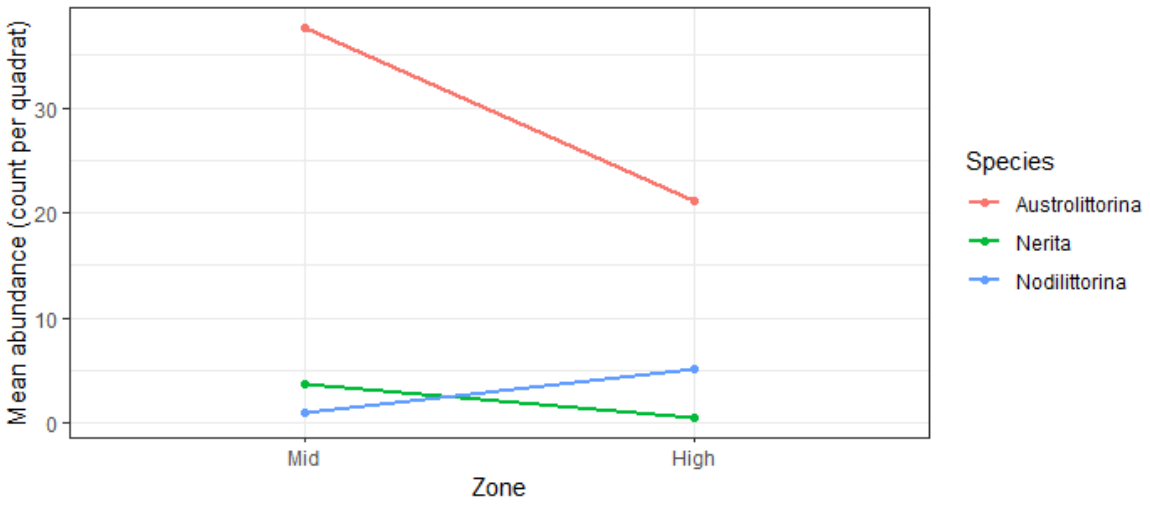

Fig. 2. Interaction plot of species and zone factors in Maroubra data showing significant interaction by intersection. Each line represents a mean abundance trend between zones for one species. Note that *Austrolittorina* and *Nerita* are visibly similar in direction and magnitude with increasing height. Note also that *Nodilittorina* distinguishes from the other two in trend direction, which intersects with *Nerita* and with *Austrolittorina* by interpolation. The slope magnitude in *Austrolittorina* is significantly larger than the other two (See also Table 2b). The overall abundance of *Austrolittorina* is higher than the other two species measured in both zones, as seen in Fig. 1 and Table 2.

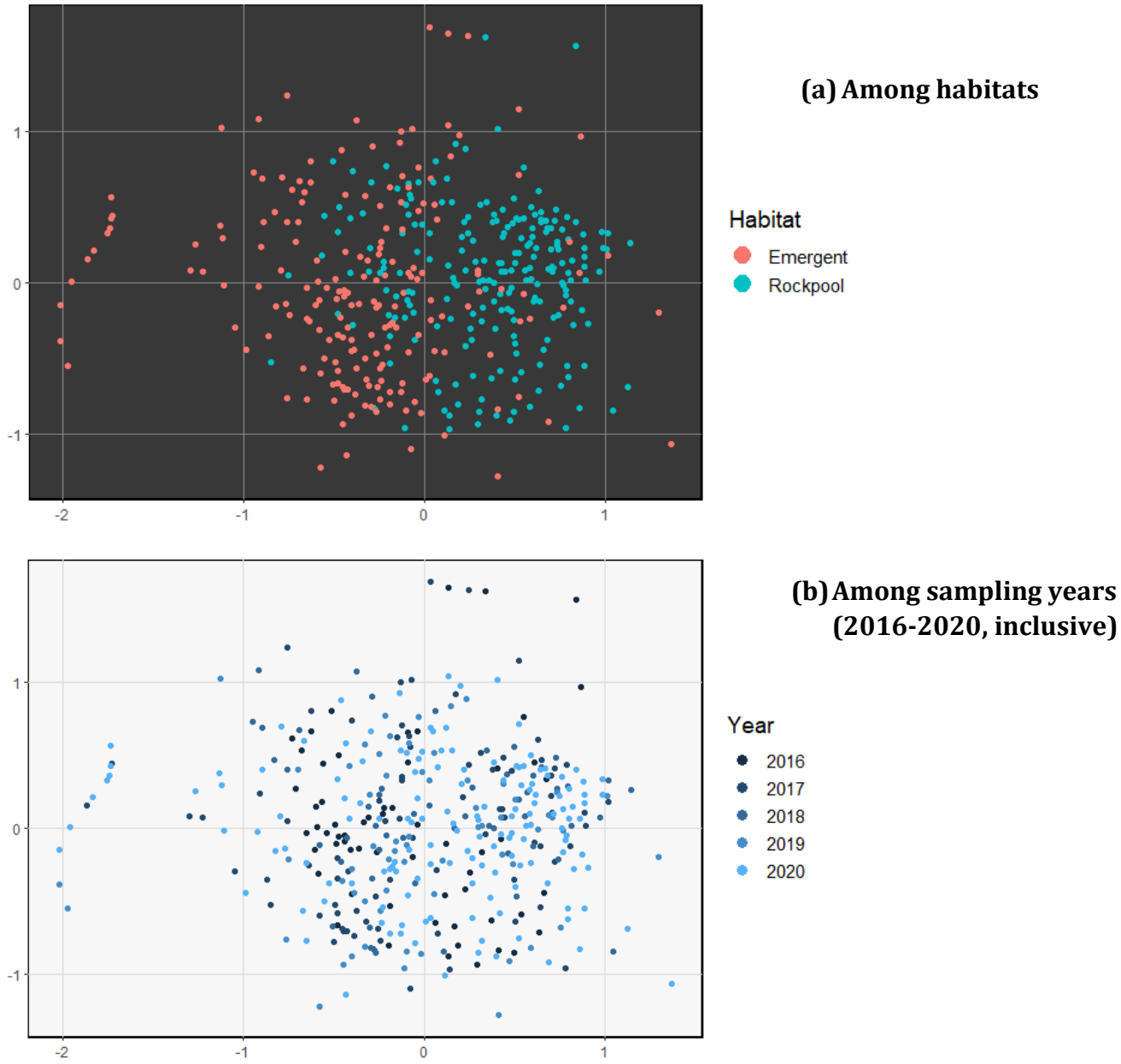

Fig. 3. Two-dimensional ordination plots contrasting multiple species composition (a) in rockpool vs emergent habitats; and (b) among sampling years (2016-2020, inclusive) at Maroubra bay (stress = 0.23 for both). Ordination of species composition are based on counts per quadrat, and percentage cover for algae recorded, with data standardised using the vegan function *decostand* in R. Both conducted with the Bray-Curtis dissimilarity index.

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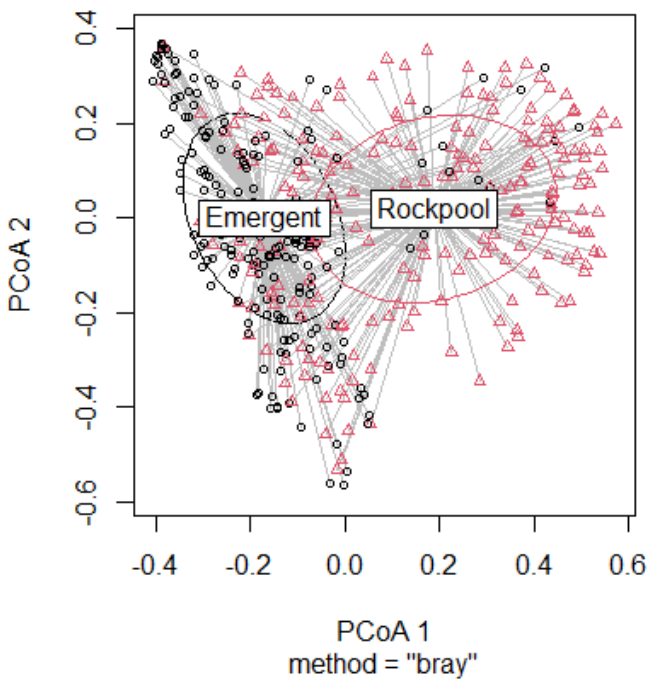

Fig. 4a. Multivariate dispersion plot of multiple species composition samples from Maroubra bay, partitioned by habitats. Plot was generated using the vegan function *betadisper*. Samples are represented as distinguished symbols. Distance to group median from each sample are represented as grey lines. 1-standard deviations ellipses are shown for each group. Bray-Curtis distance was used.

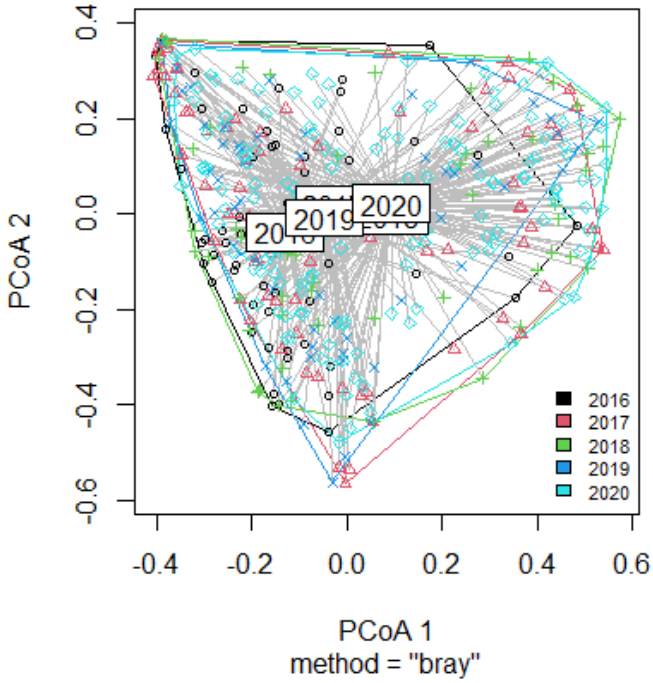

Fig. 4b. Multivariate dispersion plot of multiple species composition at Maroubra bay, partitioned by sampling years (2016-2020). Bray-Curtis distance was used. Convex hulls are boundaries of sample dissimilarity dispersion within each group. The total dispersion among years is highly overlapping compared to the contrast seen between habitats (Fig. 4a). No evident pattern of dispersion over the years.

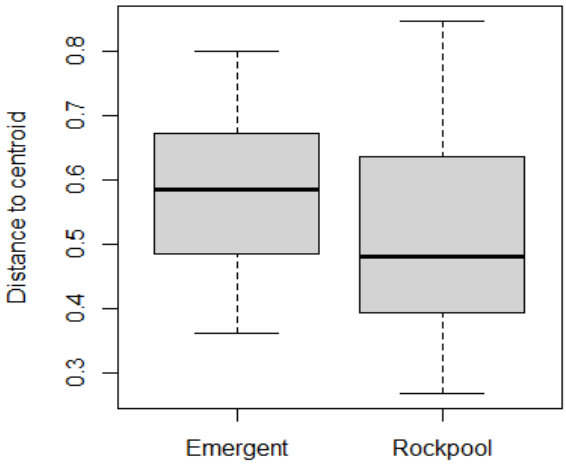

Fig. 4c. Box plot showing distance distribution of multiple species composition samples to group centroid in the two habitats at Maroubra bay. Bray-Curtis distance was used.

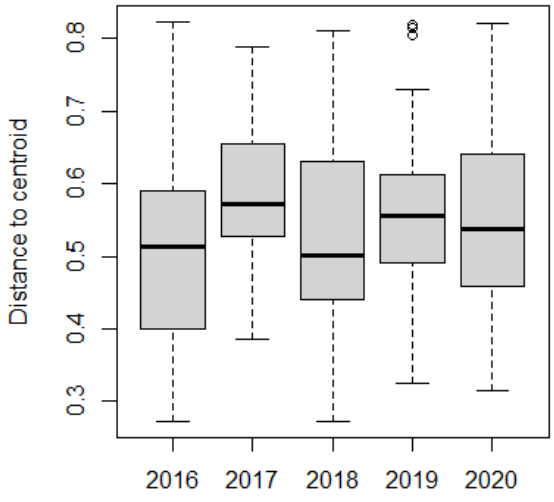

Fig. 4d. Box plot showing distance distribution of multiple species composition samples to group centroid across sampling years. Bray-Curtis distance was used.

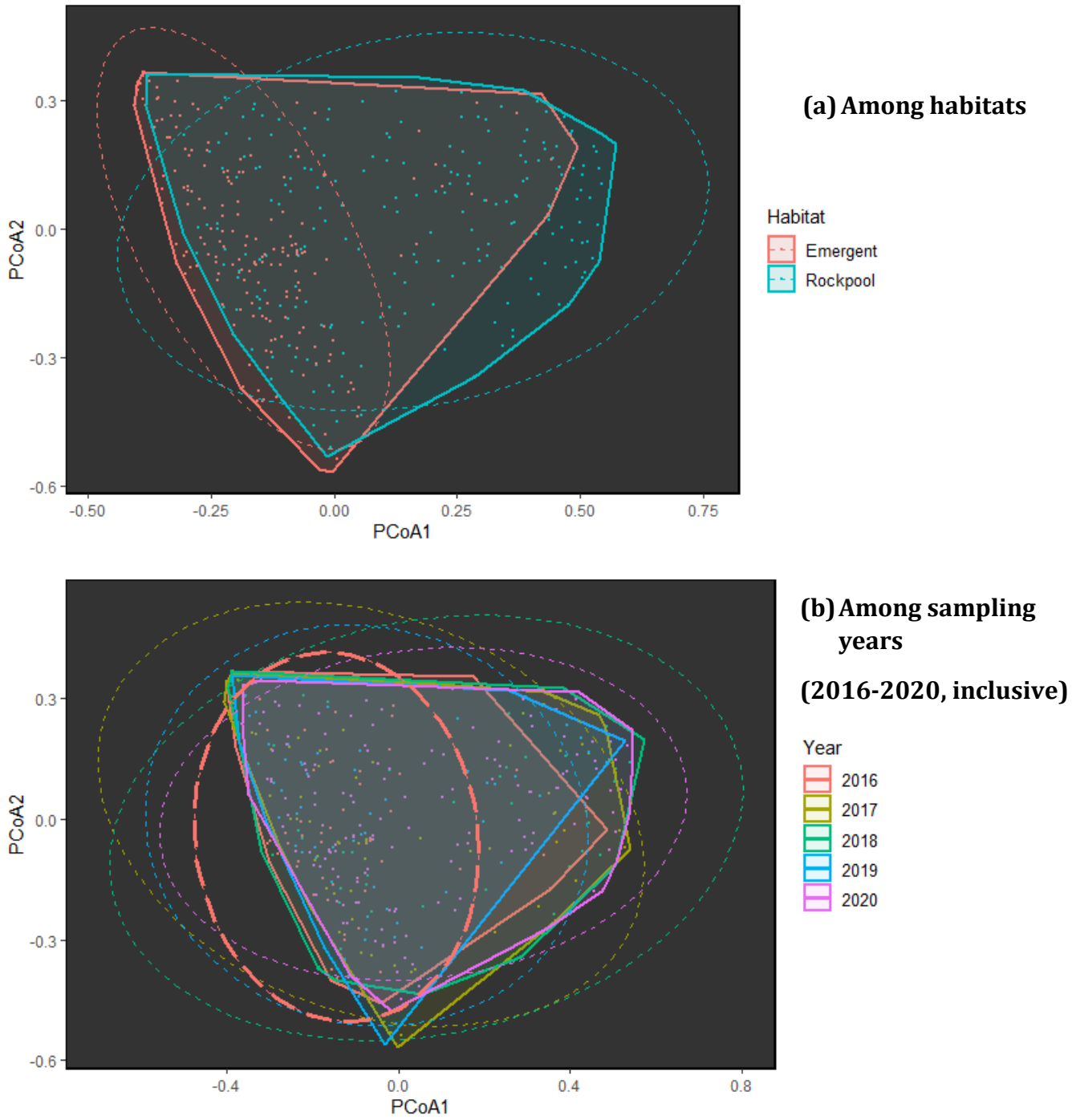

Fig. 5. Plots showing dispersion of Bray-Curtis distances in multiple species composition (a) between the two habitats; and (b) across sampling years (2016-2020). Distance based on multiple species composition sampled at Maroubra bay (standardised). Convex hulls are line-bound regions coloured by (a) habitats; and (b) sampling year, representing complete areas of dispersion. Dotted ellipses are 95% confidence intervals of Bray-Curtis distance (assumed t-distributed) between each sample point and respective group medians. Each point within the convex hull represents the Bray-Curtis distance of a sample. Variables are standardised via *decostand*, *method=max*. Note that no additional transformation was used (stress=0.23), and the Bray-Curtis distances skew towards 1 (reflecting the multiple zeros in standardised variable matrix). In Fig. 5b, the confidence ellipse of year 2016 is emphasised with thick dash lines.

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Table 1. Two-Factor ANOVA contrasting abundance of gastropods at Maroubra bay among three different species and two zone treatments with distinct levels of height. Abundance was based on counts per quadrat (n = 70 per species per zone). *Significant result ($p < 0.05$)

Source	Df	Sum Sq	Mean Sq	F value	p
Zone	1	2767	2767	12.60	< 0.001 *
Species	2	67424	33712	153.50	< 2e-16 *
Zone × Species	2	7713	3856	17.56	< 0.001 *
Residuals	414	90921	220	-	-

Table 2a. Tukey's Honest Significant Difference (HSD) test contrasting gastropods abundance at Maroubra bay between zones and species. Abundance values rounded to 2 decimal places. *Significant result ($p < 0.05$)

Zone	Diff	Lwr	Upr	p adj
High-Mid	-5.13	-7.98	-2.29	< 0.001*
Species				
Nerita-Austrolittorina	-27.35	-31.52	-23.18	< 0.001*
Nodilittorina-Austrolittorina	-26.38	-30.55	-22.21	< 0.001*
Nodilittorina-Nerita	0.97	-3.20	5.14	0.847

Table 2b. Tukey's HSD test on zone-species interaction. Abundance values rounded to 1 decimal place. Entries in bold represent mean abundance differences of the same species between zones. Note that every comparison with *Austrolittorina unifasciata* returned a significant result ($p < 0.001$ for all comparisons), while every comparison excluding *Austrolittorina unifasciata* returned non-significant results ($p > 0.41$ for all comparisons). *Significant result ($p < 0.05$)

Zone: Species Difference (Significant pairs)	Diff	Lwr	Upr	p adj
High:*Austrolittorina*-**Mid**:*Austrolittorina*	**-16.5**	-23.6	-9.3	< 0.001*
Mid:*Nerita*-**Mid**:*Austrolittorina*	-34.0	-41.1	-26.8	< 0.001*
High:*Nerita*-**Mid**:*Austrolittorina*	-37.2	-44.4	-30.0	< 0.001*
Mid:*Nodilittorina*-**Mid**:*Austrolittorina*	-36.7	-43.9	-29.6	< 0.001*
High:*Nodilittorina*-**Mid**:*Austrolittorina*	-32.5	-39.6	-25.3	< 0.001*
Mid:*Nerita*-**High**:*Austrolittorina*	-17.5	-24.7	-10.3	< 0.001*
High:*Nerita*-**High**:*Austrolittorina*	-20.7	-27.9	-13.6	< 0.001*
Mid:*Nodilittorina*-**High**:*Austrolittorina*	-20.3	-27.5	-13.1	< 0.001*
High:*Nodilittorina*-**High**:*Austrolittorina*	-16.0	-23.2	-8.8	< 0.001*

Table 2b. (continued)

Zone:Species Difference (Non-significant pairs)	**Diff**	**Lwr**	**Upr**	**p adj**
High:*Nodilittorina*-**High**:*Nerita*	4.7	-2.5	11.9	0.415
High:*Nodilittorina*-**Mid**:*Nodilittorina*	**4.3**	-2.9	11.4	0.529
High:*Nerita*-**Mid**:*Nerita*	**-3.2**	-10.4	4.0	0.794
Mid:*Nodilittorina*-**Mid**:*Nerita*	-2.8	-9.9	4.4	0.879
High:*Nodilittorina*-**Mid**:*Nerita*	1.5	-5.7	8.7	0.991
Mid:*Nodilittorina*-**High**:*Nerita*	0.4	-6.7	7.6	> 0.999

Table 3. PERMANOVA contrasting multiple species composition among (a) two intertidal habitats and (b) sampling years (2016-2020, inclusive) from previous class data collected at Maroubra bay. Analysis of species composition based on counts per quadrat of multiple gastropods and percentage cover of algae recorded in one quadrat, with data standardised via the vegan function *decostand* with marginal maximum division. The Bray-Curtis dissimilarity index was used. *Significant result ($p < 0.05$)

(a) Among habitats

Source	**Df**	**Sum Sq**	**Mean Sq**	**Pseudo-F**	**R^2**	**p**
Habitat	1	11.26	11.26	35.98	0.08	< 0.001*
Residuals	414	129.53	0.31	-	0.92	-
Total	415	140.79	-	-	1.00	-

(b) Among sampling years (2016-2020, inclusive)

Source	**Df**	**Sum Sq**	**Mean Sq**	**Pseudo-F**	**R^2**	**p**
Year	1	4.93	4.93	15.02	0.04	< 0.001*
Residuals	414	135.86	0.33	-	0.97	-
Total	415	140.79	-	-	1.00	-