eDNA metabarcoding as a biomonitoring tool for marine protected areas

Zachary Gold [1,*], Joshua Sprague [2], David J. Kushner [2], Erick Zerecero[1], Paul H. Barber [1]

[1]Department of Ecology and Evolutionary Biology, University of California – Los Angeles

[2]Channel Islands National Park Service

* Correspondence: Zachary Gold, Email: zack.gold@ucla.edu; Tel.: +01-310-795-0020, Address: 610 Charles E. Young Dr. S, Los Angeles, CA, 900095

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Abstract

Monitoring of marine protected areas (MPAs) is critical for marine ecosystem management, yet current protocols rely on SCUBA-based visual surveys that are costly and time consuming, limiting their scope and effectiveness. Environmental DNA (eDNA) metabarcoding is a promising alternative for marine ecosystem monitoring, but more direct comparisons to visual surveys are needed to understand the strengths and limitations of each approach. This study compares fish communities inside and outside the Scorpion State Marine Reserve off Santa Cruz Island, CA using eDNA metabarcoding and underwater visual census surveys. Results from eDNA captured 76% (19/25) of fish species and 95% (19/20) of fish genera observed during pairwise underwater visual census. Species missed by eDNA were due to the inability of MiFish *12S* barcodes to differentiate species of rockfishes (*Sebastes,* n=4) or low site occupancy rates of crevice-dwelling *Lythrypnus* gobies. However, eDNA detected an additional 30 fish species not recorded in paired visual surveys, but previously reported from prior visual surveys, highlighting the sensitivity of eDNA. Significant variation in eDNA signatures by location (50m) and site (~1000m) demonstrates the sensitivity of eDNA to address key questions such as community composition inside and outside MPAs. Interestingly, eDNA results recorded higher species richness outside the MPA while visual surveys observed the opposite pattern. This result is likely caused by swamping effects of high fish abundance in MPAs that reduce detection probabilities of pelagic and intertidal taxa. Results demonstrate the utility of eDNA metabarcoding for monitoring marine ecosystems, providing an important complementary tool to visual methods.

Keywords: biomonitoring; Channel Islands National Park; eDNA; environmental DNA; marine protected areas; MPAs.

Introduction

Marine Protected Areas (MPAs) promote sustainability of marine ecosystems and the ecological goods and services they provide (1). However, ensuring MPA effectiveness requires regular monitoring to document that ecosystem health is stable or improving (1). MPA monitoring also provides an essential opportunity to assess the impact of management practices, allowing resource managers to adjust management plans as required (2).

Current MPA monitoring protocols typically assess the diversity and abundance of fish and benthic invertebrates, as well as community trophic structure (3). Much of this assessment is based on underwater visual census surveys conducted on SCUBA (3), which are costly, and time and labor intensive (3). For example, to survey 33 sites within the Channel Islands National Park once per year, the National Park Service Kelp Forest Monitoring Program spends over $395,000 on ~1,000 hours of dive time, ~1,400 hours of data entry to survey 33 sites within the Channel Islands National Park once per year (J. Sprague per obs., 2020). Furthermore, SCUBA-based surveys are constrained by weather, diving conditions, and personnel (J. Sprague per. obs., 2020), and can require extended and repeated dives to accurately document marine communities that place divers at risk for dive-related injuries. SCUBA surveys can also introduce significant observer bias, as fish react differently to divers, particularly inside and outside of MPAs, potentially impacting survey results (5).

Given the above logistical and methodological constraints, MPA monitoring efforts are largely limited o shallow depths (e.g. <30m) and the most economically or ecologically important taxa as proxies for ecosystem health (6). Moreover, examining a predetermined subset of community diversity potentially excludes crucial functional groups, biasing ecosystem assessment (7). Combined, these issues restrict the scope, scale, and frequency of visual surveys, limiting the utility of SCUBA-based MPA surveys to quantify biodiversity and trophic structure, (3), data essential for assessing MPA effectiveness.

One promising new approach for assessing and monitoring marine ecosystems is environmental DNA, or "eDNA", a technique based on isolation and sequencing of freely associated DNA from soil or water samples (8). Through metabarcoding and high-throughput next generation sequencing, eDNA can broadly survey community biodiversity in a rapid, repeatable, and affordable manner (8). As such, eDNA is ideally suited to intensive biodiversity monitoring programs, such as those required for MPAs (9).

eDNA has some key advantages over traditional SCUBA-based survey methods. First, eDNA can capture a wide diversity of marine vertebrate taxa, frequently detecting more species than traditional fish survey methods (10). Second, eDNA detects rare and cryptic species that are frequently overlooked or ignored in traditional survey methods (11,12), including both endangered and invasive species (8). Third, eDNA collection is relatively simple, requiring only small volumes of seawater (< 3L) and simple filtering techniques, allowing sampling by individuals with limited training, even in remote locations (Miya et al., 2016). Forth, because eDNA doesn't require diving, there are significant worker safety advantages. Lastly, eDNA is affordable (e.g. ~$50/sample) and has the potential for automation, allowing for remote sample collection and high throughput autonomous lab processing (14).

Despite these advantages, eDNA also has limitations. Of particular concern is PCR bias that can result in preferential amplification of particular taxa (15). Additionally, detection probabilities can be influenced by species specific eDNA generation and degradation rates (8), an issue potentially further complicated by the transport of eDNA on ocean currents (16). Furthermore, primer design, bioinformatic, and reference database limitations can also affect the accuracy of taxonomic assignment from eDNA (17).

Unlike well-established visual surveys, the impact of biases in eDNA metabarcoding are not well characterized, and may be less problematic than believed. For example recent studies show that impacts of PCR bias can be mitigated by technical replicates and site

104 occupancy modelling (17–19). Similarly, because eDNA signals decay relatively rapidly
105 (e.g. hours to days; (20,21), eDNA signatures are surprisingly stable (22). As such, eDNA
106 holds tremendous promise for monitoring marine ecosystems. Realizing that promise,
107 however, requires a better understanding of how visual surveys and eDNA metabarcoding
108 approaches compare in direct field applications.

109 The Channel Islands MPA Network spans >1000 reefs across 8 islands of the coast of
110 Southern California. It is monitored by several programs including the Kelp Forest
111 Monitoring Program, which conducts visual monitoring surveys of 41 invertebrates and
112 over 100 fishes (23). In total only 94 of the >1000 Channel Island reefs are surveyed, and
113 just once per year (6), missing the seasonal dynamics in the variable Southern California
114 Bight, limiting the scope and scale of assessment (2). While born of logistical necessity, the
115 spatial and temporal limits of this survey protocol makes accurately assessing the health of
116 this MPA network difficult (2,24) and suggests the need for new approaches that produce
117 data on broader taxonomic, spatial and temporal scales.

118 This study tests the efficacy of eDNA for MPA monitoring and to better understand the
119 advantages and shortcomings of eDNA methods. We do this through a side-by-side
120 comparisons of eDNA metabarcoding and visual surveys of fish communities conducted by
121 the National Parks Service.

122 **Materials and Methods**

123 *Sample Collection*

124 We conducted our study at Scorpion State Marine Reserve within the Channel Islands
125 National Park and National Marine Sanctuary. To determine the degree to which eDNA
126 could capture documented differences inside and outside this MPA, we sampled three sites:
127 1) inside the MPA, 2) outside but adjacent (<0.5km) to the MPA ("edge site"), and 3) 2.3km
128 outside the MPA boundary ("outside site"; Figure 1). At each of these three sites, we sampled

129 directly along a 100 m fixed transect used by the Kelp Forest Monitoring Program for visual
130 monitoring, using a GPS to ensure transects overlapped (23). We collected three replicate 1L
131 water samples from three locations on each transect, totaling 9 spatially structured replicates
132 per site. Due to fieldwork logistical challenges, each site was sampled on a different day with
133 a maximum of 72 hours between sampling events.

134 **Figure 1.** Map of Scorpion State Marine Reserve off Santa Cruz Island, CA, USA.

135 We collected seawater samples from 10 m below the surface and 1 m above the benthos
136 using a 4 L Niskin bottle deployed from the UCLA RV Kodiak (25). From each Niskin
137 deployment, we transferred a single liter of seawater to an enteral feeding pouch and
138 conducted gravity filtration through a sterile 0.22 µm Sterivex cartridge (MilliporeSigma,
139 Burlington, MA, USA) in the field (Miya et al., 2016). Additionally, we processed three field
140 blanks as a negative control that consisted of 1 L of distilled water following the method
141 above. Finally, we dried Sterivex filters using a 3 mL syringe and then capped and stored the
142 filters at -20˚C for DNA laboratory work back at UCLA (Miya et al., 2015).

143 *DNA Extraction and Library Preparation*

144 We extracted eDNA from the Sterivex cartridge using the DNAeasy Tissue and Blood Kit
145 (Qiagen Inc., Germantown, MD) following modifications of Spens et al. (2017). We PCR
146 amplified the extracted eDNA using the MiFish Universal Teleost *12S* primer (Miya et al.,
147 2015) with Nextera modifications following PCR and the library preparation methods of
148 Curd et al. (2019) (See supplemental methods). All PCRs included a negative control where
149 molecular grade water replaced the DNA extraction. For positive controls, we used DNA
150 extractions of grass carp (*Ctenopharyngodon idella,* Cyprinidae*)* and Atlantic salmon (*Salmo*
151 *salar,* Salmonidae), both non-native to California. Libraries were sequenced on a MiSeq PE
152 2x300bp at the Technology Center for Genomics & Bioinformatics (University of California-
153 Los Angeles, CA, USA), using Reagent Kit V3 with 20% PhiX added to all sequencing runs.

Bioinformatics

To determine community composition, we used the *Anacapa Toolkit* to conduct quality control, amplicon sequence variant (ASV) parsing, and taxonomic assignment using user-generated custom reference databases (28). We processed sequences using the default parameters and assigned taxonomy using two reference databases. We first assigned taxonomy using the FishCARD California fish specific reference database (Gold 2020). Second, we used the *CRUX*-generated *12S* reference database supplemented with FishCARD reference sequences to assign taxonomy using all available *12S* reference barcodes to identify any non-fish taxa.

Raw ASV community table was decontaminated following Kelly et al. (2018) and McKnight et al. (2019) (See supplemental methods). We then transformed all read counts into an eDNA index for beta-diversity statistics (15). All non-fish species (mammals and birds) were removed prior to final analyses.

eDNA Data Analysis

To test for alpha diversity differences, total species richness for each site was then compared, using an Analysis of Variance (ANOVA) and subsequent Levine's test for homogeneity of dispersions (31).

To determine whether our eDNA sampling design was sufficient to fully capture fish community diversity, we created species rarefaction curves using the iNext package (32). Species coverage estimates were then compared between each site, with and without site occupancy modeling, and using all three 1L replicates taken at three locations along a 100m transect (n=9) as well as only three 1L biological replicates (n=3). We ran a piecewise regression analysis to identify breakpoints in the rate of species diversity found per sample collected using the *R* packaged *segmented* (33).

To test for differences among fish communities, we calculated Bray-Curtis similarity distances on the eDNA index scores between all samples (22). Specifically, we tested for the

180 difference in community similarity variance between our three sites using an *adonis*
181 PEMANOVA (31), followed by a companion multivariate homogeneity of group dispersions
182 test (BETADISPER) (31). Both the PERMANOVA and BETADISPER were run using the
183 following model: eDNA Index ~ Site + Location. We also visualized community beta
184 diversity using non-metric multidimensional scaling (NMDS) (31). To further investigate
185 which species were driving eDNA community differences among sites, we conducted
186 constrained analysis of principle components (CAP) (31).

187 *Visual Underwater Census Methods*

188 To assess fish communities using underwater visual census techniques, SCUBA divers from
189 the Kelp Forest Monitoring Program followed standard survey protocols following Kushner
190 et al. (2013). These protocols include survey types: visual fish transects, roving diver fish
191 counts, and 1m quadrats. The visual fish transects targeted 13 indicator species of fish on
192 visual fish transects recording the counts of adults and juveniles. This protocol consists of
193 performing 2m x 3m x 50 m transects along the 100 m permanent transect. During roving
194 diver fish count surveys all positively identified species are recorded. This protocol consists
195 of 3-6 divers counting all fish species observed during a 30 minute time period, covering as
196 much of the 2000 m^2 of bottom and entire water column as possible. The 1 m^2 quadrat
197 records three small demersal species of fish. All visual surveys occurred along a permanent
198 100 m transect at each site and were conducted within 2 weeks of eDNA sampling (See
199 supplemental methods).

200 *Comparison of eDNA and Visual Underwater Census Methods*

201 We compared species detected by eDNA and underwater visual census approaches across
202 corresponding transects at each site. We identified core taxa that were shared across all sites
203 for a given method. In addition, we identified species that eDNA methods failed to detect but

were observed in visual census surveys and vice versa. We note that given the few numbers of sites (n=3) we were unable to robustly compare abundance estimates between methods.

Results

eDNA Results

We generated over 4 million reads that passed quality control. The *Anacapa Toolkit* identified 2,906 ASVs from 3,091,063 reads representing 27 samples and 8 controls. After the second decontamination step, however, totals reduced to 931 ASVs and 2.35 million reads (Tables S1-S3).

Combined, eDNA metabarcoding successfully detected 54 fish taxa, representing 50 unique species, 48 genera, 34 families, and two classes (Tables S1-S3). eDNA detected 35 species within the MPA, 34 at the edge, and 42 species outside the MPA. The three sites shared a core group of 26 taxa including bony fish and one species of ray (Figure 2) (Table S4). Of these taxa, 15 species are associated with rocky reef habitat, five species are associated with sandy bottom habitat, four species are pelagic-neritic, and two species are pelagic-oceanic.

Figure 2. Venn Diagram of Fish Species Detected with eDNA

Species rarefaction curves showed that sampling at each site (n=9) was insufficient to capture all species diversity (Figure 3). Sample coverage estimates from eDNA results before filtering by site occupancy modeling filters were 94.0%, 88.0%, and 92.9% for the MPA, edge, and outside sites, respectively. Coverage estimates dropped to 81.0%, 80.0%, 83.6% for the MPA, edge, and outside sites, respectively, when only three 1L samples were used. Piecewise regression analysis showed a transition from exponential to linear increase in species detected per replicate between three and four replicate water samples per site (3.36-3.53) with subsequent diminishing sample coverage returns with the addition of more

228 samples. In contrast, species diversity was near saturated (>99.0%) when applying a site
229 occupancy rate above 75% and using three 1 L replicates taken at three locations along a
230 100 m transect. However, using only three samples, sample coverage dropped to 87.1%,
231 90.3%, 88.9% for the MPA, edge, and outside sites, respectively.

232 **Figure 3.** Species Rarefaction Curves. **a**) Species rarefaction curves for all fishes
233 found at each site across 3 1L replicates taken at 3 locations along a 100m transect.
234 **b**) Species rarefaction curves for fish species with occupancy rates above 75% found
235 at each site across 3 1L replicates taken at 3 locations along a 100m transect. Sample
236 coverage estimates were higher for species with occupancy rates above 75% (100%)
237 than for all species (85.8%-93.1%). For species with occupancy rates above 75%
238 sample coverage estimates ranged from 89.3-91.1% for only 3 1L replicates.

239 Analyses showed a significant difference in the total number of observed species across
240 sites, with the site outside the MPA having significantly higher diversity than both the edge
241 and MPA sites (ANOVA, $p<0.001$, Levine's test $p> 0.5$). Observed species differences
242 between sites were partially driven by the presence of non-rocky reef taxa (46.4%, 13/28),
243 primarily pelagic, mobile, sandy bottom, and intertidal species. Moreover, there were also
244 significant differences in fish communities among the three sites as well as among the three
245 sampling locations along each of the three transects (PERMANOVA $p<0.001$, betadisper
246 $p>0.05$). Location along the transect explained 26.4% of the total variance while site (e.g.
247 inside, edge and outside MPA) explained 19.0% of the total variance; 54.5% of the total
248 variance was unexplained.

249 NMDS ordination showed weak clustering of samples by both location and site
250 (NMDS, Stress 0.20; Figure 4). Constrained analysis of principle components (CAP) found
251 significant differences in species assemblages between samples collected at different sites
252 and locations (CAP, $p<0.001$) (Figure 5), further indicating difference in eDNA signatures

253 across sites and locations. CAP analysis identified seven taxa with the strongest differences
254 between sites. The MPA site had higher eDNA index scores of kelp perch (*Brachyistius*
255 *frenatus,* Embiotocidae*)*, sarcastic fringehead (*Neoclinus blanchardi,* Chaenopsidae), and
256 spotted cusk-eel (*Chilara taylori,* Ophidiidae). The edge site had higher index scores of
257 roughback sculpin (*Chitonotus pugetensis,* Cottidae). The site outside the MPA had higher
258 index scores of yellowtail amberjack (*Seriola lalandi,* Carangidae), sand bass sp.
259 (*Paralabrax* spp., Serranidae), and dog-faced witch eel (*Facciolella gilberti,*
260 Nettastomatidae).

261 **Figure 4.** NMDS of Bray-Curtis Dissimilarities. Bray-Curtis dissimilarities were
262 calculated between all samples using only species with occupancy rates over 75%.
263 Samples from Sites (colors) and locations (shapes) are similar to each other (NMDS,
264 Stress = 0.20).

265 **Figure 5.** Constrained Analysis of Principle Components (CAP) Ordination. Bray-
266 Curtis dissimilarities were calculated between all samples using only species with
267 occupancy rates over 75%. Samples from sites and locations within sites were used
268 as independent variables. Site and locations within sites are significantly more
269 similar to each other (CAP, $p<0.001$). Sites (shapes) and Locations (colors) are
270 plotted against CAP1 and CAP2 axes. Arrows correspond to direction and strength
271 (length) of each species. Only the top 7 species with CAP distances greater than 0.35
272 were plotted.

273

274

275 *Visual Census Surveys Results*

Across all three sites, 25 bony fish species were recorded using underwater visual censuses, representing 20 genera, 13 families, and one class (Figure 6) (Table S5), 11 of which were shared across all three sites (Table S6). Within the MPA site, visual census methods detected 21 unique species, 18 genera, and 11 families. At the edge site visual census methods detected 18 species, 16 genera, 11 families, and four classes. Lastly, at the outside site visual census methods detected 13 species, 13 genera, 10 families, and four classes. Of all taxa observed in visual census methods, 24 species were associated with rocky reef habitat and one species was pelagic-neritic. The pelagic-neritic species, top smelt (*Atherinops affinis,* Atherinopsidae), was only found in the MPA site.

On average, roving diver fish counts recorded 17.6 species per replicate survey (Range: 10-22). Visual fish counts recorded an average 7.8 species per replicate survey out of the 13 indicator species (Range: 5-10). 1m quadrats recorded an average 2.3 species of 3 target species (Range: 1-3).

Figure 6. Venn Diagram of Species Observed from Visual SCUBA Surveys.

Comparison of eDNA and Visual Census Surveys

eDNA detected 76% (19 out of 25) of species observed during all combined National Park Service transect surveys (Table S5-S6). eDNA failed to resolve *Lythrypnus dalli* (Gobiidae), *L. zebra, Sebastes atrovirens* (Sebastidae)*, S. auriculatus, S. chrysomelas,* and *S. serranoides* to species level. At the genus level, eDNA performed markedly better recovering 95% (19 out of 20) of genera observed during under water censuses. The remaining genus *Lythrypnus* was detected prior to site occupancy modeling, but occurred in only one replicate at two separate sites.

In addition to the above, eDNA recovered 31 species that were not recorded during the visual censuses conducted by the National Park Service. Of these, 30 were California fish

301 species previously recorded in Kelp Forest Monitoring Program surveys (Table S7), but not
302 observed during our paired surveys. In addition, eDNA detected the California native dog-
303 faced witch eel (*Facciolella gilberti,* Nettastomatidae) that had not previously been
304 observed by the Kelp Forest Monitoring Program.

305 There were few conspicuous differences in species observed across sites, with visual
306 census results identifying 11 common taxa across all sites (Table S6). Of these, 10 were
307 also found to be common across all sites using eDNA methods with one species
308 (*Lythrypnus dalli*) not detected by eDNA. Species richness from visual census data showed
309 that fish diversity was highest within MPA (n=21), lowest outside the MPA (n=13) and
310 intermediate (n=18) on the edge of the MPA, while eDNA had the opposite pattern.

311 **Discussion**

312 Results demonstrate the power of for detecting a broad range of fish biodiversity in
313 California kelp forest ecosystems, providing more detailed species inventories needed for
314 marine ecosystem monitoring (9). eDNA was able to detect significant differences in fish
315 communities inside, on the edge of, and outside of the Scorpion State Marine Reserve, even
316 though the closest sites were no more than 500 m apart. Even within each of these sampling
317 sites, eDNA distinguished among sample locations separated by only 50 m, highlighting the
318 sensitivity of eDNA in capturing local fish communities, and matching previous studies
319 showing fine-scale spatial resolution of eDNA signatures (12).

320 Importantly, eDNA captured 76% of fish diversity observed during visual surveys,
321 despite species rarefaction indicating insufficient sampling. In total, eDNA only failed to
322 identify six of 25 fish species observed during visual surveys, four of these being rockfish
323 (*Sebastes,* Sebastidae), a taxon that *12S* barcoding cannot distinguish to species (29). This
324 small deficiency was offset by detecting an 30 additional fish taxa not recorded during
325 paired Kelp Forest Monitoring Program visual monitoring, representing an important

advantage of eDNA. Because sampling can be obtained easily and processed economically, eDNA could allow for more frequent monitoring, expanding the scope of MPA monitoring programs while providing greater personnel safety.

The utility of eDNA for MPA monitoring

Despite the limited sampling design and the inability of our *12S* barcode to distinguish species of rockfish and gobies, eDNA largely recovered the same taxa observed in visual census surveys. This strong concordance likely stems from high eDNA detection probabilities lasting only a few hours (20), such that eDNA captures marine communities that were recently present (21). The similarity of eDNA and visual surveys is even more remarkable given that eDNA and visual surveys were taken two weeks apart, a result that strongly suggests that fish diversity captured by eDNA is truly representative of fish communities and their differences inside and outside the Scorpion State Marine Reserve (22).

In addition, eDNA recorded an addition 30 species not recorded from visual surveys, but have been previously reported in other Kelp Forest Monitoring Program surveys (Table S7). Importantly, these taxa included species of significant management concern such as endangered giant black seabass (*Stereolepis gigas,* Polyprionidae) and important commercial targets like yellowtail amberjack (*Seriola lalandi,* Carangidae). Additionally, although we focused on teleost fishes, our eDNA data included elasmobranchs, marine mammals, and marine birds, taxa that play important roles in nearshore rocky reef ecosystems, but that can be difficult to survey and monitor (9). The expanded taxonomic coverage and the ability to detect rare, or hard to observe taxa is a significant advantage of eDNA over traditional visual surveys, expanding the scope of MPA monitoring by capturing entire communities rather than a selected subset of taxa.

Key to MPA monitoring is the ability to distinguish among communities inside and outside of the MPA. Not only did eDNA detect significant differences inside and outside the

MPA, it could also differentiate among samples taken 50 m apart. This result adds to a growing literature that shows the fate and transport of eDNA in marine environments is relatively limited in space and time (20,34,35), and highlights the suitability of eDNA for comparing inside and outside of even relatively small MPAs (12).

While eDNA found significant differences inside and outside of the MPA and provided data on more taxa than visual survey methods, it did it for a fraction of the cost and effort. Roving fish diver counts, the most similar visual survey to eDNA monitoring methods, costs the Kelp Forest Monitoring Program ~$1,200 per site (Table S8). In contrast, the eDNA sampling design employed in this study including materials, labor, and transportation was ~$600 per site (Table S9)—and 25% of this total was just transportation. Moreover, total costs could have been significantly reduced by sampling in one day, which was not possible due to vessel logistics. Further cost efficiencies can come from automating lab methods and conducting sequencing in house (8).

In addition to the above, eDNA has other significant advantages. It can potentially detect invasive species, even when rare (8). Sequence data from eDNA provides an annual snapshot of standing genetic diversity, providing the ability to monitor changes over time (9). Similarly, in species with population structure, eDNA could provide evidence of range shifts associated with climate change (36). Importantly, given eDNA metabarcoding samples can be preserved and archived, eDNA samples can be reanalyzed in the future with improved metabarcoding methods to answer additional hypotheses and environmental monitoring goals (8). Combined, the above advantages of eDNA suggest that even if eDNA metabarcoding is not viewed as a full replacement for visual surveys, the power of this method, and it's ease of sampling and affordability argue for using eDNA as a critically important complementary tool to greatly expand current monitoring activities.

Limitations and Caveats of eDNA

377 Although this and other studies highlight the promise of eDNA for monitoring marine
378 ecosystems, there are also important limitations. One key limitation is the lack of universal
379 barcode loci. Four of the six undetected species in this study were rockfish in the genus
380 *Sebastes*. While the MiFish *12S* metabarcoding primers have broad utility in vertebrates,
381 rockfishes are a recent adaptive radiation (37) with a highly conserved 12S sequence,
382 resulting in the inability to distinguish among rockfish ASVs. Identifying rockfish to
383 species using eDNA approaches is critical for MPA monitoring efforts in California as
384 *Sebastes* are important for commercial and recreational fisheries (38) and play a wide array
385 of functional and ecological roles in nearshore ecosystems (37).
386 In addition, eDNA failed to detect two gobies, *Lythrypnus dalli* and *L. zebra*
387 (Gobiidae). Previous efforts to barcode *L. dalli* for the FishCARD reference database found
388 two insertions not found in any other California goby, including the sister species *L. zebra*
389 (29). Thus, primer mismatch may have limited the amplification and detection of some *L.*
390 *dalli* in our eDNA samples. Alternatively, the eDNA methods employed here may not be
391 suited for small, crevice-dwelling fish species such as gobies. Species of *Lythrypnus* rarely
392 leave the reef boundary layer (39). As such their eDNA maybe entrained close to the reef,
393 resulting in hyper-spatial variability of eDNA signatures (12). More work is necessary to
394 determine whether eDNA can reliably detect species living in interstitial reef habitat. This
395 limitation, however, is not unique to eDNA as the Kelp Forest Monitoring Program
396 employs 1 m^2 quadrat surveys, specifically designed to capture these taxa. Likewise eDNA
397 surveys that specifically sample within the boundary layer may be needed to survey benthic
398 cryptic species.
399 Another limitation of eDNA is standardizing processing techniques, including the
400 spatial design of field sampling, number of replicates, and sequencing depth (8,15,19). The
401 three replicate water samples taken from a single location and time recovered only 81.5%
402 of the species present based on modeled species coverage estimates of species with at least

403 75% occupancy. This value increased to near saturation (>99%) by sampling three replicate
404 water samples from three locations along a 100 m transect. That said, rarefaction curves
405 indicated that additional sampling would have recovered additional species. These results
406 provide important benchmarks for replication and sampling efficiency within nearshore
407 marine environments and highlight the need to adjust sampling intensity and replicates,
408 depending on the questions to be addressed with eDNA.

409 Despite not achieving saturation with our sampling design, we did observe a transition
410 from exponential to linear addition of species detections with additional sampling similar to
411 that previously demonstrated in mesocosm experiments (19). This shift likely reflects the
412 biological reality of eDNA within marine ecosystems, with a few taxa being abundant and a
413 long tail of low abundant species (15). As such, while only a few replicates are needed to
414 capture local core species diversity, high technical (PCR) and biological (bottle) replication
415 may be required to saturate species detection (19). Thus, if the goal is to detect rare species,
416 it is imperative to increase sample number, an unsurprising result given the reality of
417 detection probabilities of rare taxa (18). Despite this caveat and our relatively limited
418 number of sample replicates, we still detected rare species such as giant black seabass
419 (*Stereolepis gigas,* Polyprionidae) suggesting that eDNA is likely still superior to visual
420 techniques at rare species detection (10).

421 *Importance of Site Occupancy Modelling*

422 Site occupancy modeling showed that almost all species (48/50) with occupancy rates
423 higher than 75% were common Southern California kelp forest species with the exception
424 of the spotted cusk eel and dog-faced witch eel (40). In contrast, almost all pelagic and
425 intertidal species that should not be present in a kelp forest had low occupancy rates and
426 were detected only in a single bottle replicate (Tables S1-S2). These low occupancy
427 detections cannot be contamination because they did not occur in field or laboratory

428 controls; instead, they likely represent eDNA transported between habitats (16). Regardless,
429 site occupancy modeling removed the vast majority of unexpected kelp forest fishes,
430 highlighting its value for determining true species detections in a rigorous and repeatable
431 way (18,19), aiding in the interpretation and comparison of eDNA results.

432 While site occupancy modelling removed non-kelp forest taxa (e.g. Blue whale;
433 *Balaenoptera musculus* (Cetacea); California sea lion, *Zalophus californianus* (Otariidae);
434 pelagic cormorant *Urile pelagicus* (Phalacrocoracidae); Table S10), it also removed some
435 kelp forest species (e.g. zebra goby, *L. dalli,* Gobiidae; swell shark, *Cephaloscyllium*
436 *ventriosum,* Scyliorhinidae; zebra-perch *Hermosilla azurea,* Embiotocidae; California angel
437 shark, *Squatina californica,* Squatinidae). These results highlight the need for increased
438 replication depending on the management question, just as it may require more visual
439 surveys to observe numerically rarer taxa, such as sharks. Although the ability of eDNA to
440 detect marine mammals and birds is useful, visual observations maybe more effective
441 depending on the taxa, suggesting that complementary methods may yield the most
442 effective sampling regime (41).

443 *Diversity inside and outside MPAs*

444 Traditional visual surveys most often report higher biodiversity and biomass inside MPAs
445 (42), including Scorpion State Marine Reserve (6). However, our results surprisingly
446 indicate lower diversity inside the MPA. This paradoxical result is partially explained by
447 the inability of eDNA to resolve *Sebastes* species that were visually observed inside (n=3)
448 and on the edge of the MPA (n=1), but not outside. In addition, despite standardize sample
449 concentration during pooling, sites outside the MPA had ~50% more read depth. Increased
450 read depth should increase species detection, although species rarefaction curves suggest
451 that all samples had sufficient read depth to saturate species richness following site
452 occupancy modelling (Figure S1).

Instead, a more likely explanation for this unexpected result is that low density of kelp forest fishes outside the MPA increased the detection of non-kelp forest taxa advected from elsewhere. In total, 46.4% of taxa detected outside the MPA were non-rocky reef species such as California angel shark (*Squatina californica,* Squatinidae), Chub mackerel (*Scomber japonicus,* Scombridae), and Ocean sunfish (*Mola mola,* Molidae). Although these species occasionally pass through nearshore rocky reef environments, a more likely explanation is that eDNA from these species were transported from nearby pelagic, intertidal, and sandy bottom ecosystems (16,35). While such transport would be expected at all sites, high fish abundance inside the MPA would likely result in a strongly skewed ratio of endogenous kelp forest eDNA to exogenous pelagic eDNA (16), with the signal of kelp forest taxa dominating that of pelagic species.

This paradoxical pattern of species richness highlights that eDNA data must be interpreted with caution (43). Metabarcoding methods often perform unexpectedly when DNA concentrations are low, increasing the probability of sequencing rare species (8). Thus additional ecological metrics to species richness, ones that are more representative of ecological patterns and processes, are needed to optimally interpret eDNA results (15). These results ultimately highlight the value of ground truthing eDNA results with visual surveys in novel applications to ensure proper interpretation of results (41).

Conclusion

Marine protected areas are indispensable tools for protecting marine ecosystems and effective monitoring is paramount to their success (1). Our results demonstrate that eDNA can distinguish fish assemblages inside and outside MPAs, and can detect other vertebrates, like marine mammals and birds, of special conservation concern.

Given its power, ease of sampling and relative affordability, eDNA could provide critical cost-added-benefits of repeated temporal or expanded spatial sampling of marine protected

478 areas. In particular, eDNA metabarcoding can overcome many of the current limitations of
479 visual monitoring, increasing sampling frequency and expanding monitoring beyond a small
480 subset of "important" focal taxa. Such expanded monitoring would improve our ability to
481 understand the ecological processes, human impacts, and management strategies that affect
482 marine community communities that MPAs are designed to protect.

483 However, important aspects of eDNA remain unresolved, notably determining
484 abundance and biomass via eDNA (44). Given the mixed results on the efficacy of biomass
485 estimates from eDNA in marine systems (8,44), eDNA will not be viewed yet as a
486 wholesale replacement for visual monitoring, but instead as a powerful complementary
487 tool. There will always be value in the direct observation by divers in particular for
488 informing size class distributions and sex ratios (24), but eDNA provides an important way
489 to simultaneously make surveys more comprehensive and efficient. By replacing aspects of
490 underwater visual surveys, eDNA could reduce the dive time per site, allowing more sites
491 to be surveyed more frequently or improve overall biodiversity estimates. Additionally,
492 whereas it takes 3 months for the Kelp Forest Monitoring Program to complete diver-based
493 surveys, field collection of eDNA could be completed in a week, allowing for surveys to
494 occur during short periods of good weather in the winter when full visual surveys would be
495 impossible. As such, eDNA could greatly expand current monitoring activities across
496 space, time, and depth, providing resource managers critical information on the response of
497 MPAs to changing environments and management practices, and contributing greatly to
498 marine sustainability.

499

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502 analysis, Z.G.; resources, Z.G.; data curation, Z.G.; writing—original draft preparation,
503 Z.G., J.S., D.K., E.Z., and P.B.; writing—review and editing, Z.G., J.S., D.K.,E.Z., and

P.B.; visualization, Z.G.; supervision, D.K. and P.B.; project administration, Z.G. and P.B.; funding acquisition, Z.G. and P.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability: We intend to archive all data on the Dryad Digital Repository and a link will be provided upon acceptance.

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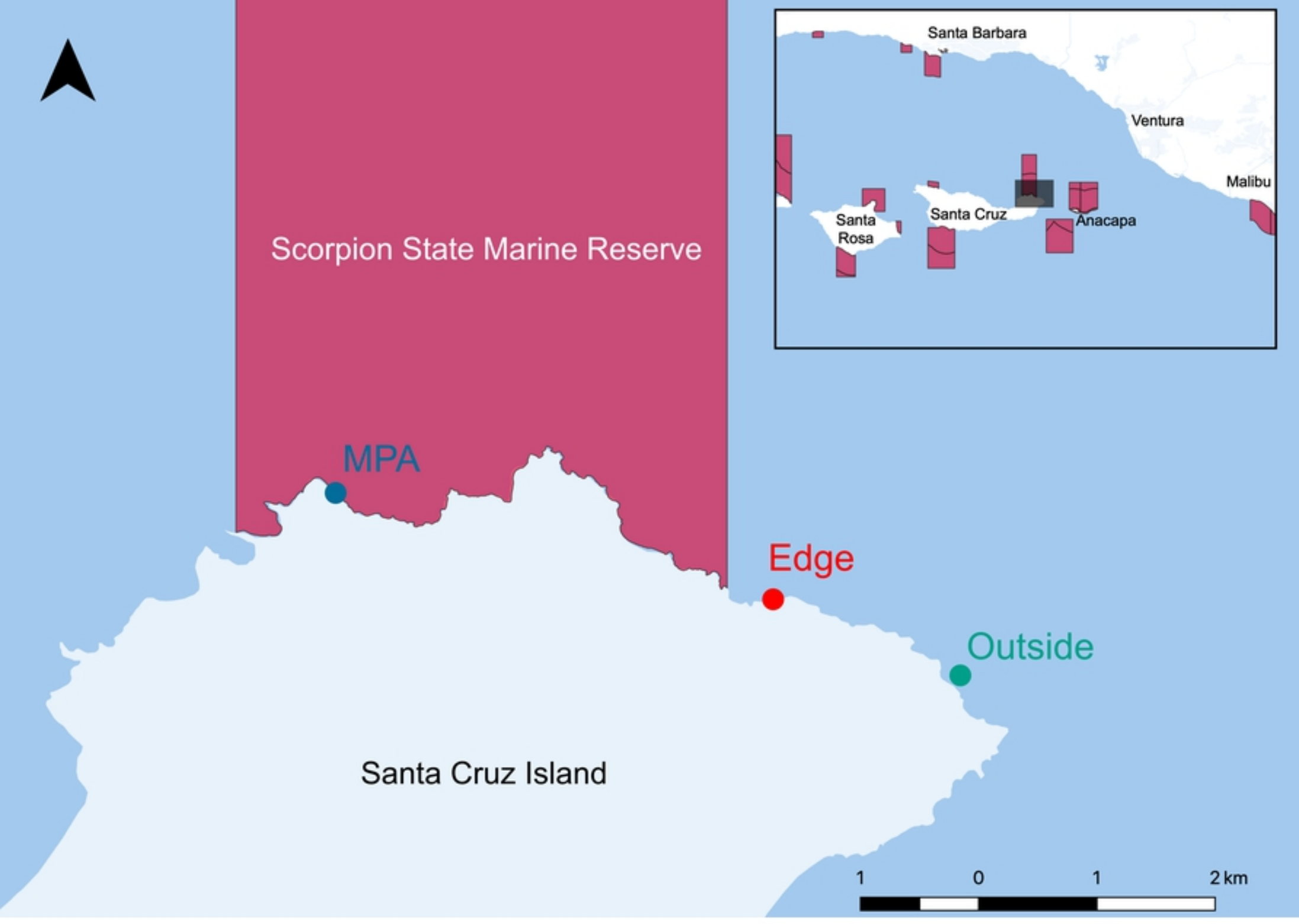
Scorpion State Marine Reserve
MPA
Edge
Outside
Santa Cruz Island
Santa Barbara
Ventura
Malibu
Santa Rosa
Santa Cruz
Anacapa
1
0
1
2 km

Figure 1

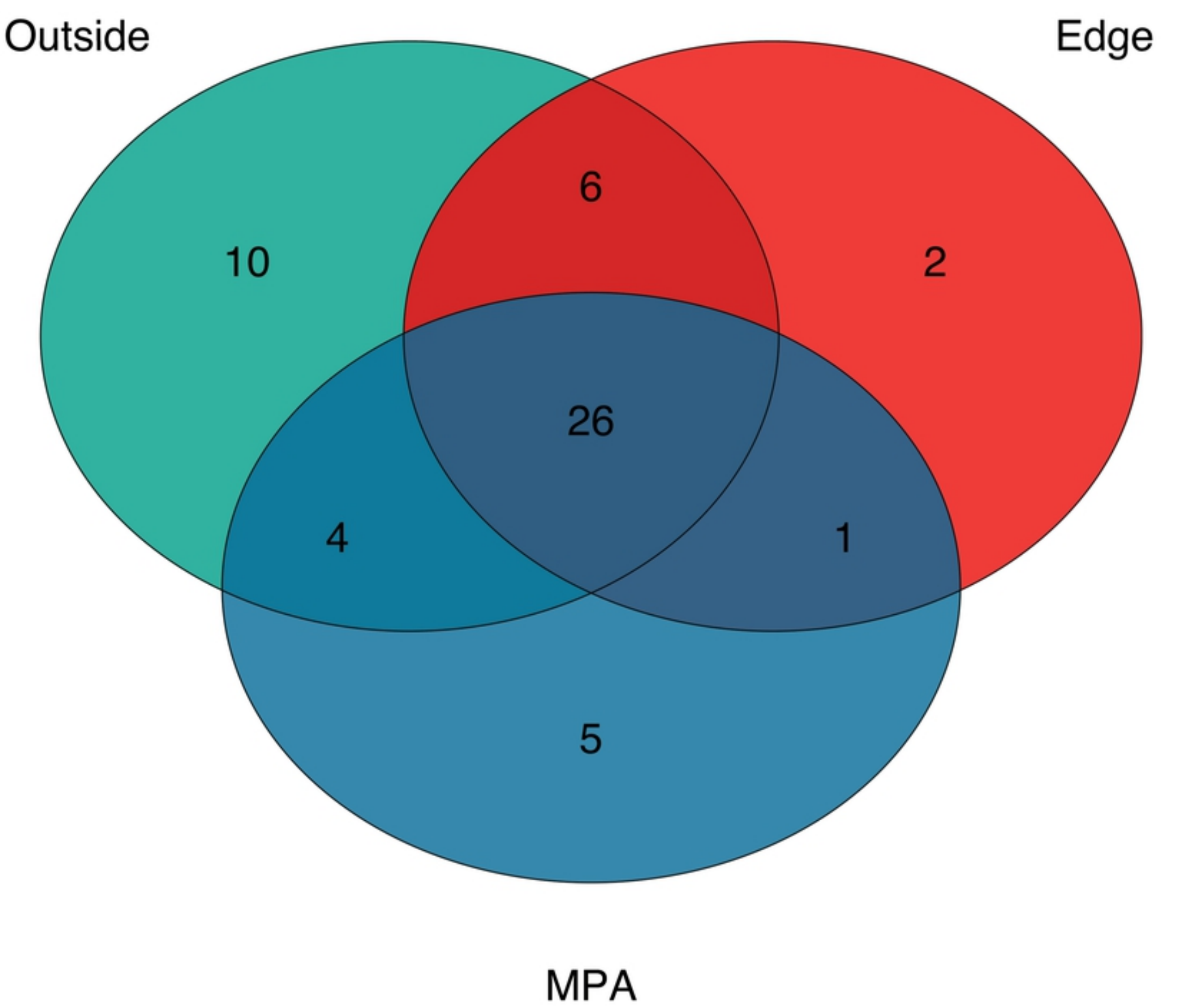

Figure 2

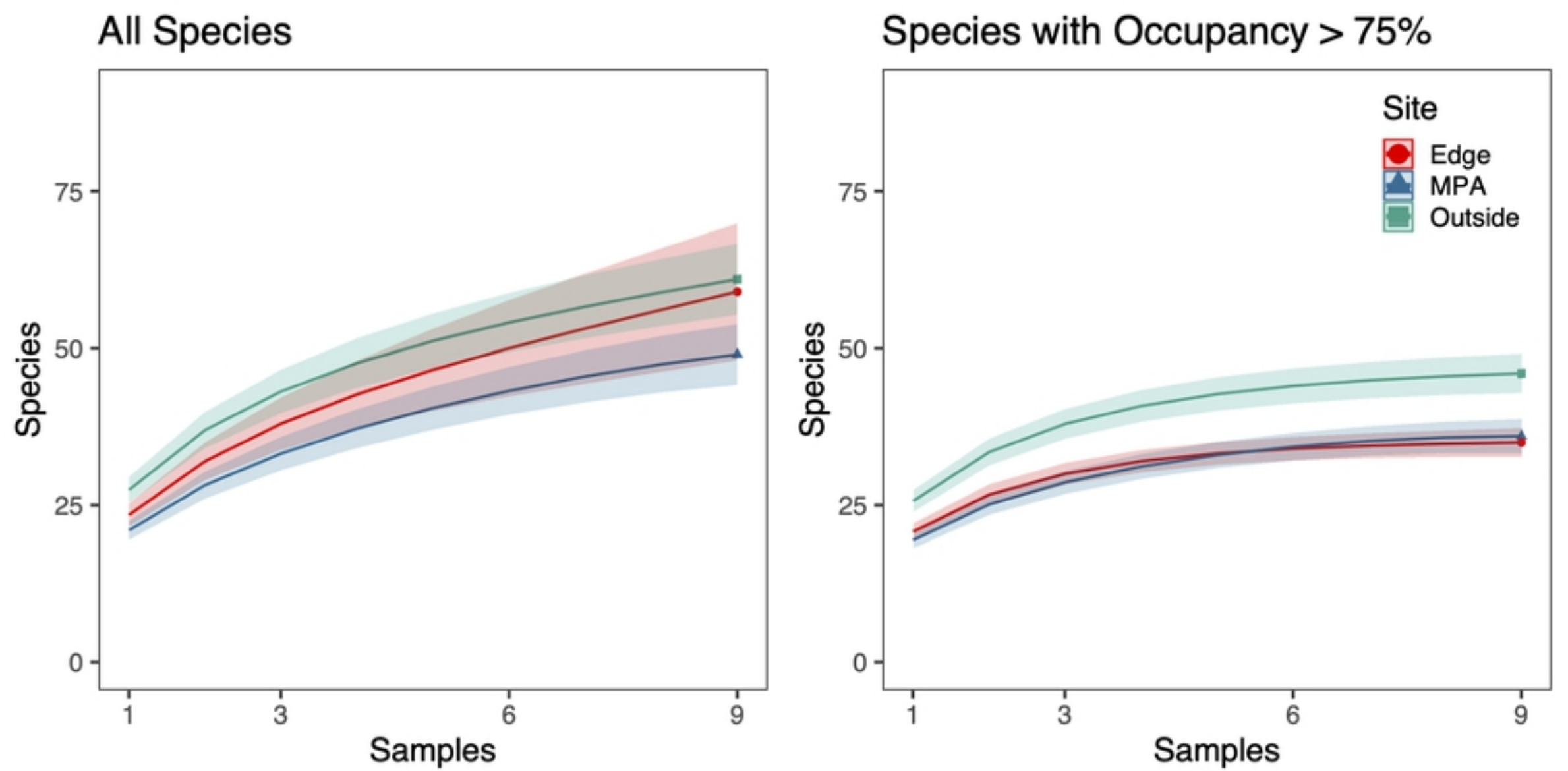
All Species
Species with Occupancy > 75%
Site
Edge
MPA
Outside
Species
Samples
0
25
50
75
1
3
6
9

Figure 3

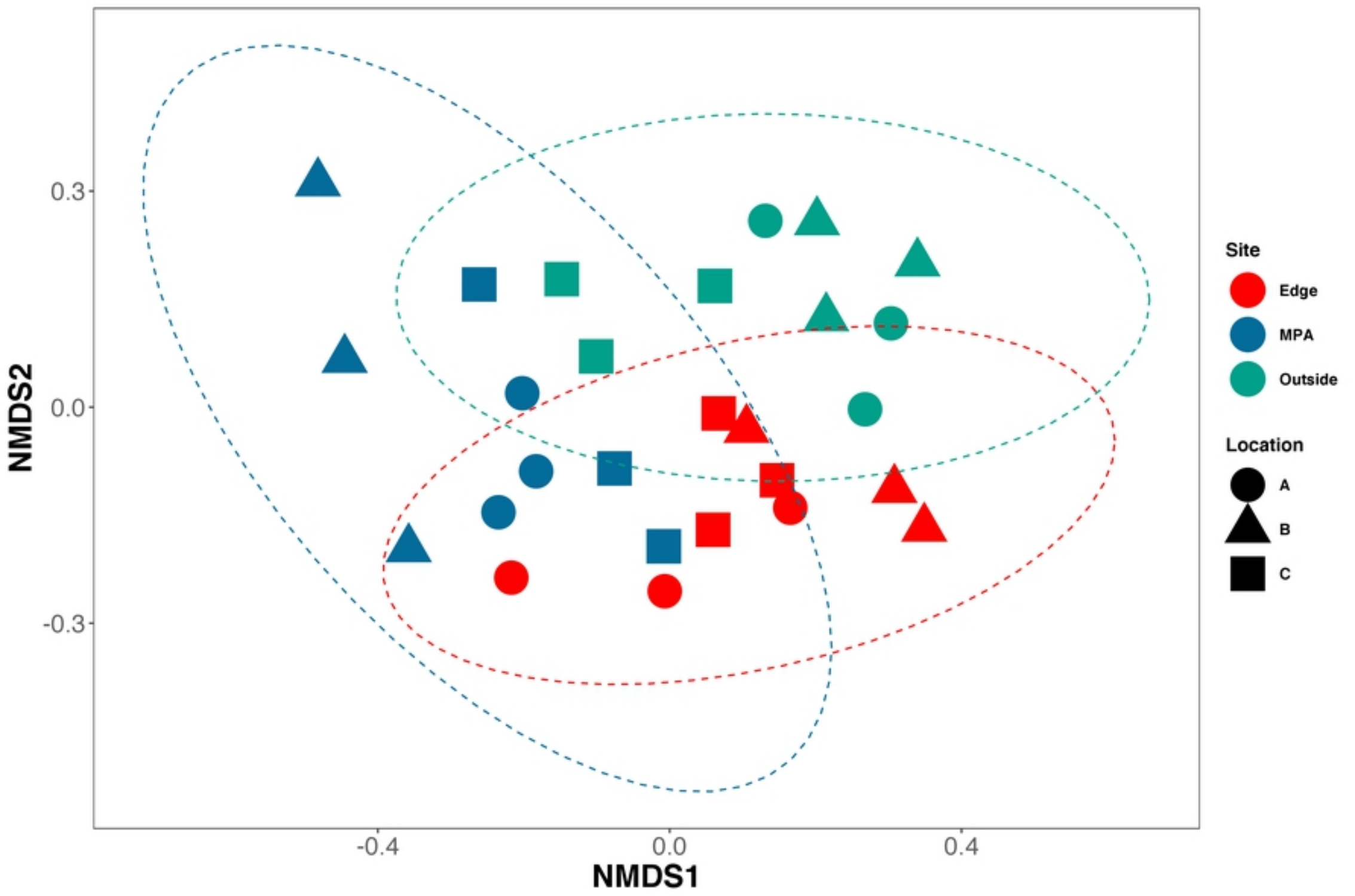
0.3
0.0
-0.3
NMDS2
-0.4
0.0
0.4
NMDS1
Site
Edge
MPA
Outside
Location
A
B
C

Figure 4

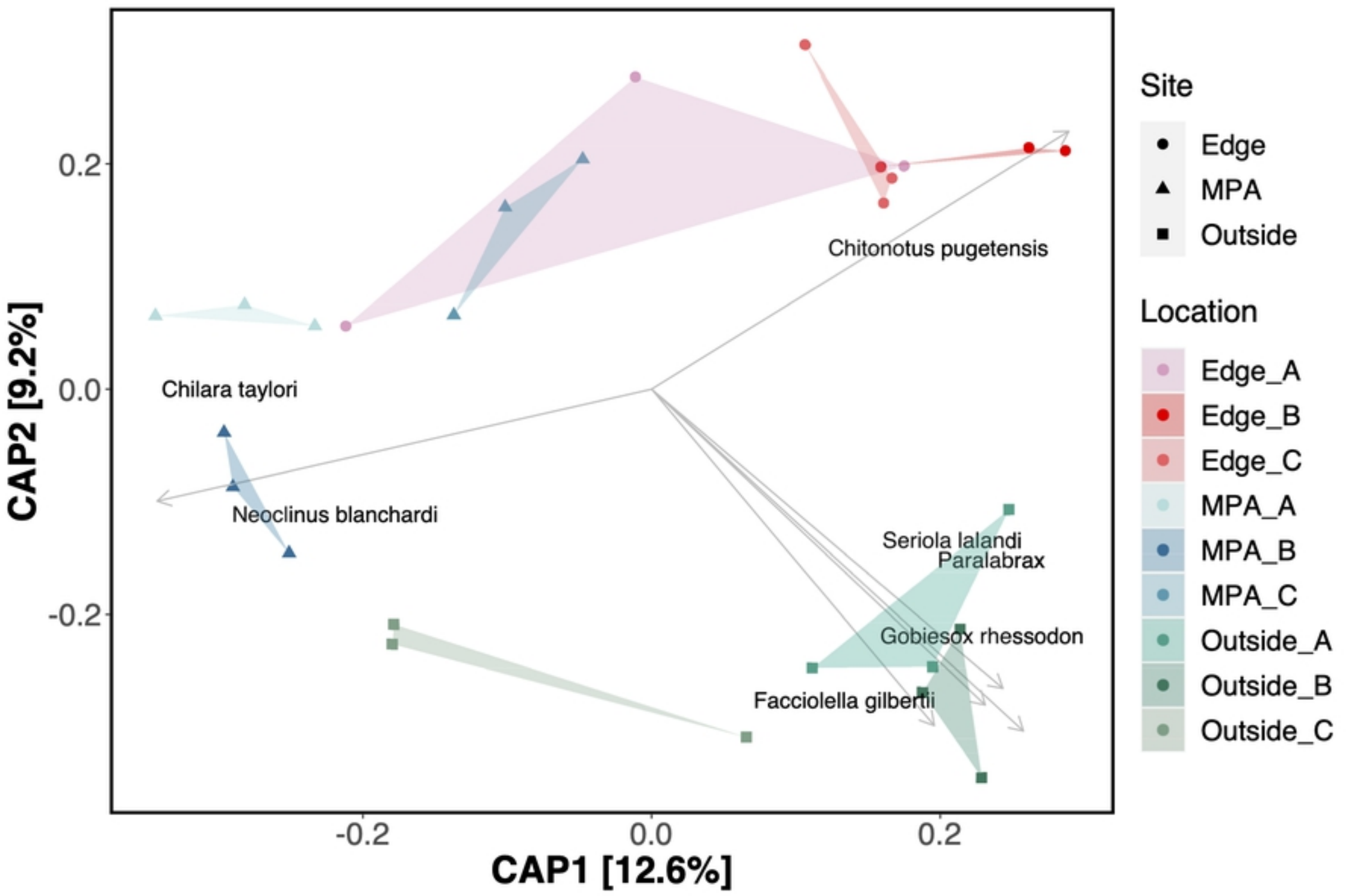
Site
Edge
MPA
Outside
Location
Edge_A
Edge_B
Edge_C
MPA_A
MPA_B
MPA_C
Outside_A
Outside_B
Outside_C
Chitonotus pugetensis
Chilara taylori
Neoclinus blanchardi
Seriola lalandi
Paralabrax
Gobiesox rhessodon
Facciolella gilbertii
0.2
0.0
-0.2
CAP2 [9.2%]
-0.2
0.0
0.2
CAP1 [12.6%]

Figure 5

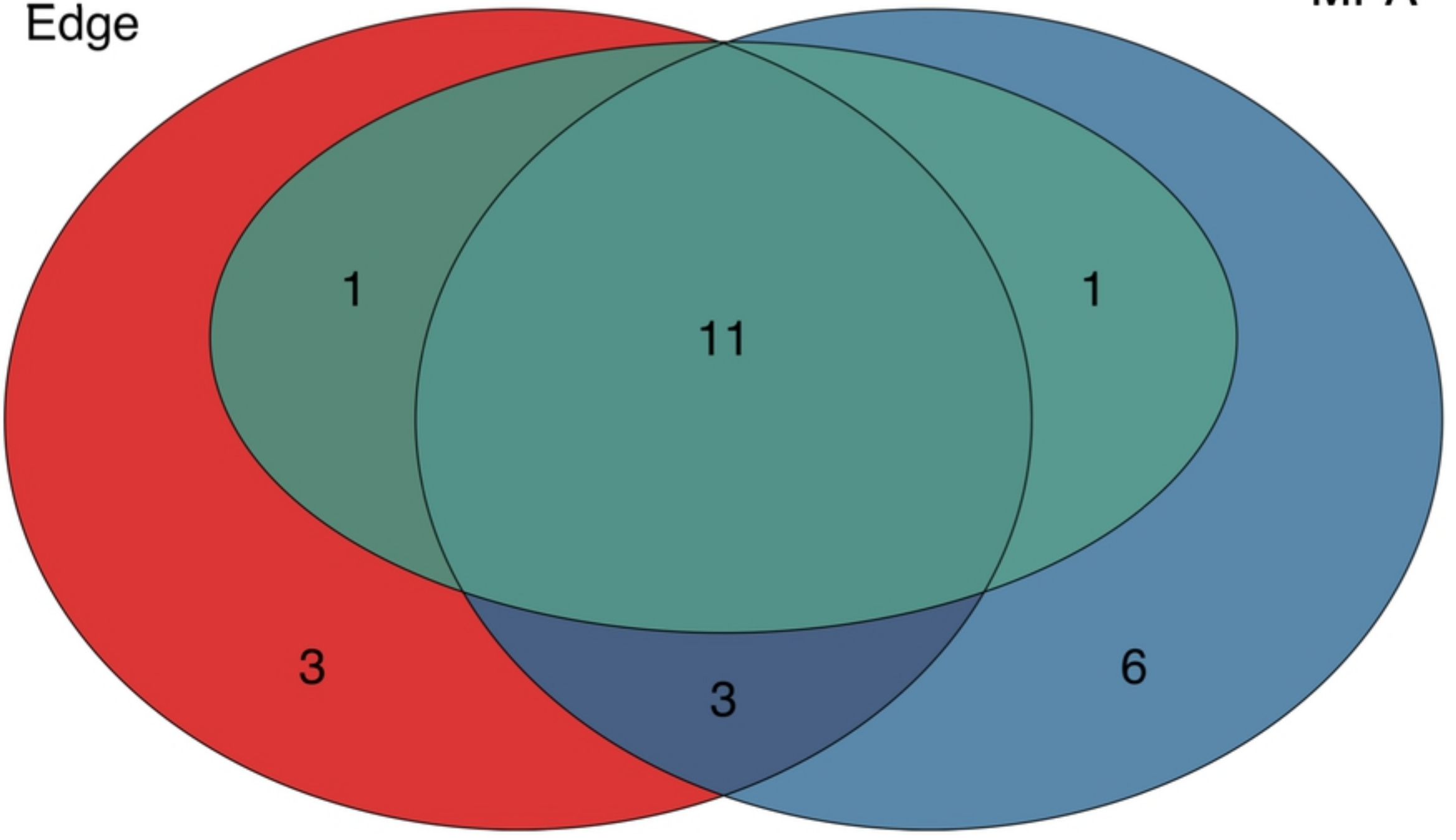

Figure 6