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Nektonic assemblages determined from baited underwater video in protected versus unprotected shallow seagrass meadows on Kangaroo Island, South Australia

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ABSTRACT: As the number of marine protected areas grows worldwide, it is important to understand how existing protected areas have functioned. Baited remote underwater video stations (BRUVS) are becoming a widely used technique for monitoring reef fish, but with few studies having so far used BRUVS in seagrass meadows, it remains unclear how they perform within these habitats. The aims of this study were to trial the use of BRUVS in shallow seagrass habitats and to compare animals observed at Pelican Lagoon Aquatic Reserve to 2 broadly similar locations on Kangaroo Island that have had no protection. BRUVS identified 47 distinguishable taxa from 5 phyla, with the majority (79%) of those being fishes. Assemblages taking into account relative abundances were not significantly different between protected and unprotected areas; however, species compositions alone varied significantly across all 3 locations. Only 2 out of 18 commercially and/or recreationally targeted species had a higher abundance within the reserve. Overall, BRUVS were found to be suitable for use in seagrass habitats; however, some limitations (particularly the potential for obstruction of the camera by seagrass blades) may exist.

KEY WORDS: BRUVS · Conservation · Fish · Habitat description · Marine protected area · Method development · *Posidonia* · Video

INTRODUCTION

As threats such as climate change, overfishing and pollution increase pressure on the world's marine ecosystems, governments are looking for ways to manage these threats. The use of marine protected areas is one option that is becoming increasingly popular as a tool to conserve biodiversity and manage oceanic resources (Warner & Pomeroy 2012). Marine protected areas are locations set aside within the ocean for conservation, which vary in protection levels. Aside from restricted-access (or 'no-go') zones, the highest level of protection for an area is generally a sanctuary (or 'no-take') zone, where fishing, dredging, mining and other potentially harmful extractive activities are prohibited (Agardy et al. 2003); these areas are often referred to as 'reserves'. Sanctuary zones have been shown, on average, to increase diversity, abundance, size and biomass for a range of fish species in both temperate and tropical areas worldwide (Halpern & Warner 2002, Halpern 2003, Lester et al. 2009, Stewart et al. 2009) and also

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increase the diversity and abundance of reef algae and invertebrates (Edgar & Barrett 1999).

An effective monitoring program is essential for any marine protected or otherwise managed area to assess whether its goals are being achieved. Monitoring is needed to ensure that a reserve is functioning as intended and to provide feedback to adapt management strategies over time where necessary. Despite the well-known need for monitoring, many protected areas fail to have adequate plans in place, and this is probably because of the perceived high cost of long-term monitoring, among other challenges (Day 2008, Warner & Pomeroy 2012). To reduce costs of monitoring, more efficient and cost-effective methods and techniques must be developed and trialled for specific habitat types. Many temperate marine reserves will include significant areas of seagrass meadow, a habitat that has been little studied in terms of its response to protection. For example, South Australia's protected areas have had minimal ongoing monitoring, and it is unclear how these reserves have been operating. Only 2 protected reef areas in South Australia have been studied for performance: Point Labatt Marine Reserve (Currie & Sorokin 2009) and Aldinga Reef Aquatic Reserve (Edgar & Stuart-Smith 2009), with neither of those containing seagrass-dominated habitats.

Seagrasses are important components of many coastal ecosystems (Hemminga & Duarte 2000). Their roots and rhizomes stabilise sediment and prevent coastal erosion (Duarte 2002, Orth et al. 2006). Seagrasses also provide shelter, habitat and foraging sites for many fish species (Hemminga & Duarte 2000, Beck et al. 2001, Jackson et al. 2001, Gillanders 2006), with studies having shown that loss of seagrass habitats can result in reductions in fish recruitment, density and biomass (Pihl et al. 2006).

Baited remote underwater video stations (BRUVS) have often been used in monitoring programs in protected areas (e.g. Cappo et al. 2004, Malcolm et al. 2007, McKinley et al. 2011, Gladstone et al. 2012). The non-destructive nature of this method along with its ease of use, replicability and ability to detect a range of fish species (Harvey et al. 2007) make BRUVS sampling suitable for such monitoring programs. Currently, BRUVS have been used mainly in coral or rocky reef habitats (e.g. Cappo et al. 2007, 2011, Watson et al. 2007), with some use in other habitats such as unconsolidated substrates or estuaries (e.g. McKinley et al. 2011, Harvey et al. 2012, Schultz et al. 2012). Overall, few studies have used BRUVS in seagrass (e.g. Gladstone et al. 2012), even though this method may have a great potential to monitor seagrass habitat. Other video techniques, however, have been used in seagrass (e.g. Smith et al. 2011).

The objectives of this study were to investigate nektonic biota in 3 shallow seagrass areas at Kangaroo Island, South Australia, using BRUVS. In particular, the aims of this paper were (1) to trial the feasibility of using of BRUVS for monitoring programs in local seagrass habitats; and (2) to determine how several decades of protection from fishing affects assemblages in a protected seagrass habitat at Kangaroo Island as compared to similar, but unprotected, areas at the same Island.

Specifically, we predicted that (1) BRUVS would observe a wide range of nektonic species, demonstrating its suitability for monitoring in seagrass; (2) assemblages observed via BRUVS would differ between sites; (3) sanctuary-zone protection status would have a different composition structure from unprotected sites; and (4) higher abundances of fisheries-targeted species would be seen in a reserve compared to unprotected sites.

METHODS

Sampling sites

Pelican Lagoon Aquatic Reserve (PLAR) on Kangaroo Island, South Australia, is one of that state's oldest protected areas, having been declared in 1971 (as the American River Aquatic Reserve); however, there has been very little research undertaken in this area to date. The reserve covers an area of 15.2 km^2 and is composed mainly of shallow, sheltered seagrass meadows and tidal flats, with some deeper pools up to 10 m in depth (PIRSA 2012). The reserve was set up primarily to protect juvenile fish stocks that used this area as a key nursery ground (PIRSA 2012). The reserve is also thought to be a refuge for adult fish and is frequented by many different species of water birds (Kinloch et al. 2007). The key economic fish species occupying this area are King George whiting *Sillaginoides punctata*, southern sea garfish *Hyporhamphus melanochir* and Western Australian salmon *Arripis truttaceus* (Kinloch et al. 2007). The likelihood of illegal poaching within the reserve is low because of vigilant oversight by residents within the community (PIRSA 2012). Other shallow seagrass-dominated embayments on northeastern Kangaroo Island include Eastern Cove (EC) and Bay of Shoals (BoS), which had no protected status when this study was done.

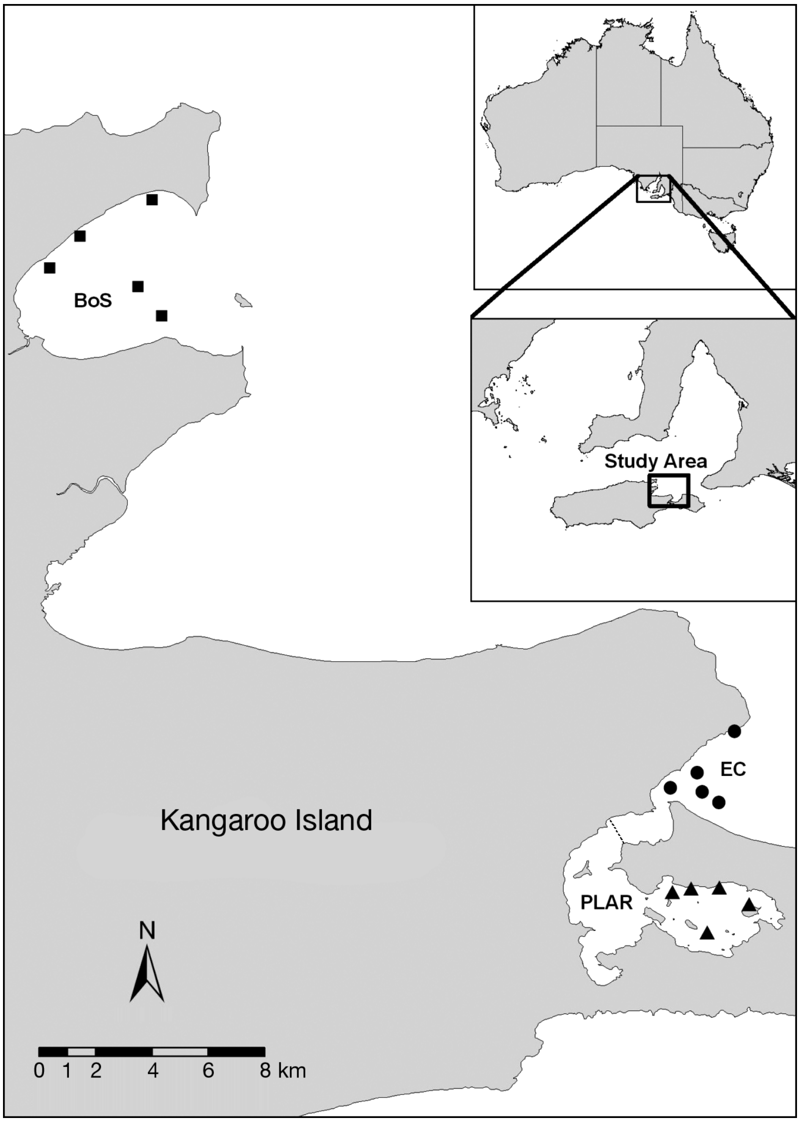

Fig. 1. Sampling locations in Kangaroo Island, South Australia, and their 5 sites selected for this study; BoS = Bay of Shoals (squares), EC = Eastern Cove (circles), PLAR = Pelican Lagoon Aquatic Reserve (triangles). The seaward limit of the aquatic reserve is shown as a dotted line between 'PLAR' and 'EC'. Only the eastern portion of PLAR could be sampled because of a lack of seagrass and very shallow tidal flats in the western portion

Sampling was conducted at 3 locations on Kangaroo Island, South Australia: BoS (35.6179° S, 137.6140° E), EC (35.7777° S, 137.7939° E) and PLAR (35.8204° S, 137.7917° E) (Fig. 1). Two of these locations have little or no protection from fishing (BoS and EC), while PLAR has strong protection, with no fishing allowed within the reserve since 1971. BoS and EC were selected for comparison with PLAR because of their broad similarities in habitat and proximity but different level of (i.e. no) protection. Five sites were selected within each location based on depth, limited proximity to each other and suitable habitat type (i.e. the seagrasses *Posidonia* spp. being present). Sampling was conducted on January 16 to 20, 2012, during mostly sunny conditions with winds up to 20 knots. Water quality variables including salinity (ppt) and temperature (°C) were measured during sampling using a TPS WP-84 conductivity-salinity probe.

Habitat description

Habitat was assessed using the BRUVS (see below) field of view. After deployment, when the BRUVS and any disturbed sediment had settled (approximately 1 to 5 min), a still image was taken, and using SeaGIS TransectMeasure (www.seagis.com.au/transect.html), the image was analysed using a dot-point method, in which 100 points were randomly applied to the frame, and the habitat type (i.e. *Posidonia* spp., *Halophila* spp., *Heterozostera* spp., macroalgae, detritus, unconsolidated bare substrate, invertebrates) beneath each point was recorded.

BRUVS sampling setup

Animal assemblages were investigated using BRUVS. Four replicate drops were conducted at each site in each location (total n = 60). Single BRUVS units, as opposed to stereo, were used in this study because the remote location compounded some logistical and vessel space restrictions. The shallow depths studied prevent larger vessels from accessing the sites, and depth was also a significant limiting constraint for camera height, making some obstruction of the lens unavoidable. Where possible, units were placed on the edge of seagrass beds, in sparse patches or in sand patches within beds, to minimise this obstruction of the lens by seagrass blades. BRUVS were set up based on Colella et al. (2010). High-definition Canon HV30 video cameras and Panasonic AY-

DVM60FF 60 min tapes were used. BRUVS units were baited with approximately 500 g of crushed pilchards. Four units were used at a time and were dropped consecutively at a site, each approximately 200 m apart, to reduce bait plume interactions. Units were left for 1 h before retrieval. The depth range of BRUVS drops was between 0.5 and 2.7 m.

Analysing nekton assemblages

Each 60 min tape constituted 1 replicate. BRUVS videos were analysed for observed fish diversity and abundances using SeaGIS EventMeasure software (www.seagis.com.au/event.html). Fish identification was carried out with the aid of reference books (Kuiter 1996, Edgar 2008, Gomon et al. 2008). Abundance was recorded as MaxN, which is the maximum of the total number of individuals (for each species or taxon) observed in a single frame throughout the duration of a single sample recording. MaxN is a conservative estimate of abundance, particularly where large numbers of fish are present (see reviews by Willis et al. 2000, Cappo et al. 2003, 2004). Three BRUVS videos (one from each location) were unreadable because of equipment failure and/or operator error and so were excluded from the analysis, leaving a final n = 57.

BRUVS video quality covariates

Observed conditions within each tape, such as water turbidity (classified as either high, medium or low depending on visibility through the water column), level of obscurity by seagrass (classified as either high, medium or low depending on visible obstructions to the camera lens), seagrass height (classified as either tall, medium or low depending on height, and, to a lesser extent, number of blades above the bait arm), relative field of view (classified as either to bait bag or beyond bait bag), whether or not the camera was zoomed in and any observed focus issues, were also recorded. Frequencies of these were used as covariates in further analyses to assess any influence on MaxN estimates.

Data analysis

The hierarchical sampling design for this study had 3 locations and 5 sites nested within each location. The general approach was to analyse whether either single or multiple variables differed across each of these nested factors, with the factors being Location (a fixed factor with 3 levels) and Site (a random factor nested within Location with 5 levels in each location). All analysis was carried out using PRIMER v.6 (Clarke & Gorley 2006) and PERMANOVA+ (Anderson et al. 2008) software.

In analyses of the BRUVS-viewed habitat, percent covers of unknown origin, the water column, bait arm or bait bag were excluded from the analysis, as they did not contribute useful information about the habitat type. This also allowed for variation in the degrees of freedom for the statistical analysis. No transformations were deemed necessary. Multivariate permutational analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity index was used to test the hypothesis that habitats differed among locations and/or sites, with the factors as per the statistical model given above. This test was performed on the habitat percent covers assessed from BRUVS with 999 permutations.

Differences between mean MaxN (all species summed or just for fished species) per location or site were tested using a univariate 2-factor nested PERMANOVA based on Euclidean distances of single variables to achieve a distribution-free version of ANOVA tested via permutation (as per Anderson et al. 2008). Significant results from this model were explored further in PERMANOVA using pair-wise tests. The same test was done on data for selected individual species to determine any significant differences between locations and/or sites.

Because of the unknown area that was sampled by the BRUVS, multivariate data were range standardised by the total (i.e. each entry was divided by the total abundance in that sample, across all species) before analysis. The Bray-Curtis dissimilarity index was used to calculate the multivariate dissimilarity among BRUVS surveys (with the input file being the MaxN values for each taxon per tape) in terms of their taxonomic composition and relative abundances of species between samples. Two-factor PERMANOVA as above was used to test the hypotheses that multispecies assemblages differed among locations and/or sites. The above analyses were also applied to presence/absence-transformed data to investigate just the species composition of each location (i.e. regardless of abundances).

Constrained ordination plots, generated by canonical analysis of principal coordinates (CAP, Anderson et al. 2008), were used to visualise assemblage similarities, explore groups in multivariate space, assess how distinct the groups were from one another and test directly the simpler hypotheses that either protection influenced fish assemblages or fish assemblages differed between locations. The leave-one-out allocation procedure provided a statistical estimate of the misclassification error between groups (Anderson et al. 2008). The influence of protection on fish assemblages was also tested using PERMANOVA with a contrast analysis added (contrast = PLAR vs. EC and BoS), with the factors as above, which was also visualised using CAP ordination. Overall significance of the trace (sum of all canonical eigenvalues over all *m* principal coordinate axes used in CAP) and delta (first squared canonical correlation) statistics were tested using 999 permutations (Anderson et al. 2008).

Contingency tables with Pearson's chi-squared tests were used to assess whether the frequency of individual variables related to the quality of video images differed between locations. These were also analysed individually by including them as a covariate within the nested design (see above) using a univariate 2-factor multivariate permutational analysis of covariance (PERMANCOVA) on the total MaxN or the sum for fished species, with the factors as above.

RESULTS

Habitat description

Temperature and salinity recorded at the time of sampling were similar across the 3 locations, and no impact on the assemblages was observed.

Approximately 20% of the BRUVS units dropped were not touching seagrass beds. Of that 20%, all were less than 1 m from the edge of a seagrass bed. Habitat assessment of BRUVS videos found that seagrass *Posidonia* spp. dominated, with between 70 and 74% cover on average per location (Fig. 2). PERMANOVA results showed no significant differences between locations, but significant differences were observed as small-scale variation (i.e. among sites, Table 1).

Nekton assemblages

BRUVS recorded 2995 ind. (summed MaxN values) from 47 taxa of nekton. Of the 47 taxa, 31 were teleosts, with 5 elasmobranchs, 1 diving bird, 2 marine mammals and 8 invertebrates (4 crustaceans, 2 cephalopods, 1 echinoderm and 1 cnidarian, Table 2). Small schooling fish within the family Atherinidae made up the greatest abundance in all 3 locations (especially at BoS and EC) but could not be distinguished to species. Eight taxa could only be identified to genus or family, and 3 taxa remain unidentifiable because of their small size combined

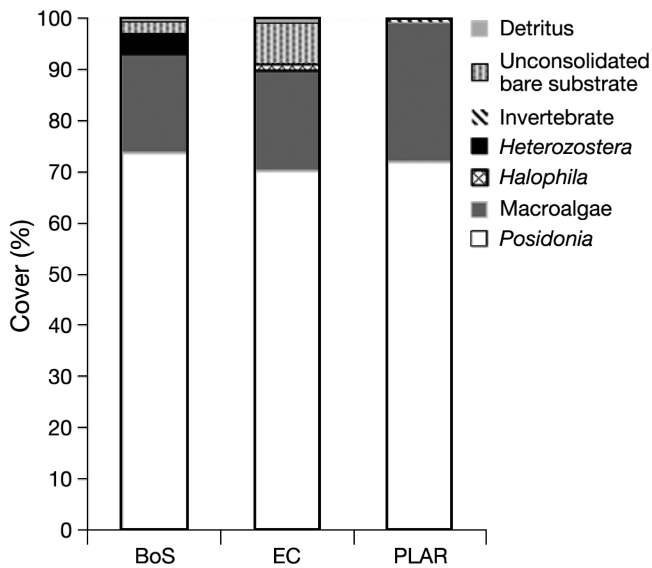

Fig. 2. Mean percentage cover estimates of 7 habitat types from 3 locations on Kangaroo Island, South Australia, taken using baited remote underwater video stations. BoS = Bay of Shoals, EC = Eastern Cove, PLAR = Pelican Lagoon Aquatic Reserve

Table 1. Multivariate PERMANOVA results from habitat assessed using baited remote underwater video station (BRUVS) videos (n = 57), total biotic assemblages observed based on standardised MaxN estimates of each species using abundances from BRUVS (n = 57) and presence/absence-transformed assemblages observed from BRUVS (n = 57), all taken from 3 shallow, sheltered seagrass locations in Kangaroo Island, South Australia. All 3 post-hoc pairwise comparisons of locations for presence/absence were significant ($t > 1.72$, $p < 0.017$). Significant results are shown in **bold**

Source	df	Habitat			Total fish assemblage			Presence/absence		
		MS	Pseudo-*F*	p(perm)	MS	Pseudo-*F*	p(perm)	MS	Pseudo-*F*	p(perm)
Location	2	833.9	0.5	0.74	8178.9	1.6	0.065	6969.7	3.4	**0.001**
Site (location)	12	1578.6	1.8	**0.03**	5077.1	2.9	**0.001**	2082.4	2.3	**0.001**
Residual	42	890.8			1767.9			913.76		

Table 2. Taxa identified using baited remote underwater video stations from n = 57 drops across 3 shallow seagrass locations on Kangaroo Island, South Australia. Numbers show how many sites (out of 5 total at each location) each species was observed at. BOS = Bay of Shoals, EC = Eastern Cove (EC), PLAR = Pelican Lagoon Aquatic Reserve

Family	Taxon		Taxonomic authority	No. of sites		
	Genus	Species		BOS	EC	PLAR
Teleosts						
Apogonidae	*Siphamia*	*cephalotes*	(Castelnau 1875)	2	5	4
	Vincentia	*conspersa*	(Klunzinger 1872)	0	0	5
Arripidae	*Arripis*	*georgianus*	(Valenciennes 1831)	3	4	5
	Arripis	*truttaceus*	(Cuvier 1829)	4	3	5
Atherinidae			–	4	2	4
Carangidae	*Pseudocaranx*	sp.	–	1	1	1
Cheilodactylidae	*Dactylophora*	*nigricans*	(Richardson 1850)	0	1	0
Enoplosidae	*Enoplosus*	*armatus*	(White 1790)	2	0	0
Gobiesocidae		Unidentified sp.	–	0	0	2
Hemiramphidae	*Hyporhamphus*	*melanochir*	(Valenciennes 1847)	3	1	1
Monacanthidae	*Acanthaluteres*	*spilomelanurus*	(Quoy & Gaimard 1824)	1	3	5
	Meuschenia	sp.	–	0	1	0
	Meuschenia	*freycineti*	(Quoy & Gaimard 1824)	0	4	0
Mugilidae	*Aldrichetta*	*forsteri*	(Valenciennes 1836)	3	0	2
Odacidae	*Haletta*	*semifasciata*	(Valenciennes 1840)	5	5	5
	Neoodax	*balteatus*	(Valenciennes 1840)	5	5	5
Ostraciidae	*Aracana*	*ornata*	(Gray 1838)	0	1	0
Paralichthyidae		Unidentified sp.	–	1	0	0
Platycephalidae	*Platycephalus*	*speculator*	Klunzinger 1872	1	4	0
	Thysanophrys	*cirronasa*	(Richardson 1848)	0	1	0
Plotosidae	*Cnidoglanis*	*macrocephalus*	(Valenciennes 1840)	0	0	1
Scorpididae	*Scorpis*	*aequipinnis*	Richardson 1848	0	0	1
Sillaginidae	*Sillaginodes*	*punctata*	(Cuvier 1829)	4	4	5
Sphyraenidae	*Sphyraena*	*novaehollandiae*	Günther 1860	1	3	5
Syngnathidae	*Leptoichthys*	*fistularius*	Kaup 1853	1	0	2
	Stigmatopora	*argus*	(Richardson 1840)	0	0	4
Terapontidae	*Pelates*	*octolineatus*	(Jenyns 1840)	5	4	3
Tetraodontidae	*Contusus*	*brevicaudus*	Hardy 1981	0	1	0
Tetrarogidae	*Gymnapistes*	*marmoratus*	(Cuvier 1829)	0	1	0
Unidentified small benthic fish			–	0	1	0
Other unidentified bony fish			–	0	0	1
Elasmobranchs						
Dasyatidae	*Dasyatis*	*brevicaudata*	(Hutton 1875)	2	0	1
Heterodontidae	*Heterodontus*	*portusjacksoni*	(Meyer 1793)	0	1	0
Myliobatidae	*Myliobatis*	*australis*	Macleay 1881	5	5	5
Rhinobatidae	*Trygonorrhina*	*fasciata*	Müller & Henle 1841	5	3	5
Urolophidae		Unidentified sp.	–	1	1	0
Aves						
Phalacrocoracidae	*Phalacrocorax*	*varius*	(Gmelin 1798)	2	0	0
Mammalia						
Delphinidae	*Tursiops*	*aduncus*	(Ehrenberg 1832)	0	0	1
Otariidae		Unidentified sp.	–	0	1	0
Cephalopoda						
Loliginidae	*Sepioteuthis*	*australis*	Quoy & Gaimard 1833	0	3	0
Sepiidae	*Sepia*	*apama*	Gray 1841	0	0	1
Decapoda						
Decapoda (prawn or shrimp)	Unidentified sp.		–	0	1	1
Majidae	*Naxia*	*aurita*	(Latreille 1825)	2	0	0
Mysidacea or larval fish schools			–	0	3	0
Portunidae	*Nectocarcinus*	*integrifrons*	(Latreille 1825)	4	5	4
Scyphozoa						
Cyaneidae	*Cyanea*	*rosea*	Quoy & Gaimard 1824	4	2	2
Holothuroidea						
Stichopodidae	*Stichopus*	*ludwigi*	Erwe 1913	0	1	0
Total taxa observed				25	31	28

with the limited quality of the video. All taxa were included separately in the data analyses because numbers were small for either unidentified or non-fish species and could influence the analysis of taxon richness of the total assemblage observed more than any analysis including abundances. Approximately one-third (16) of the taxa observed were identified from all 3 locations (Fig. 3). Fourteen taxa were unique in EC, 7 in PLAR and 4 in BoS. BoS and PLAR shared the most taxa in common, with an additional 3 species being found in both locations.

Fourteen taxa were most abundant in PLAR (Fig. 4), with most of these being small cryptic fish. Eighteen commercially and/or recreationally targeted species were identified in this study (Fig. 5). All of these species had a higher abundance outside the reserve except in the case of *Arripis truttaceus* and *Sphyraena novaehollandiae*. Additionally, 2 of the fisheries-targeted species were detected only in the reserve (*Scorpis aequipinnis* and *Sepia apama*). Two protected species were identified in this study, both pipefish from the Syngnathidae family, *Stigmatopora argus* and *Leptoichthys fistularius*. These 2 species were both more abundant within the reserve, with *S. argus* significantly so (Fig. 4, PERMANOVA, pseudo-F = 8.8818, p(perm) = 0.004). The only other species with a significantly higher abundance within PLAR was

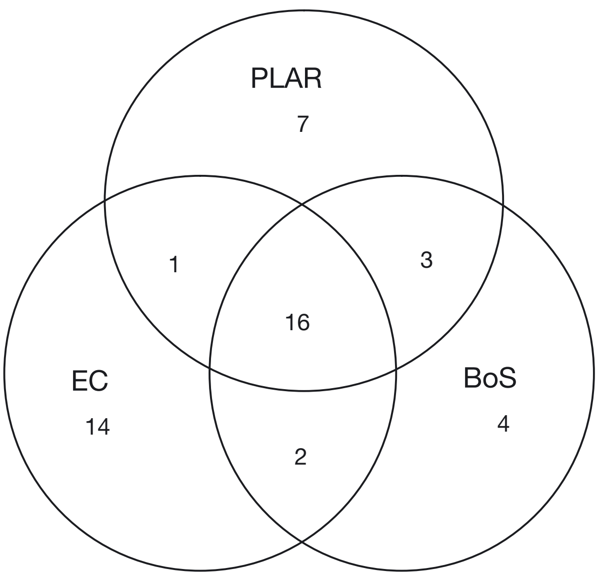

Fig. 3. Venn diagram showing overlaps across locations of the number of taxa seen visiting baited remote underwater video stations in 3 shallow seagrass locations in Kangaroo Island, South Australia. Each sector of the overlapping circles (1 per location) shows the number of species shared in 3, 2 or only 1 location(s). Species identities shown in Table 2. BoS = Bay of Shoals, EC = Eastern Cove, PLAR = Pelican Lagoon Aquatic Reserve

Vincentia conspersa (Fig. 4, PERMANOVA, pseudo-F = 14.582, p(perm) = 0.003). Four more fisheries-targeted species were significantly different between locations, with both *Meuschenia freycineti* (PERMANOVA; pseudo-F = 7.3232; probability from a Monte-Carlo test [used for available permutations < 100] p(MC) = 0.005) and *Platycephalus speculator* (PERMANOVA, pseudo-F = 3.995, p(perm) = 0.038) more abundant in EC than in the other 2 locations. The other 2 species showed significant differences between locations, i.e. *Sepioteuthis australis* (PERMANOVA, pseudo-F = 4.9861, p(MC) = 0.032) and *Sillaginoides punctata* (PERMANOVA, pseudo-F = 3.9926, p(perm) = 0.039), but pair-wise tests were unable to determine between which locations (Fig. 5).

Mean (± SE) MaxN pooled across all species was highest at BoS at 1.78 (± 0.57) followed by EC at 1.20 (± 0.26) and then PLAR at 0.48 (± 0.06), but these were not significantly different among the 3 locations (univariate pseudo-F = 1.2051, p(perm) = 0.37). However, site-to-site variation was significant (pseudo-F = 3.788, p(perm) = 0.004). Correlations between distance to seagrass and each of mean MaxN, total number of individuals and number of species were all not significant and showed no correlation (Bonferroni p > 0.33). The combined sum of the 18 fisheries-targeted species showed no significant differences between locations (univariate pseudo-F = 1.4057, p(perm) = 0.26), but small-scale variation was significant (univariate pseudo-F = 5.7629, p(perm) = 0.001).

Assemblages based on standardised abundances across the 3 locations did not differ significantly by PERMANOVA (Table 1); however, considering species composition alone (via the presence/absence transformation) yielded significant differences between locations (Table 1). Small-scale site-to-site variation was significant in both tests (Table 1). Results from the pair-wise tests of the presence/absence data indicated that significant differences in composition occurred between all pairs of locations. The more focussed CAP analysis performed with standardised abundance data used m = 10 principal coordinate axes explaining 97 % of the total variation and showed some overlap between locations (Fig. 6a), with an overall leave-one-out cross-validation allocation success rate of 64.9 % (thus, misclassification error was modest at 35.1 %), with PLAR being best at 74 %. The permutation test showed that separation between locations was highly significant (for both trace and delta statistics, p = 0.001), meaning that disregarding all other information, CAP was able to identify significant differences between the assemblages from the 3 locations.

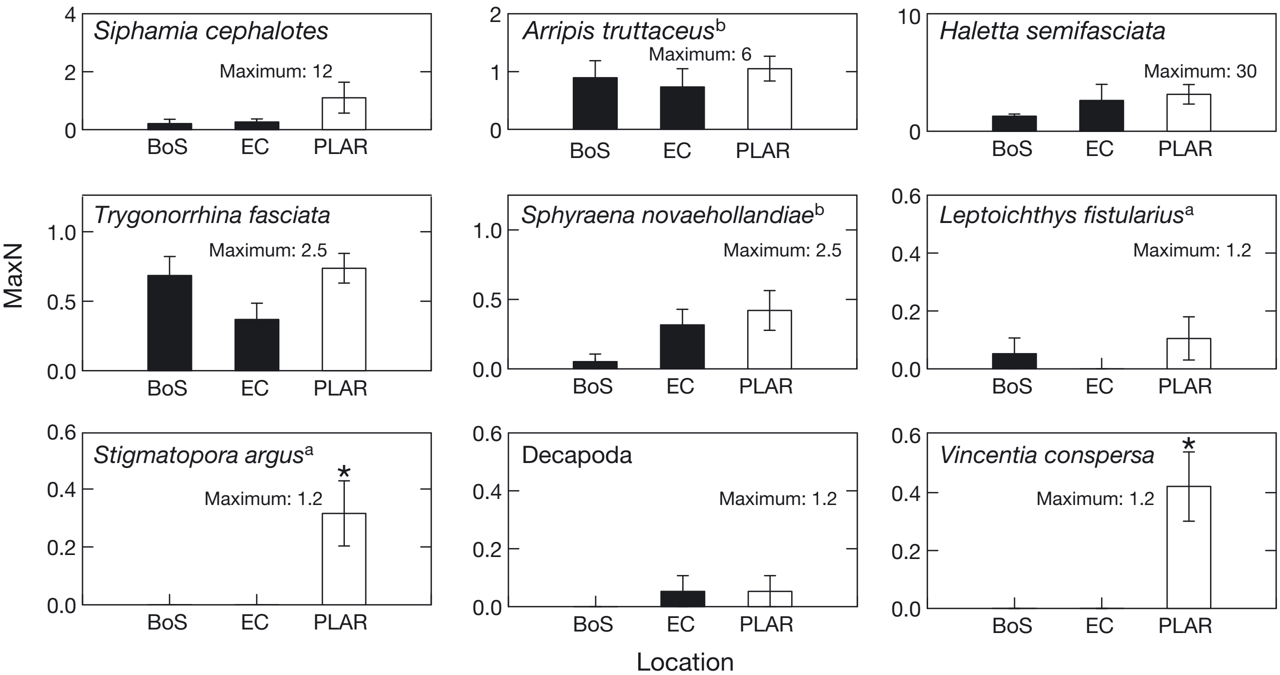

Fig. 4. Untransformed mean (± SE) MaxN values from 3 shallow seagrass locations in Kangaroo Island, South Australia, taken from baited remote underwater video stations (n = 57) for the 9 taxa that were more abundant in Pelican Lagoon Aquatic Reserve (PLAR) than in the other 2 locations, Bay of Shoals (BoS) and Eastern Cove (EC). In addition, *Tursiops aduncus*, Gobiesocidae, *Cnidoglanis macrocephalus*, *Scorpis aequipinnis*, and *Sepia apama* were also seen in PLAR but not in any other locations, and are thus not shown here. Unfilled bars = protected locations, filled bars = unprotected locations. *Significant difference among locations by univariate 2-factor PERMANOVA. [a]Protected species; [b]Fisheries-targeted species. Maximum: largest replicate data point

The fish abundance assemblage observed in PLAR did not differ significantly compared to BoS and EC by PERMANOVA (Table 3), indicating that protection did not seem to influence this result. Only some overlap between clusters of points was seen from the CAP analysis of protected vs. unprotected status (Fig. 6b), using m = 10 principal coordinate axes explaining 97% of total variation; however, the leave-one-out success rate was high at 82.5% (misclassification error = a much smaller 17.5%), with 89% correct for protected but 79% correct for unprotected, and the permutation tests again showed highly significant differences between protection status (both trace and delta p = 0.001).

BRUVS video quality covariates

At more than half of the sites in all locations, tall seagrass was observed in the horizontal field of view on BRUVS videos, with all seagrass observed in PLAR being classified as tall (Table 4). In PLAR, 63% of the videos had a medium level of obscurity, which was the result of this tall seagrass height, whereas in BoS and EC, 74% of the videos had low levels of obscurity. Turbidity levels in all 3 locations were usually low, with PLAR having 8 (out of 19) videos classified as medium or high compared to only 5 from BoS and 1 from EC. The field of view was past the bait bag in between 63% of cases in PLAR to 95% of cases in EC. EC had some trouble with zoomed-in cameras (5 out of 19), but focus issues were negligible in all locations. Results from contingency table chi-squared tests showed significant differences in frequencies for seagrass height, turbidity and obscurity between locations. Based on the PERMANCOVA analysis with covariates, it is possible that seagrass height and turbidity may have influenced the assemblages observed, but the overall results remain unchanged (Table 5).

DISCUSSION

The current study found BRUVS was an effective method for assessing assemblages in seagrass habitats, with no zero counts recorded and only a 3 out of 60 (5%) failure rate. More than 40 species of fish and

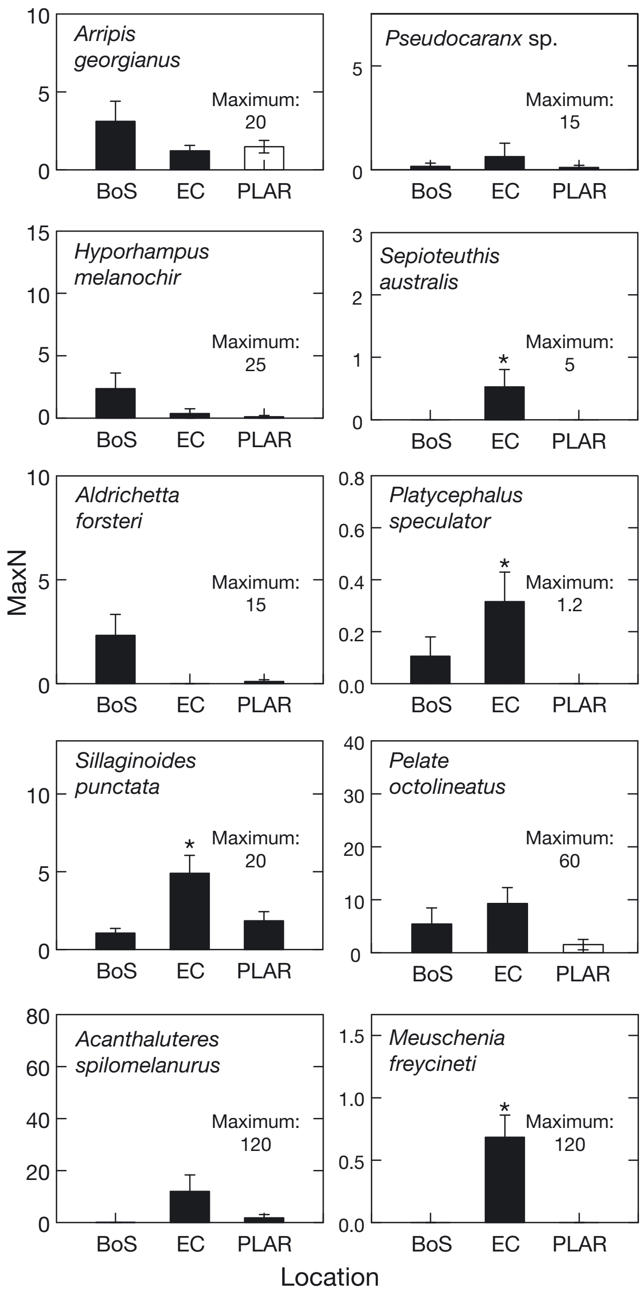

Fig. 5. Untransformed mean (± SE) MaxN values from 3 shallow seagrass locations in Kangaroo Island, South Australia, for commercially and/or recreationally targeted species observed from baited remote underwater video stations (n = 57). Several other taxa are additional fisheries species not shown here because they were present in only 1 location (viz. Monacanthidae sp. 1, EC; *Dactylophora nigricans*, EC; *Thysanophrys cirronasa*, EC; Paralichthyidae sp., BoS; *Sepia apama*, PLAR; *Scorpis aequipinnis*, PLAR) or are shown in Fig. 4 (*Arripis truttaceus* and *Sphyraena novaehollandiae*). Unfilled bars = protected locations, filled bars = unprotected locations. *Significant difference among locations by univariate 2-factor PERMANOVA. BoS = Bay of Shoals, EC = Eastern Cove, PLAR = Pelican Lagoon Aquatic Reserve. The *y*-axis in each case extends to include the largest replicate data point

other animals were recorded from the 3 locations, and almost 3000 ind. were seen, which shows that a diverse and abundant range of seagrass-associated taxa can be observed via BRUVS.

BRUVS were thus suitable for assessing nektonic communities in seagrass habitats, with a wide range of pelagic (e.g. *Arripis* spp., Atherinidae, *Sphyraena novaehollandiae*), demersal (e.g. *Platycephalus speculator*, *Myliobatis australis*, *Naxia aurita*) and cryptic (e.g. *Stigmatopora argus*, *Leptoichthys fistularius, Neoodax balteatus*) fish species being observed. Comparisons with other methods such as trawls have shown elsewhere that BRUVS observed more mobile species than the trawls but missed smaller cryptic and more sedentary species (Cappo et al. 2011). This may mean that the species from these groups could have been under-represented or missed in the present study. Evidence from previous beam trawling in PLAR supports this possibility, with great numbers of *Vincentia conspersa*, Clinidae, Gobiesocidae, Gobiidae, Odacidae, *Gymnapistes marmoratus* and 6 pipefish species in the family Syngnathidae being caught by nocturnal trawls (Kinloch et al. 2007). Apart from the odacids, these species were either absent (Clinidae, Gobiidae and many syngnathids) or only seen in low numbers (Gobiesocidae, *Vincentia conspersa, Gymnapistes marmoratus*) in this study.

A study by Bryars (2003), based on fisheries data and correlations with habitat, predicted the important fisheries-targeted species likely to be seen in each location. For BoS, Bryars (2003) predicted 12 adult species that we considered likely to be observed from BRUVS, 4 of which were not observed in this study; these included red mullet *Upeneichtys vlamingii*, yellowtail kingfish *Seriola lalandi*, whaler sharks from the *Carcharhinus* genus (all of the above 3 were not observed in any of our locations by BRUVS) and the southern calamari *Sepioteuthis australis*. With the exception of *S. lalandi*, the same species were predicted for EC, where only 2 species were not observed by BRUVS (*U. vlamingii* and *Carcharhinus* spp. as listed above). PLAR had 10 potential species predicted, the same as for EC but without *Carcharhinus* spp. Three of these species were not observed by BRUVS, *S. australis*, flathead from the *Platycephalus* genus, and *U. vlamingii*. Reasons for BRUVS failing to record sightings of *U. vlamingii*, *Carcharhinus* spp. and *S. lalandi* may be related to the shallow depths sampled or the true absence of these species from the locations chosen. It is unlikely to be because of an inability of BRUVS to sample these species because they have been recorded by BRUVS in other studies (Malcolm et al. 2007, McKin-

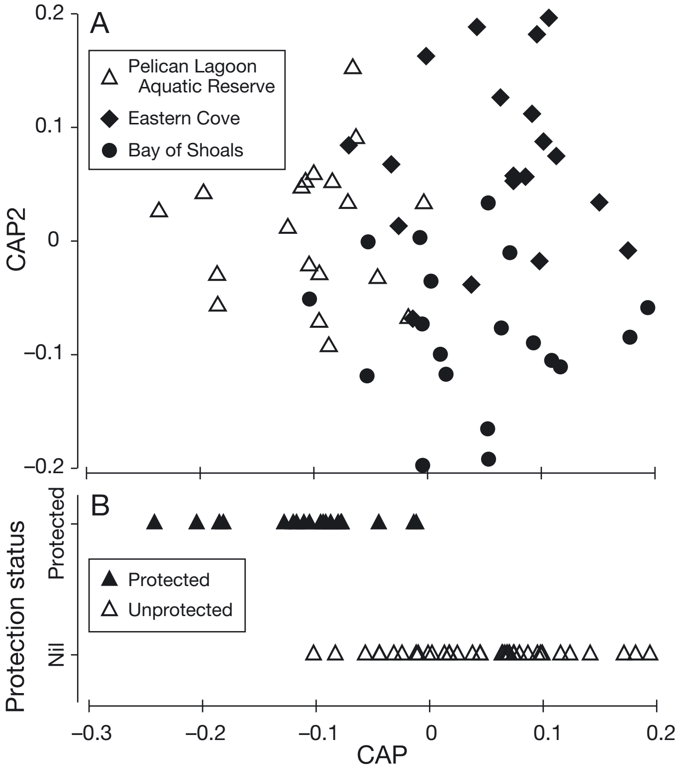

Fig. 6. Results of canonical analysis of principal coordinates (CAP) analyses showing similarities of assemblages from baited remote underwater video stations (n = 57) in Kangaroo Island, South Australia, and tests of the hypothesis of differences (A) among 3 shallow seagrass locations and (B) between2 levels of protection status

ley et al. 2011). Despite the short temporal sampling period, this study demonstrates that BRUVS are effective at recording the majority of fisheries-targeted species in each location.

The non-destructive nature of BRUVS is suited for seagrass habitats because the units did not damage fragile seagrass plants. Problems associated with seagrass habitats for BRUVS include obstruction of the camera by dense seagrass in areas with either long blades or dense macroalgal cover and a restricted field of view from potentially increased water turbidity (because of the shallow depths in which seagrasses can be found). In this study, we chose to deploy BRUVS in sparser patches or on the edge of beds, where possible, to minimise the obstruction risk, whereas Gladstone et al. (2012) modified the BRUVS unit with a mesh bottom to flatten nearby seagrass blades. It is also possible to modify the BRUVS design by making the base taller to elevate the camera above the canopy, but because of the shallow depths in the present study, this was not an option. To try to minimise the effect of water turbidity in the current study, we chose to use only high-definition video recorders to obtain the clearest image possible. Wind, weather and wave action often contribute to increases in turbidity, so selecting the appropriate conditions, where possible, could also decrease the effects of this factor. Despite these considerations, these covariates may still have had some effect on the assemblages observed. PLAR had long and dense seagrass blades with thick epiphyte loads, and it was often difficult to find sand patches or edges in which to place the BRUVS. The turbidity in PLAR was also higher, because of less water movement. These factors may have contributed to the assemblages observed and could also have hampered bait plume dispersion and/or fish coming to the bait. The longer seagrass blades, and the closer position of the BRUVS to them, could also have accounted for the significantly higher numbers of the pipefish *Stigmatopora argus* being observed within the reserve. *S. argus* and other syngnathids are usually strongly associated with their resident habitat (Kendrick & Hyndes 2003).

Table 3. Multivariate PERMANOVA results for a contrast analysis of the protection status of assemblages based on standardised MaxN values from baited remote underwater video stations (n = 57) in shallow seagrass habitats at 3 locations on Kangaroo Island, South Australia. **Bold:** significant. BoS = Bay of Shoals, EC = Eastern Cove, PLAR = Pelican Lagoon Aquatic Reserve

Source	df	MS	Pseudo-*F*	p(perm)
Location	2	8178.9	1.62	0.069
(PLAR) vs. (BoS, EC) or Protection status	1	8009.6	1.49	0.163
Site (Location)	12	5077.1	2.87	**0.001**
Site (Protection status)	13	5426.3	3.07	**0.001**
Residual	42	1767.9		

Investigations into the use of BRUVS in protected areas have previously occurred, especially in Australia (e.g. Cappo et al. 2003, 2004, 2007, Malcolm et al. 2007, Watson et al. 2007, Gladstone et al. 2012), but little work has been done on investigating their use in seagrass habitats. In this study, 47 taxa comprising mainly fish (37 taxa, with 32 bony fish and 5 elasmobranchs) were recorded by BRUVS across all sites. These findings were similar to Gladstone et al. (2012), who assessed seagrass and sand habitats in an estuarine environment of New South Wales (NSW) and found 35 fish species (31 bony fish and 4 elasmobranchs), while McKinley et al. (2011) assessed shallow bare sand habitat adjacent to rocky reefs in NSW estuaries and found more species, a

Table 4. Variables potentially affecting the quality of videos (defined in 'Methods: BRUVS video quality covariates') assessed from baited remote underwater video stations in 3 shallow, sheltered seagrass locations in Kangaroo Island, South Australia. Entries are numbers of videos displaying this characteristic (out of n = 19 per location). BoS = Bay of Shoals, EC = Eastern Cove, PLAR = Pelican Lagoon Aquatic Reserve

Location	Seagrass height			Obscurities			Turbidity			Field of view		Zoomed in?			Focus issues
	Low	Medium	Tall	Low	Medium	High	Low	Medium	High	To bait bag	Beyond bait bag	Yes	Little	No	
BoS	6	1	12	14	2	3	14	4	1	4	15	0	2	17	1
EC	7	2	10	14	4	1	18	1	0	1	18	2	3	14	0
PLAR	0	0	19	6	12	1	11	6	2	7	12	0	0	19	1

Table 5. Results from the univariate 2-factor PERMANOVA using baited remote underwater video station (BRUVS) video quality covariates (see Table 4) and mean MaxN abundance data with the results of contingency tables and Pearson chi-squared tests for each covariate measured from the BRUVS (n = 57). Where too few entries were recorded for each category within a covariate, categories were combined to give larger sample sizes (see the 'Groupings' column for where this was applied). **Bold**: significant. N/A = not applicable

Covariate tested	PERMANOVA: Covariate significant?	Location factor significant?	Site factor significant?	Interpretation	Contingency tables testing whether frequencies differed across categories and locations for covariate: Pearson chi-squared test Value	df	p	Groupings
None	N/A	N	**Y**	N/A	–	–	–	–
Seagrass height	**Y**	N	**Y**	Height could be important but overall result unchanged	11.64	2	**0.003**	Low/Medium < Tall
Obscurities	N	N	**Y**	Obscurity unlikely to have affected outcome	9.33	2	**0.009**	Medium/High < High
Turbidity	**Y**	N	**Y**	Turbidity could be important but overall result unchanged	7.00	2	**0.030**	Medium/High < High
Field of view	N	N	**Y**	Field of view unlikely to have affected outcome	5.70	2	0.058[a]	Nil
Zoomed in	N	N	**Y**	Zoom unlikely to have affected outcome	7.56	4	0.109[a]	Nil
Focus issues	N	N	**Y**	Focus unlikely to have affected outcome	Too few to run		No effect to test	

[a]Suspect; too many cells empty, so categories were concatenated

total of 59 (9 elasmobranchs and 50 bony fish). Sampling effort was similar between the current study (n = 57) and Gladstone et al. (2012) (n = 72); however, McKinley et al. (2011) replicated their study in time and thus had a larger overall sampling effort of n = 128 (they did state that they found no differences in species composition between time periods, so we still find these studies to be comparable).

BRUVS deployed in seagrass habitats may be a useful tool for monitoring marine reserves, as they are non-destructive and have an ability to detect a wide range of species including many of those targeted by fisheries. They are easy to use and provide replicated samples, store a hard copy of the images that can be re-examined at a later date if needed, are suitable for a wide range of habitats including seagrass and avoid many safety hazards because personnel are not required in the water. Procedures for the use of BRUVS can also be standardised (in terms of bait type and amount, soak time, high definition or standard definition camera use, unit design) across areas and habitats to provide accurate results for interpretation (Cappo et al. 2003, 2004, Harvey et al. 2007, Gladstone et al. 2012).

While no significant differences were found between PLAR and EC and BoS for relative abundances, significant differences in species composition alone may show some influence of protection since the habitats investigated were matched. The community of PLAR was composed of a variety of species, with some smaller sedentary and cryptic species more abundant there compared to EC and BoS. The unusual topography of PLAR, along with its small mouth (see Fig. 1) and high likelihood of limited water exchange and low flow within, may promote a unique cryptic fish assemblage, and therefore more-destructive methods specifically targeting small sedentary species (such as beam trawls, seine nets or electrofishing) could be used to further explore and identify these communities. BRUVS used in different habitats within the reserve could also yield different results and may identify more targeted species or hotspots of biodiversity, e.g. in deeper holes.

Contrary to expectation, commercially and recreationally targeted species were often less abundant in the fully protected PLAR compared to the unprotected BoS and EC. It is unlikely that habitat types, salinity or temperature were affecting this result because these variables were not significantly different between PLAR and EC/BoS. The unusual topography of Pelican Lagoon may have influenced the fish assemblages observed. The distance of the BRUVS from the lagoon entrance is likely to have been great enough that fish from outside the reserve were not attracted to the bait. Also, this study did not investigate the seasonal, tidal or diurnal changes in fish assemblages that may occur and that could also influence results for all 3 locations.

PLAR was initially set up because the area was thought to be a nursery ground for juvenile fish species (PIRSA 2012), unlike modern reserve design that usually projects multiple habitats. It may be that juvenile fish species were not attracted to the bait in the present study or were scared off by the foraging activity surrounding it. This study could not measure the length of fish, so juveniles could not be objectively identified; however, investigations into numbers of juveniles within the reserve compared to outside could be done using stereo BRUVS, which allow for the measurement of fish individuals. Unfortunately, the larger vessels required for the use of stereo-BRUVS may have issues navigating some shallow areas within the lagoon.

Species that were significantly more abundant within the reserve were mainly smaller cryptic species (e.g. *Vincentia conspersa, Stigmatopora argus*). The higher loads of epiphytic cover observed within the reserve (see Fig. 2) could indicate less water movement, both of which may be preferred by smaller sedentary species. More epiphytic cover could also mean an increase in food for herbivorous and carnivorous fish, with some studies having shown an increase in abundance of mobile epifauna with increased epiphyte load (e.g. Hall & Bell 1988). Structural habitat complexity is also important for many fish species (Hemminga & Duarte 2000), and with more epiphytic algae in PLAR, available habitat will also be more complex and so may be preferable for some species that are closely associated with such structure. Other species, such as the King George whiting *Sillaginoides punctata*, have been shown to shun *Posidonia* spp. beds as adults, most likely because the blades and canopy hamper their movement (Hyndes et al. 1996). This may explain the significantly higher numbers of *S. punctata* seen in EC, where seagrass height was often shorter with more bare sand (see Fig. 2).

One factor affecting fish assemblages observed in the present study may be the placement of the BRUVS on the edge of beds or in sand patches within beds. Studies have shown that fish may be more abundant on the edge of beds compared to the middle (Smith et al. 2008) and that food availability is likely to be the main contributing factor to that pattern of abundance for some fish species (Macreadie et al. 2010). This may mean that any BRUVS placed in the middle of beds (where no edges or sand patches were available) could be potentially biased toward lower MaxN values. However, for many fish species (especially those likely to be seen on BRUVS), the presence of bait may overcome any previous dispositions the fish had toward locations within a bed. To truly understand the effects of bait in relation to edge effects of seagrass beds, future research investigating these factors is needed, such as comparing fish assemblages observed by BRUVS from the middle of patches to those on the edge.

In conclusion, a diverse range of species was identified from these shallow seagrass areas studied using BRUVS. In addition, few problems were encountered while using the BRUVS among seagrass, and little impact was observed from potential issues with obstruction of views or turbidity. Overall, BRUVS appears to be a suitable method for use in shallow seagrass habitats and, with its non-destructive nature, would suit monitoring or research in protected areas, but may miss some smaller sedentary or cryptic species. While some differences in species assemblages were observed between PLAR and the unprotected areas, it is unclear whether these are the result of sanctuary-zone protected status, because of the generally lower numbers of commercially and recreationally targeted species within the reserve.

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