

Artificial reefs create distinct fish assemblages

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ABSTRACT: We conducted a before-after-control-impact (BACI) experiment to evaluate the effects of deploying small-scale artificial patch reefs on fish assemblages in a temperate bay (Port Phillip Bay) in south-eastern Australia. Three replicate artificial reef treatments were placed on sandy substratum and the response of the fish assemblage was compared with 3 control sites with no reef, and 3 nearby natural reef comparison sites. All habitats were sampled using baited remote underwater video (BRUV) and underwater visual census (UVC) multiple times, 6 mo before and 22 mo after artificial reef deployment. Fish species rapidly colonised artificial reefs, with 30 new species detected in the first year post deployment and only 4 in the following year. Both sampling methods captured a shift in assemblage structure, albeit with different species compositions, as individual species numbers varied dependent upon method used. BRUV provided better estimates of the important recreational species snapper *Chrysophrys auratus* (family Sparidae), with more snapper recorded on artificial and natural reefs compared to sediment. The artificial reef assemblages were dominated by species that favour the reef/sediment interface. Several reef-associated species were detected in the juvenile stages; however, adults of obligate reef species were not observed on artificial reefs. Sediment-associated species present before artificial reef deployment persisted within the artificial patch reef area over the course of the study. Overall, the deployment of patchwork artificial reefs increased local species diversity and abundance of fish, and did not impact existing sediment fish assemblages.

KEY WORDS: Before-after-control-impact · BACI · Reef edge · Artificial reef · Baited video · Underwater visual census · *Chrysophrys auratus* · Victoria · Australia

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INTRODUCTION

Artificial structures are widespread in coastal environments throughout the world, providing infrastructure for shipping, protection of shorelines, and enhanced tourism and fisheries (Bombace 1989, Monteiro et al. 1994, Baine & Side 2003, Kheawwongjan & Kim 2012). Artificial structures can be comprised of artificial and natural materials (e.g. steel, concrete, rock and wood) and are deployed in a range of depths, from intertidal seawalls, to offshore oil rigs in deep water (Bulleri & Chapman 2010, Macreadie et al. 2011). Irrespective of the myriad of ways in which artificial structures differ, they have long been recognised as places where fish and other marine biota live

or congregate (Bohnsack & Sutherland 1985, Ambrose & Swarbrick 1989, DeMartini et al. 1989, Pickering & Whitmarsh 1997).

In Australia and other developed nations, artificial reefs are increasingly being created to provide enhanced recreational fishing opportunities (Milon 1989b, McGlennon & Branden 1994, Baine 2001, Folpp et al. 2013). The repeated observations of higher density, diversity and biomass of fish at artificial structures compared to nearby sediment habitats (Bohnsack 1989, Fabi & Fiorentini 1994) is the primary reason for their deployment for fisheries enhancement objectives (Milon 1989a). Artificial reefs deployed for recreational fisheries enhancement are often small in scale compared to surrounding natural

habitat and artificial reefs designed for commercial fisheries enhancement (Santos & Monteiro 1998). However, they may enhance recreational fisheries by increasing local production, diversity and aggregation of fish, resulting in localised increases in catch rates and angler satisfaction (Bohnsack 1989, Lindberg 1997).

Irrespective of the relative contributions that attraction, production and increased diversity make to fisheries enhancement (Lindberg 1997, Brickhill et al. 2005), the ongoing deployment of artificial structures, particularly in smaller sheltered bays and estuaries where natural reef may be sparse, clearly has implications for local fish assemblages and ecological interactions (Waltham & Sheaves 2015). Implications are likely to be viewed as positive, such as enhancing regional biomass and biodiversity (Bohnsack et al. 1997, Carr & Hixon 1997, Pickering & Whitmarsh 1997), or negative, such as increased 'fish harvesting' due to the concentration of target species at greater densities than those occurring naturally (McGlennon & Branden 1994). Other risks may include the attraction of reef fish from nearby natural reef habitats, thereby impacting natural reef assemblages and altering adjacent sediment assemblages due to increased predation and competition (Bohnsack 1989, Grossman et al. 1997, Powers et al. 2003).

Studies have shown that fish assemblages associated with artificial structures are generally different to nearby natural reef and sediment habitats (Edwards & Smith 2005, Clynick et al. 2008, Folpp et al. 2013, Lowry et al. 2014). Many of these studies have used replicate artificial habitats for comparisons with nearby natural habitats (Alevizon et al. 1985, Ambrose & Swarbrick 1989, McGlennon & Branden 1994, Carr & Hixon 1997, Santos & Monteiro 1998, 2007, Sánchez-Jerez & Ramos-Esplá 2000, Burt et al. 2009), and some have included both sediment controls and natural reef comparison sites (Bohnsack et al. 1994, Folpp et al. 2013, Lowry et al. 2014). However, studies of fish assemblage response to addition of artificial structures have rarely used formal before-after-control-impact (BACI) designs, or sampled multiple habitats over multiple climatic seasons and years (Bergström et al. 2013). The BACI design allows changes detected at the artificial reef deployment sites to be differentiated from more general changes that might be occurring in the region of the study and are not due to reef deployment. Conducting before sampling at control and reef deployment sites also ensures conclusions are not confounded by pre-existing differences between the reef deployment sites and the controls or the seasons/years in

which comparisons are made. Additionally, if the goal is to use artificial structures to restore lost or damaged natural habitat, or to compensate for loss of angler access to natural reefs, natural reef comparison sites are also important.

Obtaining a comprehensive picture of fish assemblages will most often require multiple survey approaches due to the different behaviour of species and survey environments, particularly levels of crypsis, avoidance of divers and visibility (Colton & Swearer 2010, Lowry et al. 2012). Baited remote underwater video (BRUV) has been shown to detect mobile predators and recreational fishery species with increased frequency compared to underwater visual census (UVC) (Colton & Swearer 2010, Lowry et al. 2012), while UVC records more species, particularly cryptic species (Colton & Swearer 2010, Lowry et al. 2012). While some artificial reef studies have focussed on UVC (Burchmore et al. 1985, Bohnsack et al. 1994, Edwards & Smith 2005, Cenci et al. 2011) and others on BRUV (Caselle et al. 2002, Folpp et al. 2011) as the primary techniques to sample fish assemblages, few have combined the 2 methods to achieve a more complete picture of how an artificial reef alters fish assemblage composition (Seaman et al. 1989, Lowry et al. 2014).

Sparidae species are frequently recorded on artificial structures, often in numbers exceeding nearby natural reefs (Clynick et al. 2008, Lowry et al. 2014). Port Phillip Bay is the centre of the snapper *Chrysophrys auratus* fishery in Victoria. Adult snapper (>40 cm) form spawning aggregations in the deeper silt/clay bottom waters of Port Phillip Bay in spring and summer (October to January) where they are targeted by both commercial and recreational fishers (Coutin et al. 2003). Smaller sub-adult (<40 cm) snapper are also targeted by recreational anglers on shallow natural reefs, mostly in autumn (February to May) (Grixti et al. 2010). There is limited natural reef habitat in Port Phillip Bay (<1% of the sub-tidal substrate, Hamer et al. 2011). The deployment of artificial reefs has the potential to increase the reef habitat area for young snapper. While the main impetus for creation of artificial reefs in Port Phillip Bay is to provide habitat for recreational species such as snapper, no artificial reefs have been deployed in Port Phillip Bay since the late 1960s to early 1970s (Beinssen 1976), and these earlier reef deployments were not formally assessed for fish community responses.

Recent deployment of artificial reefs in Australia have used Reef Balls® arranged as a continuous structure (Folpp et al. 2013). In this study, we used clusters of 2 to 5 Reef Balls®, arranged in a regular

patchwork array to increase the overall footprint of each reef and provide a larger sediment to reef edge ratio. The design is considered suitable for species such as snapper, as they utilise both sediment and reef habitat (Ross et al. 2007). Additionally, we wanted to create artificial reefs that would potentially increase snapper abundance and species diversity, but not at the expense of displacing local sediment species that provide prey for piscivores and are targeted by recreational anglers.

This project was designed to evaluate the impacts of small-scale artificial patch reefs on fish community assemblages, including fishery target species. We used a BACI design, with sediment control and natural reef comparison sites, and a combination of UVC and BRUV sampling methods to (1) determine the impact of small-scale artificial patch reef deployments on existing fish assemblages, (2) identify fish species that characterise community differences between artificial reef, sediment controls and reference natural reef habitats, and (3) identify changes in the abundance of key species, including fishery species, in response to artificial patch reef deployment. The outcomes provide baseline information that can inform consideration of a broader program of artificial reef creation.

MATERIALS AND METHODS

Study area and artificial reef structure

This study took place in Port Phillip Bay, southeast Australia (Fig. 1). Site selection for artificial reef deployments was initially guided by a constraint mapping exercise to identify suitable areas that were free of existing marine assets and or other uses or habitats that would conflict with the deployment of artificial reefs. The constraint mapping identified Carrum Bight, a large area of intermediate diversity sands (Cohen et al. 2000) on the eastern side of Port Phillip Bay ($-38.071523^{\circ}\text{S}$, $145.093713^{\circ}\text{E}$), as free of conflicts and highly suitable for artificial reef deployment. Once this area was identified, consultation with recreational angler

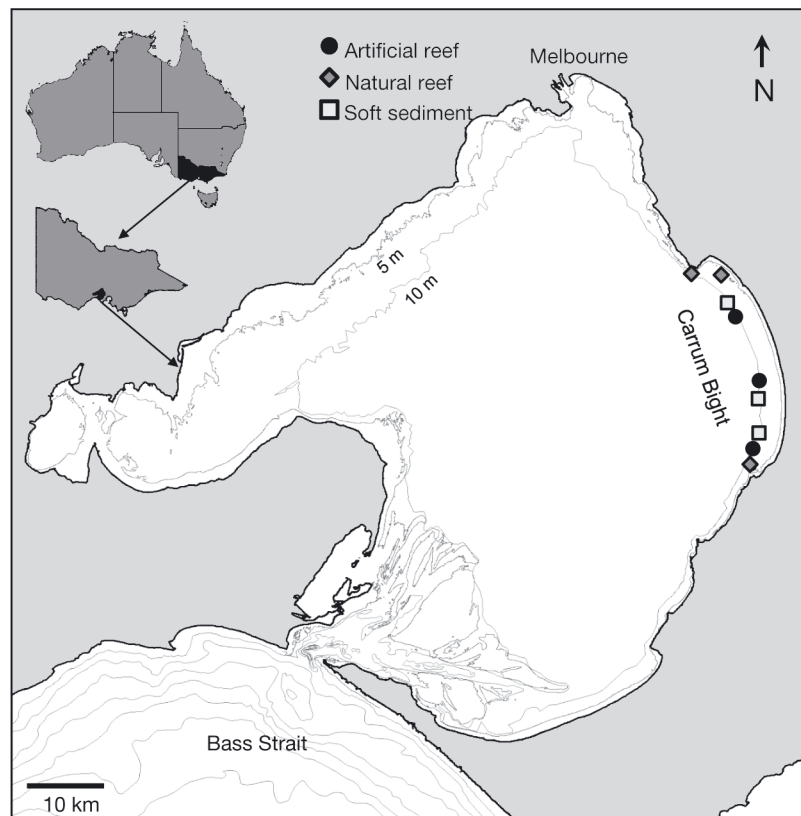


Fig. 1. Location of study sites in Port Phillip Bay (38.1732°S , 144.8731°E), southeast Australia. The 5 and 10 m depth contours are shown. (Locations are approximate and symbols are not to scale)

stakeholders determined that the reefs should be placed in locations directly accessible to boat ramps, and not shallower than 10 m, as anglers mainly target *Chrysophrys auratus* in intermediate depth water. This further constrained the area in which the artificial reefs could be deployed. Field investigations within the area identified that the depth zone of 10 to 12 m provided a stable firm substrate for the artificial reefs, with low risk of burial or subsidence of the reef structures.

Six sediment and 3 natural reef locations were chosen in Carrum Bight. Three of the 6 sediment sites were randomly chosen to have artificial reefs deployed and were paired with 3 sediment control sites. Each artificial reef deployment site was approximately 1.5 km from a sediment control site at a similar depth of 10 to 12 m and located approximately 2 km offshore from boat launching facilities. The 3 artificial reef–sediment control pairs were placed with approximately 8 km separating the pair in the north of the bight from the pair in the middle, which was approximately 7 km from the pair to the south (Fig. 1). The nearest 3 natural reef sites were chosen

as comparison sites. However, natural reefs do not occur deeper than 9 m in this area of Port Phillip Bay or immediately adjacent to the artificial reef–sediment pairs, therefore natural reefs could not be matched to the other habitat treatment pairs. Two natural reefs in the north of the bight (approximately 4 to 5 km from the nearest artificial reef–sediment pair) and 1 natural reef in the south (approximately 2 to 3 km from the nearest artificial reef–sediment pair) were used (Fig. 1). In summary, there were 3 habitat treatments each with 3 replicates: pre- and post-artificial reef sites and the control sediments, which were paired at 3 locations, and the unpaired natural reef comparison sites.

Artificial reef design and materials

Each artificial patch reef consisted of 96 Reef Balls®, comprised of 16 Pallet Balls (0.88 m high × 1.22 m diameter, ~750 kg), 56 Bay Balls (0.61 m high × 0.91 m diameter, ~200 kg), and 24 Mini-Bay Balls (0.53 m high × 0.76 m diameter, ~120 kg). Because snapper move freely between reefs and sediments and are common along reef margins (Ross et al. 2007), the reef balls were laid out in a regular patchwork array instead of clumping them together to form a contiguous structure (Folpp et al. 2013). This increased the ratio of structure to sand with clusters placed over a square area of 50 × 50 m (Fig. 2). The reefs were all deployed in May 2009.

Survey methods

Fish assemblages were surveyed using BRUV and UVC. BRUV systems were constructed based on the design of Cappo et al. (2004), and 4 systems were used to allow all sites of all habitat treatments to be sampled in 1 d. Each BRUV unit consisted of an underwater housing fixed to an aluminium frame, with a wooden bait pole that had a plastic mesh bait cage fixed at 1.2 m from the face of the camera housing. A standardised bait consisting of a mixture of 200 g chicken feed pellets, 200 g crushed pilchards *Sardinops sagax* (family Clupeidae) and 100 ml tuna oil was placed within the bait cage. Two small strips of squid *Nototodarus gouldi* (family Ommastrephidae, ca. 2 × 5 cm) were attached to the outside of the bait

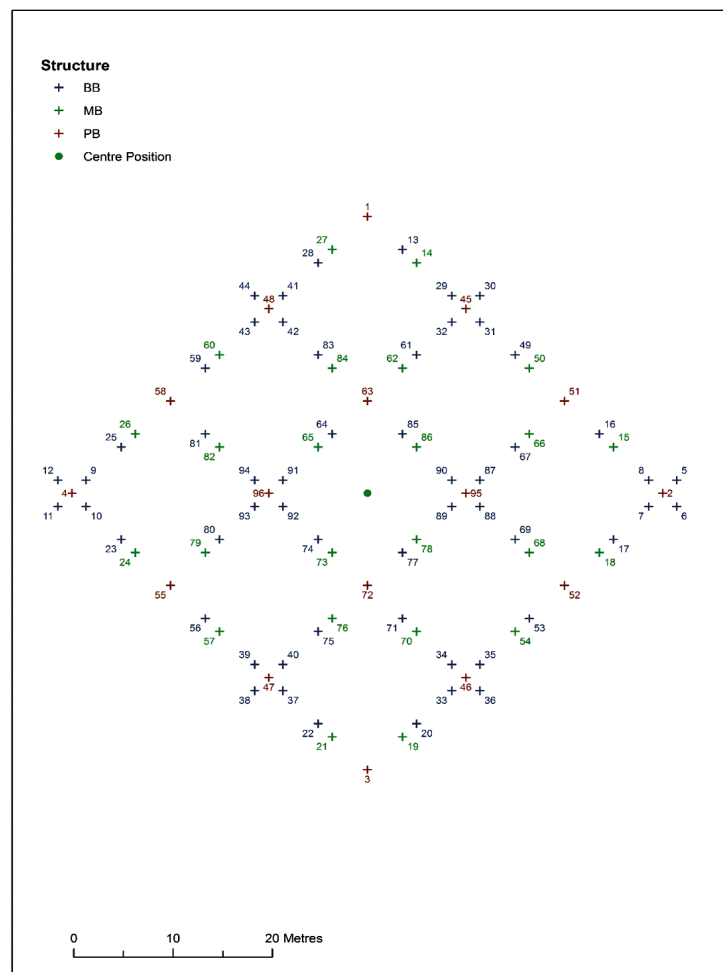


Fig. 2. Layout of artificial reefs. BB: Bay Ball; MB: Mini-Bay Ball; PB: Pallet Ball. Individual balls were numbered to aid diver navigation of the artificial reef

cage to attract individual fish closer to the camera to assist with identification. BRUVs were lowered into the central region of each reef complex and the sediment controls and natural reef sites. Footage was recorded using a high definition video camera (Canon HF100) with a Canon ×0.7 wide-angle lens. The focus was manually set to infinity to maximise the distance fish remain in focus and ensure consistent focus across all recordings, and 60 min of footage was recorded per drop. Footage was analysed by the same person in Pinnacle Studio 12® using a time code overlay. Species were identified using Gomon et al. (2008) and the index of relative abundance (MaxN) was recorded. MaxN is the maximum number of individuals of a species recorded in the frame at any one time during the 60 min recording period and is considered a conservative estimation of a species relative abundance (Willis et al. 2000, Cappo et al. 2004).

UVC of fish assemblages involved three 50-m transects at each location. At the sediment, natural reef and artificial reef sites prior to reef deployment, a shot line was lowered from a boat at a fixed GPS point (adjacent to a corner of the reef [for the artificial reefs]). Each transect started at approximately 5 m from the shot line on a random bearing, with subsequent transects at bearings of approximately 90° intervals from each other to avoid transects overlapping. After artificial reef deployment, individual transects were allocated to 3 randomly selected parallel rows of reef balls (each row covering 50 m length and 18 modules) at each artificial reef site. For each 50 m UVC transect, a diver swam out deploying a transect tape and counting larger mobile benthic and water column fish approximately 3 m either side of the tape to obtain density per 300 m². On the return swim along the transect tape, the diver counted cryptic and smaller benthic fish associated with reef balls, and sediments/natural reef 1 m to one side of the tape for 5 × 5 m segments each separated by 5 m intervals (i.e. counts were done at 50–45, 40–35, 30–25, 20–15 and 10–5 m) to obtain density per 25 m². To prevent variable diver biases, the same person conducted all UVC fish counts throughout the project.

Mean daily sea surface water temperatures were derived from satellite remote sensing data supplied by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) for the central region of Port Phillip Bay.

Sampling design

BRUV and UVC surveys were done twice before artificial reef deployment and 6 times after reef deployment. In a single year, sampling was allocated to 3 × 2 mo periods based on annual water temperature regimes and prior knowledge of seasonality of fish assemblages in the Port Phillip Bay (Jenkins et al. 1997, Jenkins & Wheatley 1998). These were summer: November to December (typical temperature range 18 to 21°C, increasing), autumn: February to March (typical temperature range 21 to 19°C, decreasing) and winter: June to July (typical temperature range 9 to 12°C, bottom of annual cycle). All locations were sampled in the period of peak fish abundance in November to December 2008 and February to March 2009, prior to the reefs being deployed in May 2009. After deployment, 6 sample periods were completed: June to July 2009, November to December 2009, February to March 2010, June

to July 2010, November to December 2010 and February to March 2011.

For each survey period, UVC was done once at each site for a total of 72 samples and BRUV was done 4 times with approximately 2 wk between each BRUV sampling event for a total of 288 samples. Sampling times within a sampling day were randomised among sites and all were completed between 09:00 and 16:00 h (full daylight) to reduce bias from natural diurnal changes in fish behaviour (Willis et al. 2006) and tidal influence (Taylor et al. 2013). BRUV and UVC surveys were completed at least 1 wk apart.

Data analysis

Species richness

To provide a comprehensive picture of the species found at each habitat, data from the BRUV and UVC sampling were combined at each site and converted to presence/absence for each location at each time of sampling. To show the detection of new species at each habitat over time, the cumulative number of species was plotted over time.

Statistical analysis of species richness and fish assemblage composition only used data from November to December and February to March, as there was no sampling in July to August before the artificial reefs were installed (July to August results are presented graphically). Two separate linear models were required due to the paired set up for artificial reefs and sediment controls with separate randomly arranged natural reef comparison locations. The interaction between habitat and before versus after reef deployment is the key test for this type of BACI design.

Surface water temperature was plotted for comparison with temporal variation in fish species numbers.

Model 1: Artificial reefs vs. sediments

In this model, location was a blocking factor for the comparison of artificial reefs and sediments. Habitat treatments (artificial reefs, sediment controls) and time were analysed as fixed factors crossed with location as a random effect.

Model 2: Artificial reefs vs. natural reefs

In this model, location was nested for the comparison of artificial reefs and natural reefs. Habitat treat-

ments (artificial reefs, natural reefs) and time were analysed as fixed factors with location as a random effect nested within habitat treatment.

To reduce the likelihood of a Type I error, statistical tests were only done on specific contrasts to test the BACI interactions between habitat treatment and before vs. after (Year 1), and habitat treatment and before vs. after (Year 2). For both models, data were checked using visual inspections of boxplots for assumptions of linear models with no transformation required. Paired comparisons were done for significant interactions. Analysis was done in R 3.3.1 (R Core Team 2016).

Fish assemblage composition

BRUV and UVC data were analysed separately because each method samples species differently. The results from each method were compared to investigate the impact of artificial reef deployment on fish assemblages. All data were fourth-root transformed to downweigh the contributions of quantitatively dominant species (Clarke & Warwick 2001) and were analysed using a distance-based permutational multivariate analysis of variance (PERMANOVA) applied to a Bray-Curtis similarity matrix (Anderson 2001). To test for differences in fish assemblages between habitats and over time, the linear models used for species richness were applied, as were the specific contrasts that tested the interactions between treatment and before vs. after (Year 1) and before vs. after (Year 2). Significant contrasts were investigated further using pairwise tests comparing times before vs. after (Year 1) and before vs. after (Year 2) separately for each habitat. Interpretation of the pairwise tests used Monte Carlo p-values, as there were not enough possible permutations of the raw data to make statistical inferences at a significance level of 0.05 (Anderson & Gorley 2008).

Differences in fish assemblages identified by PERMANOVA between habitats over time for BRUV and UVC were visualised using multi-dimensional scaling (MDS) of fourth-root transformed data (Anderson 2001, Clarke & Warwick 2001). For the MDS plots, the centroids of the replicate samples within each 2 mo sampling period were plotted for each location of each habitat treatment. To highlight species responsible for differences between habitats, a canonical analysis of principal coordinates (CAP, Anderson & Willis 2003) was used. Individual species likely to be responsible for observed differences between habitats were determined by examining Pearson cor-

relations between abundance data and canonical axes. A correlation of $r > 0.8$ was used to identify strong relationships between individual species and the canonical axis (Anderson & Gorley 2008).

Individual species

Individual species identified by CAP as likely to be responsible for the observed differences between habitats were plotted over time.

RESULTS

Species richness

The combined BRUV and UVC surveys across the entire survey period recorded 70 fish species, 56 species were recorded in the 288 BRUV samples and 53 species in the 72 UVC samples (Table A1 in the Appendix). Of these, 42 species were recorded by both methods, 13 species were only recorded by BRUV and 15 species were only recorded by UVC. A cumulative total of 56 species (42 families) were recorded on natural reefs, 53 species (38 families) on artificial reefs and 29 species (29 families) on sediments. Thirteen species were recorded only on natural reefs: *Seriola lalandi* (family Carangidae), *Girella zebra* (family Girellidae), *Tilodon sexfasciatum* (family Microcanthidae), *Scorpius lineolata* (family Kypnosidae), *Enoplosus armatus* (family Enoplosidae), *Cheilodactylus nigripes* (family Cheilodactylidae), *Parma victoriae* (family Pomacentridae), *Notolabrus tetricus*, *Pictilabrus laticlavus* (family Labridae), *Sphyræna novaehollandiae* (family Sphyrænidae), *Thyrsites atun* (family Gempylidae), *Meuschenia flaviolineata* and *M. hippocrepis* (family Monocanthidae). With the exception of *Thyrsites atun*, *Sphyræna novaehollandiae* and *Seriola lalandi*, these are all considered reef-obligate species (Norman & Jones 1984, Barrett 1995, Shepherd & Clarkson 2001, Connell 2002, Ross et al. 2007, Gomon et al. 2008, Ferguson et al. 2016). Nine species were recorded only on the artificial reefs: *Noorynchus cepedianus* (family Hexanchidae), *Genypterus tigerinus* (family Ophidiidae), *Atherinason* spp. (family Atherinidae), *Cyttus australis* (family Cyttidae), *Maxillocosta scabriceps*, *Nesosebastes scorpaenoides* (family Neobastidae), *Nemadactylus macropterus* (family Cheilodactylidae), *Seriolella brama* (family Carangidae) and *Thamnaconus degeni* (family Monocanthidae). Of these, only 4 species were detected more than once:

Nemadactylus macropterus, *Thamnaconus degeni*, *Genypterus tigerinus* and *Nesosebastes scorpaenoides*. Four species were unique to sediment controls: *Mustelus antarcticus* (family Heterodontus), *Hyporhamphus melnochir* (family Hemiramphidae), *Pegasus lancifer* (family Pagasidae) and unidentified Plueronectiformes (order Plueronectiformes). Of these species, only the cryptic sediment dwelling *Pegasus lancifer* was recorded more than once.

The detection of new species (i.e. cumulative species number) followed a similar trajectory on natural reef and sediment controls, albeit with considerably more species on natural reefs (Fig. 3a). On artificial reefs, there was a rapid increase in new species detected during the first year after reef deployment

(17), followed by a plateauing in the subsequent year, similar to natural reefs with only 4 new species detected in both habitats in the last year of the study (Fig. 3a). The cumulative number of species recorded at artificial reefs did not exceed that of natural reefs by the end of the study period (Fig. 3a).

Seasonal variation of species richness was consistent among all habitats. There was a decline in species richness recorded during winter (June to July), except for the artificial reef treatment immediately following reef deployment when the number of species was increasing (Fig. 3b).

Artificial reefs vs. sediments

The linear model for artificial reefs and sediment controls showed a significant interaction between habitat treatment \times before vs after (Year 1) ($F_{(1,10)} = 30.08$, $p < 0.001$) and before vs. after (Year 2) ($F_{(1,10)} = 18.75$, $p < 0.01$). Interactions were driven by an increase in species richness at the artificial reef locations (before vs. after 1: $p < 0.001$, before vs. after 2: $p < 0.001$). Species richness did not change at sediment controls (before vs. after 1: $p = 0.81$, before vs. after 2: $p = 0.87$). Fig. 3b clearly shows an increase in species richness at both times (Year 1 and Year 2) after artificial reef deployment compared to before the artificial reefs were deployed. Artificial reef treatments were similar to sediments before artificial reef deployment, whereas after artificial reef deployment, a higher number of species were detected on artificial reefs at all times while species richness on sediments showed comparatively less variation (Fig. 3b).

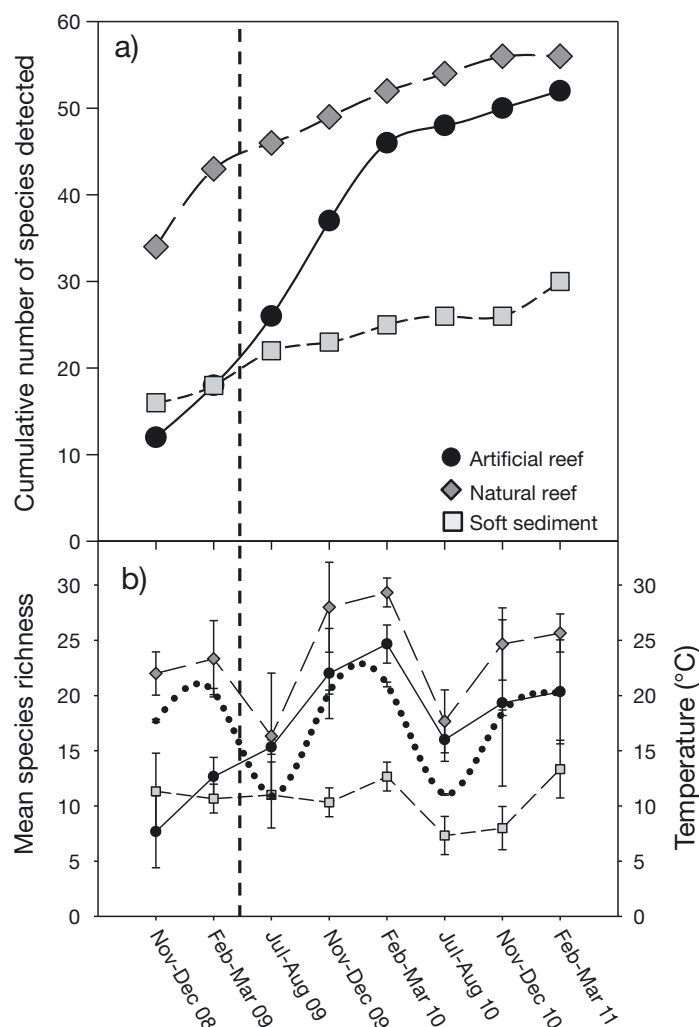


Fig. 3. Data from combined baited remote underwater video (BRUV) and underwater visual census (UVC). (a) Cumulative number of species, (b) mean species richness of data pooled across all sites (\pm SE) and water temperature (dotted line). Reef deployment in May 2009 is indicated by the vertical dashed line

Artificial reefs vs. natural reefs

The linear model for artificial reefs and natural reefs showed a significant interaction between habitat treatment \times before vs. after (Year 1) ($F_{(1,20)} = 77$, $p < 0.01$) and before vs. after (Year 2) ($F_{(1,20)} = 77$, $p < 0.01$). Interactions were mostly driven by an increase in species richness at the artificial reef locations following artificial reef deployment (Fig. 3b, before vs. after 1: $p < 0.001$, before vs. after 2: $p < 0.001$). Species richness increased significantly at natural reefs in the first year post artificial reef deployment (Fig. 3b, before vs. after 1: $p < 0.01$) but not in the second year (before vs. after 2: $p = 0.20$). Species richness at artificial reefs was lower than natural reefs at all times except during winter (Fig. 3b).

Fish assemblage composition

Artificial reefs vs. sediments

The PERMANOVA for BRUV comparing artificial reefs to sediments showed a significant habitat treatment \times before vs. after (Year 1) interaction (pseudo- $F_{(1,2)} = 6.5373$, $p = 0.026$) and a significant habitat treatment \times before vs. after (Year 2) interaction (pseudo- $F_{(1,2)} = 4.8823$, $p = 0.044$). Pairwise comparisons within the level of habitat for time found significant differences for sediments (before vs. after 1: $p = 0.009$ and before vs. after 2: $p = 0.02$) and artificial reefs (before vs. after 1: $p = 0.021$ and before vs. after 2: $p = 0.006$).

For UVC, the comparison was significant for both habitat treatment \times before vs. after (Year 1) interactions (pseudo- $F_{(1,4)} = 13.001$, $p = 0.002$) and habitat treatment \times before vs. after (Year 2) interactions (pseudo- $F_{(1,4)} = 16.862$, $p = 0.001$). Pairwise comparisons found significant differences at artificial reefs (before vs. after 1: $p = 0.002$ and before vs. after 2: $p = 0.002$). Sediments were not significantly different for the first comparison (before vs. after 1: $p = 0.5526$), but were for the second (before vs. after 2: $p = 0.0423$).

The MDS for both survey techniques illustrated the divergence of the artificial reef sites after reef deployment to form their own unique assemblage (Fig. 4a,b). The stress for both survey methods was however relatively high, 0.14 and 0.13 for BRUV and UVC, respec-

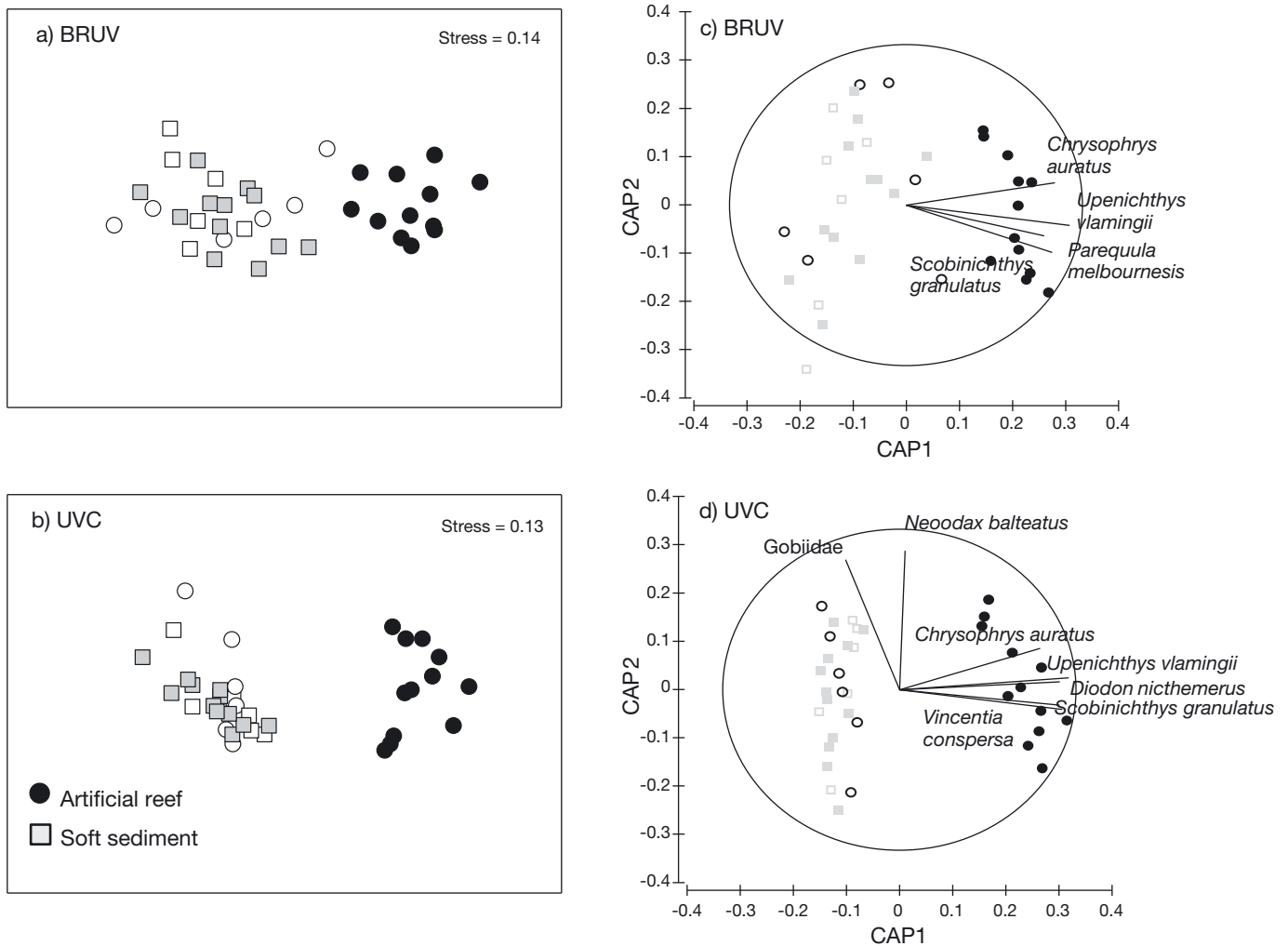


Fig. 4. (a,b) Multi-dimensional scaling of fish communities from sediments and artificial reefs using fourth-root transformed data from each location at each time. (c,d) Canonical analysis of principal coordinates (CAP) of fish communities from sediments and artificial reefs using fourth-root transformed data from each location at each time, showing individual species that were positively correlated with an axis (Pearson correlation > 0.8). The length of vector indicates the strength of the relationship. Open symbols: before artificial reef deployment; filled symbols: after artificial reef deployment (May 2009). (a,c) Baited remote underwater video (BRUV), (b,d) underwater visual census (UVC)

tively (Fig. 4a,b). Pearson's correlation of CAP variables ($r > 0.8$) for BRUV indicated that *Chrysophrys auratus* (family Sparidae), *Parequula melbournensis* (family Gerridae), *Upenichthys vlamingii* (family Mullidae) and *Scobinichthys granulatus* (family Monacanthidae) were positively correlated with artificial reefs (Fig. 4c). Except for *P. melbournensis*, the above species were also identified using UVC. In addition, *Diodon nithemerus* (family Diodontidae) and *Vincentia conspersa* (family Apogonidae) were also positively correlated with artificial reefs using UVC (Fig. 4d). Gobiidae (family Gobiidae) were found to be more closely associated with sediments, while *Neodax balteatus* (family Labridae) was between sediments and artificial reefs (Fig. 4d).

Artificial reefs vs. natural reefs

For BRUV, the comparison of artificial reefs to natural reefs was significant for both interactions: habitat treatment \times before vs. after (Year 1) (pseudo- $F_{(1,4)} = 4.0985$, $p = 0.025$) and habitat treatment \times before vs. after (Year 2) (pseudo- $F_{(1,4)} = 4.0303$, $p = 0.016$). Pairwise comparisons found that this was driven by significant differences at artificial reefs (before vs. after 1: $p = 0.01$ and before vs. after 2: $p = 0.011$). Natural reefs were not significantly different (before vs. after 1: $p = 0.056$ and before vs. after 2: $p = 0.147$).

For UVC, habitat treatment \times before vs. after (Year 1) (pseudo- $F_{(1,4)} = 8.3289$, $p = 0.003$) and habitat treatment \times before vs. after (Year 2) (pseudo- $F_{(1,4)} = 7.9978$, $p = 0.007$) interactions were significant for artificial reef and natural reef comparisons. Pairwise comparisons found that this was driven by significant differences at artificial reefs (before vs. after 1: $p = 0.004$ and before vs. after 2: $p = 0.001$). Natural reefs were not significantly different (before vs. after 1: $p = 0.176$ and before vs. after 2: $p = 0.651$).

The MDS for BRUV and UVC shows that the artificial reef sites after reef deployment are more like natural reefs (Fig. 5a,b). The pattern was more evident for UVC than BRUV, with BRUV having a higher stress of 0.14 (Fig. 5a) compared to 0.05 for UVC (Fig. 5b). Pearson's correlation of CAP variables ($r > 0.8$) for BRUV indicated *U. vlamingii* (family Mullidae) and *Platycephalus* spp. (family Platycephalidae) were associated with artificial reefs (Fig. 5c). *Trachinops caudimaculatus* (family Plesiopidae) and *Meuschenia hippocrepsis* (family Monacanthidae) were strongly associated with natural reefs (Fig. 5c). *S. granulatus* was between artificial and natural reefs (Fig. 5c). Pearson's correlation of CAP variables ($|r| > 0.8$) for UVC

identified *T. caudimaculatus*, *Parma victoriae* (family, Pomacentridae) and *Trinorfolkia* spp. (family Tripterygiidae) were strongly associated with natural reefs (Fig. 5d). *V. conspersa* and *U. vlamingii* were between artificial and natural reefs, with no species found to be strongly associated with artificial reefs (Fig. 5d).

Individual species

***Chrysophrys auratus*.** *C. auratus*, the main fishery species, were observed mostly as juveniles (estimated < 30 cm total length, data not shown) and were detected at all times/seasons on the natural reef and artificial reef habitats, but were only detected on sediments during the summer and autumn periods using BRUV (Fig. 6a). *C. auratus* were observed in higher abundances at the artificial reef sites compared to sediments both before and after artificial reefs were deployed, but the differences were more pronounced after deployment (Fig. 6a). UVC recorded more *C. auratus* at artificial reefs compared to both sediments and natural reefs from the first summer after artificial reef deployment until the end of the study (Fig. 6b).

***Platycephalus* spp.** *Platycephalus* spp. were generally recorded in higher average abundances using BRUV than UVC, with very few detections on natural reefs (Fig. 6c). With BRUV, *Platycephalus* spp. abundances decreased at artificial reefs compared to sediments for the last 4 sample times (Fig. 6c). However, the relative abundance of *Platycephalus* spp. at artificial reefs and sediments detected using UVC varied across time (Fig. 6d).

***Upenichthys vlamingii*.** Both methods detected *U. vlamingii* in higher abundances at the artificial reef sites following reef deployment compared to sediments and natural reefs, with UVC detecting higher numbers than BRUV (Fig. 6e,f). The number of *U. vlamingii* detected by BRUV on artificial reefs was always larger than sediment controls and increased after artificial reef deployment (Fig. 6e,f). UVC consistently detected more *U. vlamingii* on artificial reef locations from the first summer after reef deployment until the end of the study.

***Scobinichthys granulatus*.** *S. granulatus* were detected by BRUV and UVC at the artificial reef sites only after reef deployment (Fig. 6g,h). For BRUV, *S. granulatus* were detected at the artificial reefs post reef deployment in abundances similar to natural reefs. More *S. granulatus* were detected in autumn in both years after reef deployment than on sediments (Fig. 6g). Using UVC, low numbers of *S. granulatus* were detected until February to March 2010 when

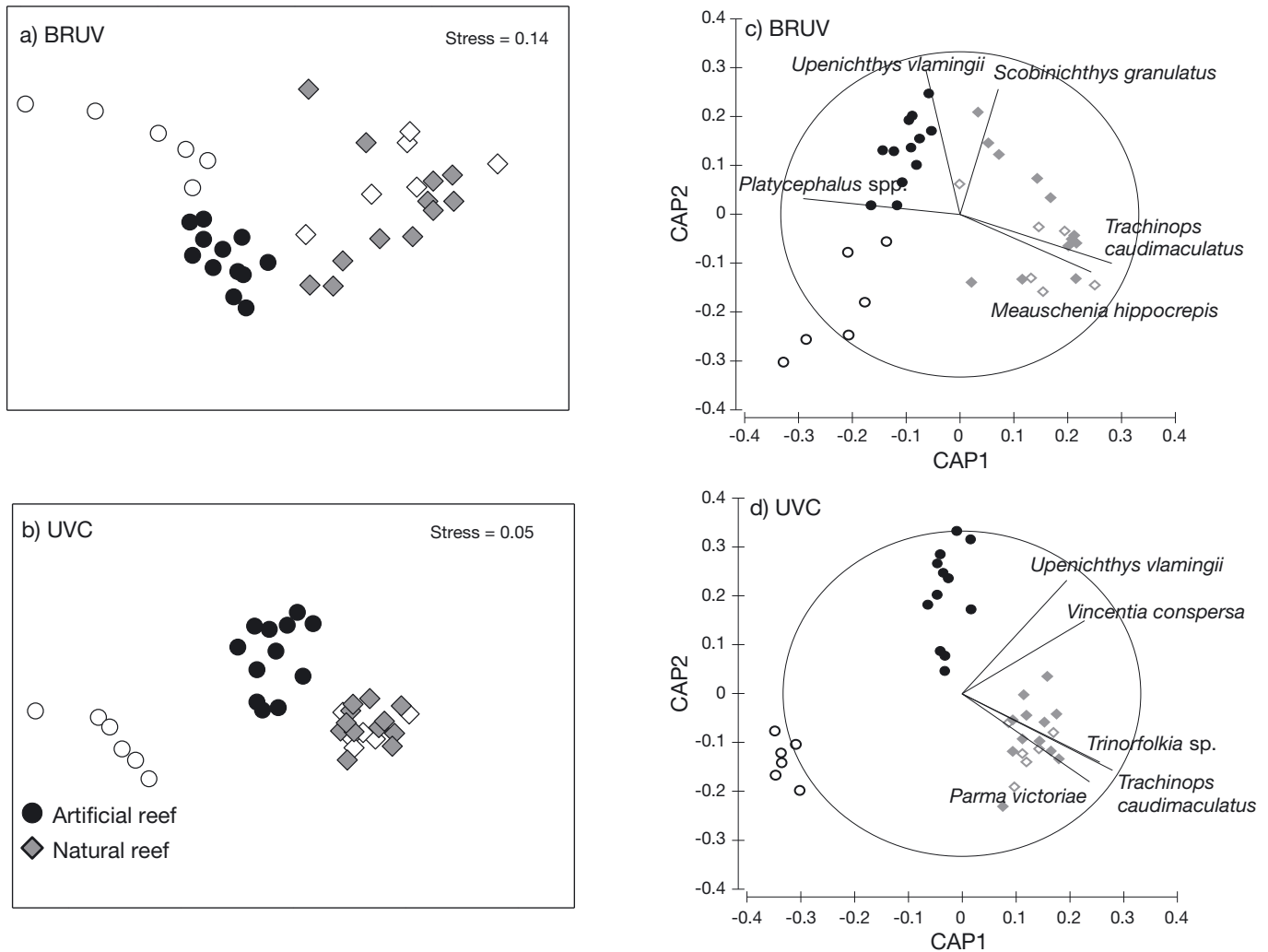


Fig. 5. (a,b) Multi-dimensional scaling of fish communities from natural and artificial reefs using fourth-root transformed data from each location at each time. (c,d) Canonical analysis of principal coordinates (CAP) of fish communities from natural and artificial reefs using fourth-root transformed data from each location at each time. See Fig. 4 for details. (a,c) Baited remote underwater video (BRUV), (b,d) underwater visual census (UVC)

more *S. granulatus* were recorded at artificial reefs than natural reefs and sediments. The numbers of *S. granulatus* remained higher at artificial reefs for the remainder of the study (Fig. 6h).

***Neodax balteatus*.** *N. balteatus* were detected in higher abundances at natural reefs than sediments or artificial reefs using BRUV, with no consistent pattern of increase after artificial reef deployment (Fig. 6i). Using UVC, *N. balteatus* were mostly detected in higher abundances at natural reefs than artificial reefs or soft sediments (Fig. 6j). Abundances of *N. balteatus* remained similar between sediment and artificial reefs throughout the study (Fig. 6j).

***Vincentia conspersa*.** Using BRUV, *V. conspersa* were only detected on natural reefs and in low numbers (Fig. 6k). *V. conspersa* were detected at artificial

reefs following reef deployment in numbers similar to natural reefs using UVC (Fig. 6l). *V. conspersa* were only detected on sediment at the last sampling occasion (Fig. 6l).

***Trinorfolkia* spp.** *Trinorfolkia* spp. were not detected using BRUV (Fig. 6m). Using UVC, *Trinorfolkia* spp. were detected at all times on natural reefs and on 2 occasions on artificial reefs following reef deployment (Fig. 6n).

***Diodon nictemerus*.** *D. nictemerus* were detected in similar numbers in all habitats using BRUV (Fig. 6o). The abundance of *D. nictemerus* detected using UVC on artificial reefs increased after reef deployment and remained similar to natural reefs for the remainder of the study (Fig. 6p). *D. nictemerus* were rarely detected on sediments using UVC (Fig. 6p).

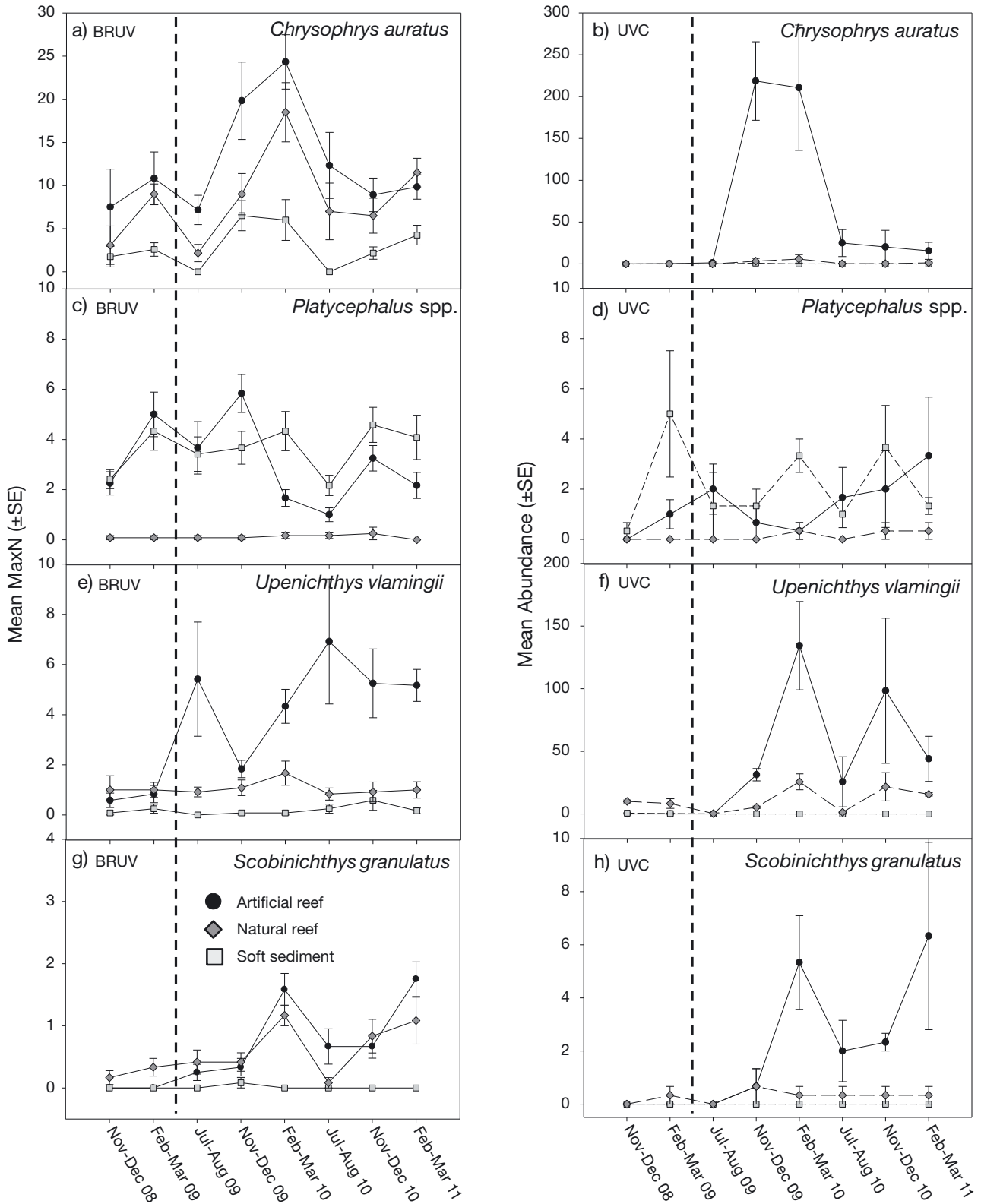


Fig. 6. Relative abundance (\pm SE) of individual species pooled across all sites, sampled by (a, c, e, g, i, k, m, o, q, s, u) baited remote underwater video (BRUV) and (b, d, f, h, j, l, n, p, r, t, v) underwater visual census (UVC). Vertical dashed line indicates artificial reef deployment. (a,b) *Chrysophrys auratus*, (c,d) *Platycephalus* spp., (e,f) *Upenichthys vlamingii*, (g,h) *Scobinichthys granulatus*, (i,j) *Neodax balteatus*, (k,l) *Vincentia conspersa*, (m,n) *Trinorfolkia* spp., (o,p) *Diodon nichthemerus*, (q,r) *Parequula melbournensis*, (s,t) *Trachinops caudimaculatus*, (u,v) Gobiidae

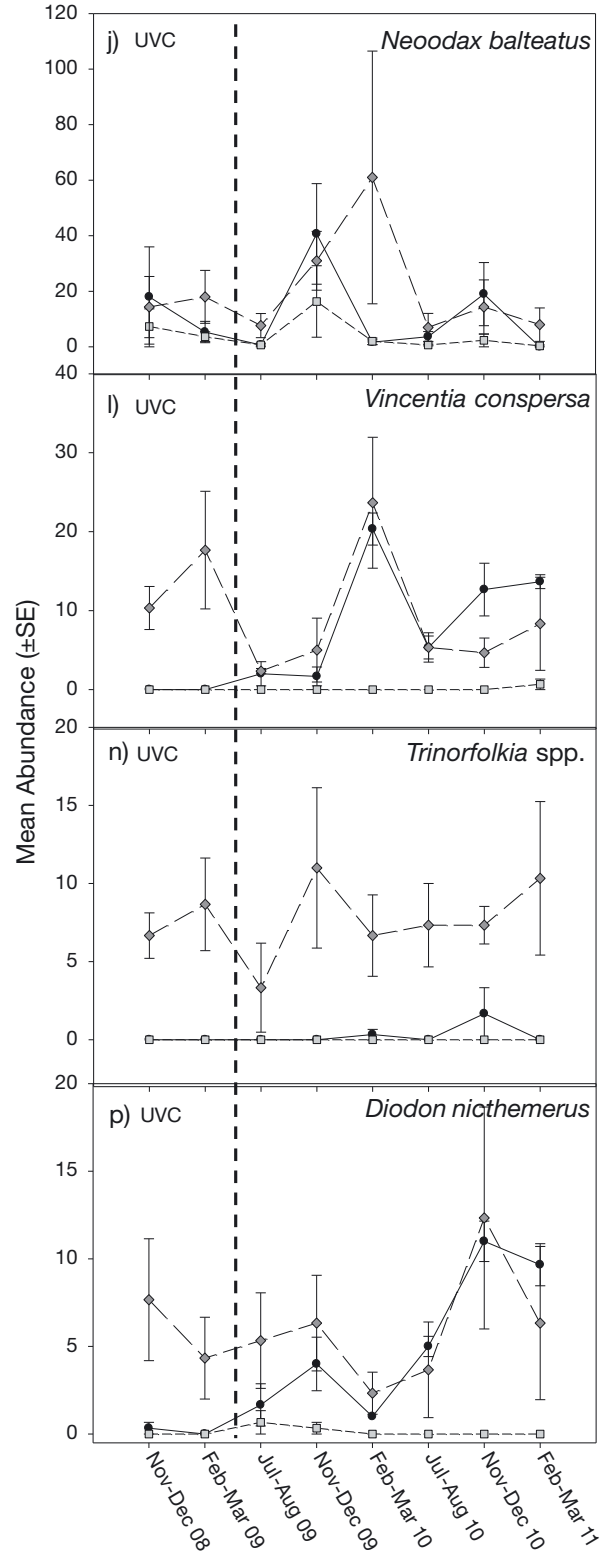
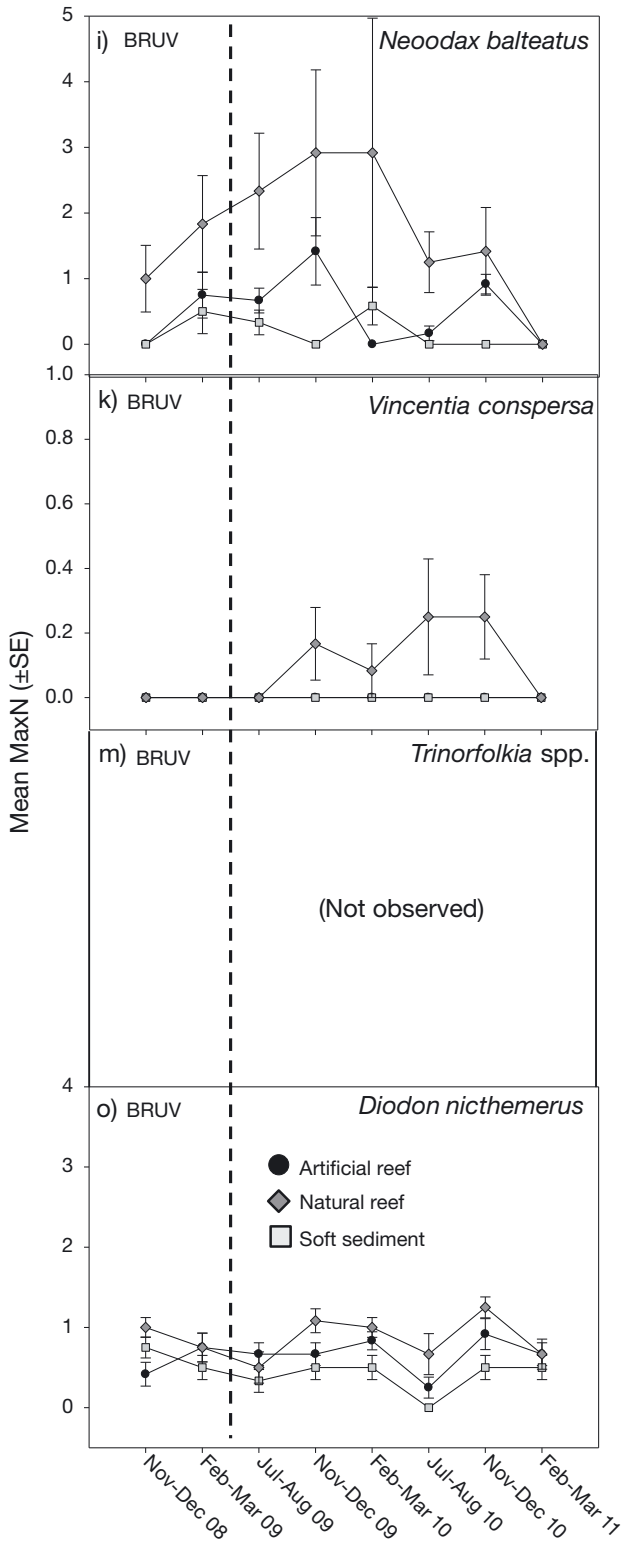


Fig. 6 (continued)

***Parequula melbournensis*.** *P. melbournensis* were mostly detected on artificial reefs after artificial reef deployment (Fig. 6q,r). More *P. melbournensis* were

recorded at artificial reefs on the last 3 sampling times using BRUV (Fig. 6q) and on 3 of the last 4 sampling times using UVC (Fig. 6r). No *P. melbournensis*

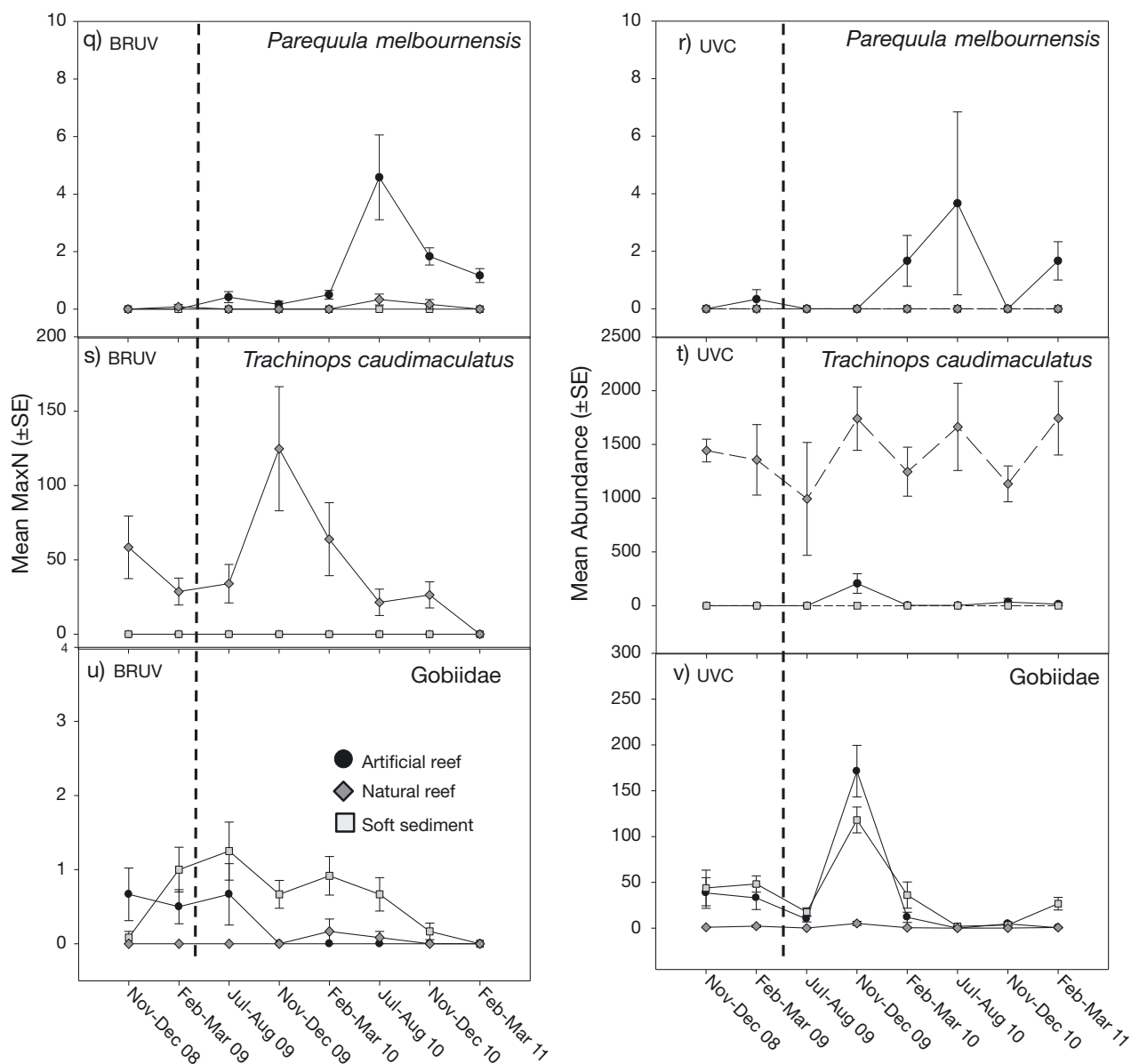


Fig. 6 (continued)

were detected on sediments and natural reefs using UVC and only a few were observed at the natural reefs using BRUV (Fig. 6q,r).

***Trachinops caudimaculatus*.** Using BRUV, *T. caudimaculatus* were only detected on natural reefs (Fig. 6s). Using UVC, however, *T. caudimaculatus* were also detected on artificial reefs, although in lower numbers than natural reefs with persistent populations failing to establish on the artificial reefs (Fig. 6t).

Gobiidae. Using BRUV, Gobiidae were only detected in small numbers during the survey. Numbers decreased at artificial reef sites following reef deployment, with none being detected in the last 5 sampling periods (Fig. 6u). UVC showed similar patterns

of abundance of Gobiidae on artificial reefs and sediments with a large recruitment event occurring in both habitats in November to December 2009 (Fig. 6v). Gobiidae were rarely detected on natural reefs using either method (Fig. 6v).

DISCUSSION

The deployment of patchwork artificial reefs on sandy substrate in Port Phillip Bay resulted in major increases in fish abundance and species numbers over the first 6 mo post-deployment. Species richness at artificial reefs was similar to natural reefs after

10 mo, with the detection rate of new species on artificial reefs plateauing 2 yr after deployment. The fish assemblages that developed on the artificial reefs were different to those found on sediment controls and natural reef comparison sites. The patchwork layout of the reefs facilitated an assemblage that was intermediate between a natural reef and sediment habitat. The patchwork artificial reefs were characterised by species that favour the natural reef/sediment interface, e.g. *Chrysophrys auratus* and *Upenichthys vlamingii* (Ross et al. 2007), and species that used the artificial reef structures for shelter, e.g. *Vincentia conspersa* and *Diodon nithemerus*, which were mostly observed inside the reef balls or in small cavities between the sediment and the reef ball bases.

Similar to many other studies, the patchwork artificial reefs in Port Phillip Bay supported elevated abundances and diversity of fish compared to nearby sediment controls (Bohnsack et al. 1994, Santos & Monteiro 2007, Santos et al. 2011, Folpp et al. 2013, Lowry et al. 2014). *Parequula melbournensis*, *Scobinichthys granulatus*, *U. vlamingii* and juvenile *C. auratus* were positively associated with artificial reefs and all derive most of their food from benthic sources (Platell et al. 1997, Ross et al. 2007, Svane et al. 2007, Currie & Sorokin 2010). The deployment of artificial reefs would likely have opened up new, under-exploited foraging areas and potentially increased foraging success (Bohnsack & Sutherland 1985). Additionally, the numerically dominant *C. auratus* and *U. vlamingii* have been found to be more abundant in areas of complex habitats (in this case a patchwork of artificial reef), due to the provision of a balance of food acquisition and refuge for predator avoidance (Ross et al. 2007, Parsons et al. 2013, 2014).

There is minimal information on the impact of artificial reefs on the pre-existing sediment fish assemblages because artificial reefs studies have rarely sampled before artificial reefs have been deployed (the exception is Lowry et al. 2014 who only sampled at the artificial reef deployment sites). Using a BACI design, we found that the abundance of the 2 most common sediment dwelling species, *Platycephalus* spp. and Gobiidae spp., remained consistent with the controls after artificial reef deployment. The patchwork arrangement of the reefs provided enough sediment habitat for the local populations to persist. The numerically dominant sediment-dwelling Gobiidae spp. are comprised of over 20 species in Port Phillip Bay, including 3 introduced species (Hewitt et al. 2004). Gobiidae spp. spawn most of the year with peaks observed in winter and summer (Jenkins 1986), are generally small (60 to 140 mm) and are

known to have short life spans of 12 to 14 mo with high adult mortality and annual survivorship of ≤ 2 to 3% (Hernaman & Munday 2005a,b). The fluctuations in Gobiidae spp. abundance that we observed with UVC can be attributed to their life history and episodic recruitment rather than habitat type, with 1 successful recruitment event detected at artificial reefs and sediments during the study. The different pattern of abundance found for Gobiidae spp. using BRUV is due to their small size and not being attracted off the sea bed to the bait cage. Individual fish were difficult to detect unless close to the camera, and interspecies interactions associated with the presence of bait potentially kept Gobiidae spp. outside the field of view (Lowry et al. 2012).

Unlike recent studies on the east coast of Australia (Edwards & Smith 2005, Folpp et al. 2013, Lowry et al. 2014) and elsewhere (Ambrose & Swarbrick 1989, Bohnsack et al. 1994, Carr & Hixon 1997, Clark & Edwards 1999, Burt et al. 2009, Granneman & Steele 2015), artificial reefs in our study did not support more species than nearby natural reefs. Possible reasons for the difference in species numbers are topographic complexity, proximity to natural reefs, age, fouling assemblages and depth between study sites (Burchmore et al. 1985, Thanner et al. 2006). In our study, the lack of structural complexity (ledges, crevices, small holes, etc.) provided by the reef balls and the limited algal growth on them due to their depth and the water clarity were likely to have affected the uptake and retention of cryptic and/or herbivorous reef obligate species (Charbonnel et al. 2002, Sherman et al. 2002). This is highlighted by the large number of highly site attached *Trachinops caudimaculatus* (Ford & Swearer 2013) that recruited and then subsequently almost completely disappeared from artificial reefs. Insufficient habitat availability and structure can negatively affect the survival of prey species on artificial reefs (Hixon & Beets 1993). The lack of cryptic refuge afforded by the reef balls may have resulted in high predation rates of *T. caudimaculatus* (Ford & Swearer 2013). Additionally, 9 reef-obligate species detected on natural reefs were not observed on the artificial reefs, indicating that for some species there was little movement or recruitment from natural reefs. This may be due to the isolation of the reefs from natural reefs and the non-contiguous design of the patch reefs being unable to sustain a population of reef-obligate species (Walsh 1985, Bohnsack et al. 1994).

C. auratus in Port Phillip Bay support the state's largest marine recreational fishery (Hamer & Jenkins 2004, Hamer et al. 2005). Sexually mature *C. auratus* (6+ yr old) spawn during the warmer months (Nov-

ember to February) in Port Phillip Bay, with successful recruits settling to the sediment after 3 to 4 wk in the water column (Hamer & Jenkins 2004, Hamer et al. 2005). Newly settled recruits and juvenile (1 to 2 yr old) *C. auratus* rapidly colonised the artificial reefs in our study (K.A. Mills unpubl. data). Sparids have been frequently observed around artificial structures in higher numbers than natural reefs (Clynick et al. 2008, Folpp et al. 2013, Lowry et al. 2014), with the increased access to food afforded by the larger 'edge-to-area' ratio (amount of sand/reef interface) suggested as a possible reason (Folpp et al. 2013). The patch reef design used in our study provided a greater edge-to-area ratio than placing the reef balls closer together. Additionally, structured habitat is known to be preferred by recently settled *C. auratus* and juveniles during this critical life stage (Ross et al. 2007, Parsons et al. 2014). It is during this critical life stage that artificial reefs may potentially increase survival of juveniles through the provision of additional habitat and food resources.

The combination of BRUV and UVC provided a comprehensive picture of spatial and temporal variation in species assemblages (Lowry et al. 2014). The use of BRUV on artificial reefs has been found to complement UVC by providing increased coverage of species known to be diver averse (Lowry et al. 2012), while UVC samples cryptic and territorial species more effectively (Colton & Swearer 2010, Folpp et al. 2013, Lowry et al. 2014). In this study, direct comparisons of methods were not made due to the inequality in sampling intensity, yet there are clear benefits of a particular method when sampling some species. For example, *T. caudimaculatus* were not detected by BRUV at artificial reefs despite 100s recruiting 6 mo after reef deployment, while *C. auratus* were less frequently, and more variably, detected by UVC. Studies on artificial reefs need to carefully consider key species of interest before choosing a sampling method, both to maximise cost-effectiveness of sampling approaches and to detect important variations in abundance.

In our study, artificial reef deployment increased fish diversity, but the resulting assemblage and abundance dynamics were influenced by large scale processes such as recruitment and migration. This was evident in the seasonal fluctuations of species numbers on natural and artificial reefs, as many species migrate into Port Phillip Bay from coastal waters during spring and summer, as the water warms, and out again in autumn and winter as the water cools (Parry et al. 1995). Further, the use of a BACI design caters for the spatial and temporal variation which

can be high in fish assemblages, while the time frame of 2 yr was sufficient to determine the impact of artificial reef deployment on species accumulation and fish assemblages.

Artificial reefs are becoming increasingly popular with anglers and fisheries agencies. Their continued deployment should consider their potential impact on existing assemblages. The results from our study suggest that small patch reefs can be deployed without negatively impacting existing sediment fish assemblages while also increasing species diversity and abundance on the artificial reefs. The contribution that small-scale artificial reefs make to the increased production of the key target species, snapper, that utilise reefs for components of their life-history and are not dependent on the structure 'per se', may be difficult to measure and cannot simply be inferred from higher abundances at the artificial reefs than at the other habitats (Osenberg et al. 2002). Indirect approaches to inferring production benefits of reefs to snapper might involve measuring growth rates, feeding success, and condition indices for small juveniles that recruit to the reefs compared to sediment and natural reefs (Brickhill et al. 2005).

The increase in *C. auratus* numbers in our study due to artificial reef deployment has the potential to increase their catch rate and fishing-related mortality, as anglers have been found to fish artificial reefs more intensely than surrounding sediment areas (McGlenon & Branden 1994, Keller et al. 2016). As artificial reefs are increasingly being built for recreational angling, future research needs to be directed at constructing artificial reefs that benefit individual species of interest, whether to improve survival and growth of species or enhance fisheries. It should be remembered that any enhancement of fisheries through increasing catch rates or making species easier to target may negatively impact the population and this needs to be incorporated into the management of the species.

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Appendix. Table A1. Species sampled by baited remote underwater video (BRUV) and underwater visual census (UVC). *Species only detected once

Family	Species	ARTIFICIAL REEFS		SOFT SEDIMENT		NATURAL REEF	
		BRUV	UVC	BRUV	UVC	BRUV	UVC
Heterodontus	<i>Heterodontus portjacksoni</i>	●		●		●*	
	<i>Mustelus antarcticus</i>			●*			
Hexanchidae	<i>Notorynchus cepedianus</i>	●*					
Rhinobatidae	<i>Trygonorrhina fasciata</i>	●	■	●	■	●	
Urolophidae	<i>Urolophidae</i> sp.	●	■	●	■	●	■
Dasyatidae	<i>Dasyatis brevicaudata</i>	●		●		●	■
Myliobatidae	<i>Myliobatis australis</i>	●		●		●	
	<i>Pseudophycis</i> sp.	●	■	●*		●	■*
Ophidiidae	<i>Genypterus tigerinus</i>		■				
Atherinidae	<i>Atherinason</i> spp.		■*				
Hemiramphidae	<i>Hyporhamphus melanochir</i>			●*			
Zeidae	<i>Cyttus australis</i>	●*					
Syngnathidae	<i>Hippocampus bleekeri</i>		■				■*
Pegasidae	<i>Pegasus lancifer</i>				■		
Scorpaenidae	<i>Scorpaena papillosa</i>		■*				■*
Neosebastidae	<i>Maxillocosta scabriceps</i>		■*				
	<i>Nesosebastes scorpaenoides</i>	●					
Tetrarogidae	<i>Gymnapistes marmoratus</i>		■		■*		■*
Triglidae	<i>Lepidotrigla mulhali</i>		■		■		
Platycephalidae	<i>Platycephalus</i> spp.	●	■	●	■	●	■
Serranidae	<i>Caesioerca rasor</i>		■				■
Plesiopidae	<i>Trachinops caudimaculatus</i>		■			●	■
Apogonidae	<i>Siphamia cephalotes</i>		■			●	■
	<i>Vincentia conspersa</i>		■		■	●	■
Dinolestidae	<i>Dinolestes lewini</i>	●*				●	
Sillaginidae	<i>Sillaginodes punctata</i>	●				●	
Carangidae	<i>Pseudocarax dentex</i>	●	■	●		●	
	<i>Seriola lalandi</i>					●*	
	<i>Trachurus novaezelandiae</i>	●	■	●		●	
Gerreidae	<i>Parequula melbournensis</i>	●	■			●	
Sparidae	<i>Chrysophrys auratus</i>	●	■	●	■	●	■
Mullidae	<i>Upenichthys vlamingii</i>	●	■	●	■	●	■
Arripidae	<i>Arripis georgianus</i>		■*				■
Girellidae	<i>Girella zebra</i>					●	■
Microcanthidae	<i>Tilodon sexfasciatum</i>					●	■
Scorpididae	<i>Scorpis lineolata</i>					●	■
Enoplosidae	<i>Enoplosus armatus</i>					●	■
Pentacerotidae	<i>Pentaceropsis recurvirostris</i>			●*		●	■*
Cheilodactylidae	<i>Cheilodactylus nigripes</i>					●*	

Appendix. Table A1 (continued)

Family	Species	ARTIFICIAL REEFS		SOFT SEDIMENT		NATURAL REEF	
		BRUV	UVC	BRUV	UVC	BRUV	UVC
Cheilodactylidae	<i>Nemadactylus macropterus</i>	●					
	<i>Dactylophora nigricans</i>		■*			●	■
Pomacentridae	<i>Parma victoriae</i>					●	■
Labridae	<i>Notolabrus tetricus</i>					●	■
	<i>Pictolabrus laticlavus</i>					●*	■
Odacidae	<i>Neoodax balteatus</i>	●	■	●	■	●	■
Tripterygiidae	<i>Trinorfolkia</i> spp.		■				■
Blenniidae	Unidentified Blenniidae		■*	●*		●*	■
Clinidae	<i>Cristiceps</i> spp.		■*				■
Bovichtidae	<i>Bovichtus angustifrons</i>	●	■		■		■
Gobiidae	Unidentified Gobiidae	●	■	●	■	●	■
Sphyrinae	<i>Sphyræna novaehollandiae</i>					●	■
Gempylidae	<i>Thyrsites atun</i>					●*	
Centrolophidae	<i>Seriola brama</i>	●*					
Order Pleuronectiformes	Unidentified Pleuronectiformes			●*			
Monacanthidae	<i>Acanthaluteres spilomelanurus</i>	●	■	●	■*	●	■
	<i>Acanthaluteres vittiger</i>	●				●	
	<i>Brachaluteres jacksonianus</i>	●*	■	●*	■	●	■
	<i>Eubalichthys mosaicus</i>	●	■			●*	■
	<i>Meschenia flavolineata</i>					●	■
	<i>Meschenia freycineti</i>	●				●	■
	<i>Meschenia hippocrepis</i>					●	■
	<i>Meschenia scaber</i>	●	■			●	
	<i>Nelusetta ayraudi</i>	●	■	●*		●	
	<i>Scobinichthys granulatus</i>	●	■	●*		●	■
	<i>Thamnaconus dageni</i>	●	■				
Tetraodontidae	<i>Contusus brevicaudus</i>	●		●		●	
	<i>Tetractenos glaber</i>		■*		■*	●	■
	<i>Diodon nictemerus</i>	●	■	●	■	●	■
Engraulidae	<i>Engraulis australis</i>	●		●		●	
Arripidae	<i>Arripis</i> sp.	●	■	●		●	
	Number of species	35	38	26	16	48	39
	Total number of species		52		32		56

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