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# Application of non-destructive methods for assessing rock pool fish assemblages on Lord Howe Island, Australia

Tom R. Davis [a,b,*], Meryl F. Larkin [b], David Harasti [a]

[a] Fisheries Research, Marine Ecosystems, NSW Department of Primary Industries, Locked Bag 1, Nelson Bay, NSW 2315, Australia
[b] National Marine Science Centre, Southern Cross University, PO Box 4321, Coffs Harbour, NSW 2450, Australia

## HIGHLIGHTS

- Fish assemblages were evaluated in rock pools using three non-destructive techniques.
- Performance of evaluation techniques varied with the size of the rock pool examined.
- Results demonstrated that non-destructive methods can be used to give valuable data on rock pool fishes.
- Data analysis showed the unique nature of rock pool fish assemblages on Lord Howe Island, Australia.

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## ABSTRACT

Rock pool fish assemblages can be highly diverse, though are poorly studied in many locations. Where rock pool fishes have been studied, sampling has often been undertaken using destructive techniques, causing fish mortality and damage to pool ecosystems. There is, therefore, a need for greater understanding of non-destructive methods for evaluating rock pool fish assemblages. To improve knowledge in this area, we tested three non-destructive techniques: mini baited remote underwater videos (mini-BRUVs) which utilised a stationary video camera to record fish, visual censuses (VCs) where a roving observer recorded fish using a slate and observer operated videos (OOVs) where fish were recorded by an observer using a video camera.

These methods were tested for their effectiveness in assessing fish assemblages, using data from rock pools on Lord Howe Island (LHI), in New South Wales (NSW), Australia. VCs and OOVs required significantly less total survey time than mini-BRUVs, whereas mini-BRUVs provided advantages in the detection of fishes in small pools ($<25$ m$^3$). Examination of mini-BRUVs' data, for 30 min samples, identified that the vast majority of fish species were detected within 15 min and indicated that a shorter 15 min sampling period would generally be suitable for collecting fish data from rock pools. The baseline data collected on fishes in LHI rock pools demonstrated the uniqueness of LHI assemblages, which were significantly different from those on the NSW coast. This study demonstrates that non-destructive sampling methods can be used in rock pool environments, making these methods suitable for sampling in inter-tidal habitats, especially within marine protected areas.



## 1. Introduction

Pools in intertidal areas of rocky reefs (rock pools) can be highly variable in size, geometry, substrate and water chemistry, which is in part due to their being exposed to harsh environmental conditions (most notably sun and waves) (Griffiths et al., 2003; Harasti et al., 2014). Consequently, these pools provide a wide variety of habitats, and are known to contain diverse fish assemblages (Mahon and Mahon, 1994; Harasti et al., 2016). Numerous studies have been conducted on rock pool fish assemblages to quantify local species diversity (Bennett, 1987; González-Murcia et al., 2012), assess biogeographical patterns (Griffiths, 2003; Okada et al., 2015; Harasti et al., 2016), evaluate the importance of rock pools for juvenile and threatened fish species (Bennett, 1987; Harasti et al., 2014; Dias et al., 2016) and understand the effects of intertidal zonation and habitat (Griffiths et al., 2003, 2006).

Rock pool fish studies have implemented a range of sampling techniques, with some methods considered to have detrimental impacts on rock pool ecosystems. Methods such as removing

* Corresponding author at: Fisheries Research, Marine Ecosystems, NSW Department of Primary Industries, Locked Bag 1, Nelson Bay, NSW 2315, Australia.
*E-mail address:* davistn1@gmail.com (T.R. Davis).

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fish using nets (Griffiths, 2003; Okada et al., 2015), introducing chemicals (e.g. clove oil or Rotenone) to pools to tranquilise or kill fish (Bennett, 1987; Prochazka, 1996; Griffiths, 2000, 2003; González-Murcia et al., 2012) and pumping pools dry to capture fish (Griffiths, 2000; Griffiths et al., 2003) are all considered to be destructive sampling techniques. These sampling methods are frequently used with the intent of sampling all of the fish in pools (Griffiths, 2000; González-Murcia et al., 2012), although the ability of these methods to capture all fish present has been questioned (Griffiths, 2000). The degree to which all species need to be identified and enumerated is study question specific and, in studies of ecological patterns, methods often only need to reliably sample the more prevalent species in order to answer a range of ecological questions (Andrew and Mapstone, 1987).

With increasingly stringent ethical standards applied in scientific research, there is a need for improved understanding of the performance of non-destructive sampling methods, for rock pool fish assemblages, in order to better understand where they can be successfully applied. This is especially important in highly protected locations such as marine protected areas (MPAs), where research permits frequently require the use of methods that minimise disturbances to marine organisms (NSW DPI, 2018). Less intrusive sampling methods applied in rock pool studies, with minimal impact on pool ecosystems (non-destructive sampling methods), include using visual censuses (VCs, Griffiths, 2003; Harasti et al., 2014) and mini baited remote underwater videos (mini-BRUVs, Harasti et al., 2014; Ebner and Morgan, 2013). Another method that is considered to be non-destructive is the application of observer operated videos (OOVs), which are widely used for surveying subtidal fish assemblages (Watson et al., 2005; Holmes et al., 2013; Davis et al., 2015); however, there is no documented evidence of this method being applied within rock pool environments. In rock pools, OOVs have the potential to provide a rapid and effective method for assessing fish assemblages, combining some of the benefits of both VCs and mini-BRUVs, with relatively short times for data collection (similar to VCs) while giving a permanent visual record of fishes present (as per mini-BRUVs).

To evaluate the application of non-destructive sampling methods for assessing fish assemblages in rock pools, a study was implemented within the Lord Howe Island Marine Park (LHI) in New South Wales (NSW), Australia. Lord Howe Island was selected for this study as this World Heritage listed island has several rock platforms containing rock pools, with very limited existing information on the fish species that these contain. The only previous published quantitative research examining fish in rock pools at LHI was focussed on the detection of juveniles of the threatened black cod *Epinephelus daemelii* (Harasti et al., 2014) and did not examine other species present.

An additional important consideration in ecological studies is the selection of suitable sampling units (Gladstone et al., 2012; Unsworth et al., 2014) and here we further explored different sampling durations for studying fishes in rock pools using mini-BRUVs. In a previous study on mini-BRUV durations in rock pools, Harasti et al. (2014) found no significant difference in fish species richness between 30 min and 60 min samples; however, no examination of shorter sampling durations was documented. It is inarguable that longer durations will, on average, detect a greater number of fish species, however, shorter times allow the collection of more samples and thereby potentially provide increased power for subsequent analyses (Harasti et al., 2015).

The primary focus of this study was to compare three different non-destructive survey methods (mini-BRUVs, VCs and OOVs) for their ability to assess rock pool fish assemblages. Secondly, we hypothesised that, within the relatively confined volume of rock pools, mini-BRUVs with sample durations of 15 min would provide representations of fish assemblages, comparable to 30 min samples, at reduced cost in terms of total set-up, sampling and post-processing times. Thirdly, as no data have been previously collected on LHI rock pool fish assemblages, data were examined to identify whether rock pools on LHI provide nursery areas for threatened and endemic species and species of importance to fishers. Data were also compared with a study from the northern NSW coast (Harasti et al., 2016) to identify whether fish assemblages on LHI were significantly different from those on the NSW mainland.

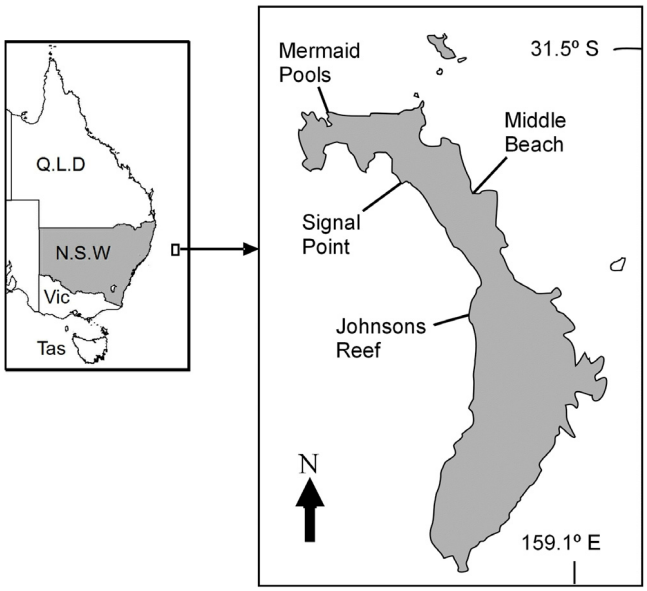


**Fig. 1.** Study sites on Lord Howe Island.

## 2. Methodology

Surveys of fish assemblages were conducted in six rock pools at each of four sites on LHI (Mermaid Pools, Middle Beach, Signal Point, Johnsons Reef; Fig. 1). These sites were selected for their extensive and easily accessible intertidal rock platforms, with rock pools at each site selected to have depths >0.20 m and areas >1 $m^2$, to allow comparison with data from the study by Harasti et al. (2016) along the NSW coast. Rock pools were completely separated from the ocean when surveyed (Fig. 2a, b), to prevent fish entering or leaving during surveys and to ensure minimal ocean influence from waves entering pools. The depth (D), length (L), width (W), substrate and dominant benthic habitat type of each pool was recorded along with the time of each survey and the low tide time. The approximate volume (V) of pools was estimated from these measurements using the relationship $V = L \times W \times D/2$ proposed by Mahon and Mahon (1994) with selected pools varying from 1.5–270 $m^3$ with depths ranging from 0.3–2.0 m (Table 1).

### 2.1. Sampling methods

#### 2.1.1. Difference in sampling methods

Sampling of fish assemblages was conducted using three alternate methods: mini-BRUVs, VCs and OOVs. Fish assemblage data was collected as the abundance of each species, in each pool, for each method. All sampling, in each pool, was conducted on the same tide to prevent fish assemblages changing between sampling methods. Preliminary testing identified that it was not feasible to test all three methods, in each pool, within a single tidal cycle. Sampling with VCs was, therefore, only conducted at Signal Point and Middle Beach and sampling with OOVs was only conducted at

**Table 1**
Substrates, habitats, sizes and sampling methods for pools surveyed on intertidal rock platforms at sites on Lord Howe Island.

| Site | Johnsons Reef | Mermaid Pools | Middle Beach | Signal Point |
|---|---|---|---|---|
| Size range ($m^3$) | 1.5–240 | 12–42 | 4.8–60 | 4.4–270 |
| Depth range (m) | 0.3–1.2 | 1.0–2.0 | 0.4–1.0 | 0.5–1.0 |
| Pool substrates | sand/flat rock | boulders | sand/flat rock | sand/rubble |
| Dominant benthic habitats | macroalgae, seagrass | hard corals, macroalgae | macroalgae, hard corals | macroalgae, hard corals |
| Sampling methods (pools sampled) | mini-BRUVs (n = 6), Observer operated Video (n = 6) | mini-BRUVs (n = 6), Observer operated Video (n = 6) | mini-BRUVs (n = 6), Visual census (n = 6) | mini-BRUVs (n = 6), Visual census (n = 6) |

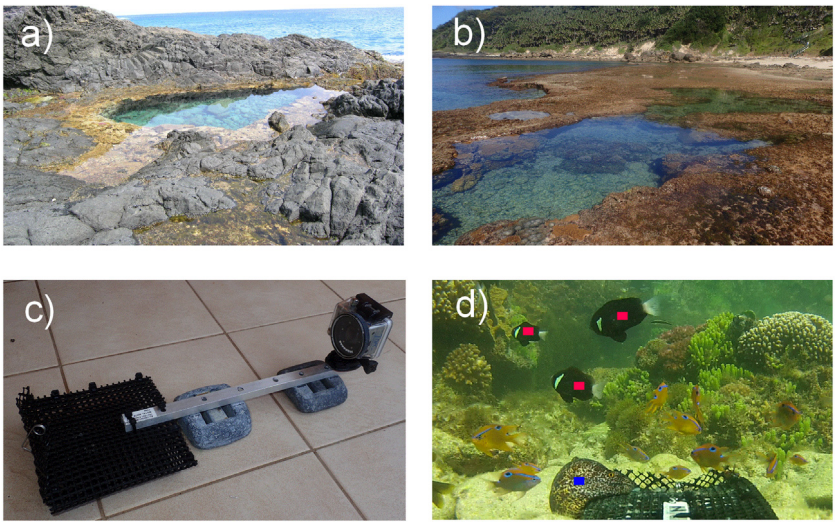


**Fig. 2.** (a) Rock platform and pool at Mermaid Pools; (b) rock platform and pool at Middle Beach; (c) mini-BRUV camera arrangement; (d) Video frame with endemic *Gymnothorax annasona* (blue square) and *Amphiprion mccullochi* (red squares) in a pool at Middle Beach.

Mermaid Pools and Johnsons Reef (Table 1). Sampling using mini-BRUVs was conducted at all sites to provide data for comparison with the other two methods and to provide additional replicates for comparisons between different mini-BRUV sampling durations. Sampling involved conducting either the VC or OOV method first and then subsequently deploying the mini-BRUV. All sampling was conducted within 2 h either side of low tide, between 06.00 and 18.00 hrs. A period of at least 5 min was taken between sampling using the different methods to allow fish to recover from any disturbances caused by the previous sampling method. The total survey time, as combined set-up, in-field sampling and post processing times, for VC, OOV and mini-BRUV surveys, were recorded to enable assessment of their relative time efficiency.

*2.1.2. Sampling method for mini-BRUVs*

The methodology used for mini-BRUVs followed that of Harasti et al. (2016), with each mini-BRUV consisting of a GoPro HERO 2 camera facing along a 30 cm weighted arm towards a mesh bag containing 3 pilchards (Fig. 2c). Cameras were set to record a 170° field of view at a resolution of 1920 × 1080. Mini-BRUVs were deployed for 30 min and positioned on the bottom of each pool, facing away from the sun (where possible) to improve video quality for analysis. All post-processing was conducted by a single person, familiar with LHI fishes (lead author), using SeaGIS 5.01 Eventmeasure software.

*2.1.3. Sampling duration comparison for mini-BRUVs*

Comparisons between sampling durations (i.e. 15 and 30 min) for mini-BRUVs were conducted by re-sampling three randomly selected pools at each site, using 15 min sampling durations, and comparing these data with data from the remaining three pools, at the same site, with 30 min sampling durations.

*2.1.4. Sampling method for visual censuses*

Surveys using VCs involved an observer (lead author) with mask and snorkel conducting a thorough search in each pool, with all fish species recorded on an underwater slate. Searches included inspecting under ledges with a torch to detect cryptic and cave-dwelling species. To standardise effort VCs were conducted by completing a circuit of each pool, thoroughly examining all areas until no additional fish species were being located.

*2.1.5. Sampling method for observer operated videos*

Surveys using OOVs involved observers (lead and second author) conducting a thorough search in each pool, recording all fish observed using a submerged GoPro HERO 2 camera mounted on a pole, with the camera set up as per the mini-BRUVs. To standardise effort OOVs were conducted by completing a circuit of each pool, thoroughly examining all areas until no additional fish species were being located. Importantly this methodology did not require expert knowledge of fish species for fieldwork, as was required for VCs, allowing data collection by less-experienced personnel (Holmes et al., 2013; Davis et al., 2015). All post-processing, however, was conducted by a single person familiar with LHI fishes (lead author), using SeaGIS Eventmeasure software.

*2.2. Statistical analysis*

*2.2.1. Difference in sampling methods*

To assess the ability of each of the three methods to detect species richness (total number of recorded species in each rock pool), a comparison was conducted using paired t-tests, for repeated measures of species richness, with rock pools as replicates.

While total survey times varied among methods, comparisons between methods were based on pairs of samples from the same spatially constrained area (i.e. a single rock pool for each comparison), thereby ensuring sampling consistency between methods. Comparisons between mini-BRUVs and VCs were conducted using data from Signal Point and Middle Beach (n = 12), while comparisons between mini-BRUVs and OOVs were conducted using data from Mermaid Pools and Johnsons Reef (n = 12). Permutational ANOVA (PERMANOVA), using PRIMER 7 PERMANOVA+ software (Anderson et al., 2008), was used to test for differences in fish assemblages (as presence–absence data), using a two-factor design, with factors of method (2 levels, fixed) and site (2 levels, random). Comparisons of assemblages, amongst methods, used species presence–absence data due to inherent differences in abundance measures obtained by the differing methods. A non-metric multidimensional scaling (nMDS) analysis using Primer 7 software package (Clarke and Gorley, 2015) was conducted to visualise similarities in fish assemblages between sampling methods across study sites. Comparisons of the total survey time, amongst different sampling methods, were conducted using paired t-tests.

#### 2.2.2. Effect of pool volume on species richness

Tests for correlations between fish species richness and pool volume were conducted using the "cor.test" function in R (R Core Team, 2014), with pool volume log transformed as recommended by Mahon and Mahon (1994). Paired t-tests were used to compare the relative ability of stationary sampling (i.e. mini-BRUVs) and roving sampling (i.e. VCs and OOVs) to detect fish species in pools of different sizes (i.e. for small pools with volume $<25$ m$^3$, n = 13 and for large pools with volume $\geq 25$ m$^3$, n = 11).

#### 2.2.3. Sampling duration comparison

Testing for differences in species assemblages, between sampling durations (15 and 30 min), for mini-BRUVs was conducted using a two-factor PERMANOVA design, with factors of sampling duration (2 levels, fixed) and site (4 levels, random). Testing used abundance data (as MaxN) that was square-root transformed to reduce the effect of highly abundant species, thereby taking into account the relative ability of the different sampling durations to measure fish abundances. The comparison was conducted using data from separate pools, to maintain independence of datasets, allowing the evaluation of whether differences between sampling durations were significant compared to the natural variability that occurred among pools and among sites. The comparison between total survey times, for 15 min and 30 min sampling durations, was conducted using a paired t-test with data from all sites (n = 12). For 15 min and 30 min samples set-up times were generally the same, but post-processing times were typically longer for 30 min sampling, due to the increased time required to watch the longer videos and record additional species data.

#### 2.2.4. Differences in fish assemblages between LHI and northern NSW

Differences in fish assemblages were assessed between sites at LHI (31.55°S, n = 4) and those on the northern NSW coast (i.e. Sandon 29.67°S to Boat Harbour 32.79°S, n = 14, Harasti et al., 2016). A non-metric multidimensional scaling (nMDS) analysis, using the Primer 7 software package (Clarke and Gorley, 2015), was conducted to visualise similarities in fish assemblages, using 30 min mini-BRUV data for sites on LHI and for sites on the NSW coast (extracted from supplementary material from Harasti et al., 2016). Tests for differences in species assemblages, between LHI and the NSW coast, were conducted using a single factor PERMANOVA design, with location (i.e. LHI or NSW) as a fixed factor with two levels, sites as replicates and site assemblages based on the average assemblage across all pools at each site. Similarity Percentages analyses were conducted in Primer 7, using the SIMPER subroutine, to identify the sites on the NSW coast which had the greatest similarity in fish assemblages to sites on LHI. Comparisons between species richness in pools at LHI and the estimated richness at the same latitude on the NSW coast, obtained from the relationship established by Harasti et al. (2016), were conducted by calculating average species richness across five pools at each site, using the EstimateS software (Colwell, 2013), as specified in Harasti et al. (2016).

## 3. Results

A total of 1219 fish from 66 species (22 families) were detected in 24 LHI rock pools (see Supplementary Table S1). The most common species across sites were: *Girella cyanea*, *Neoglyphidodon polyacanthus* and *Stegastes fasciolatus*, accounting for 33% of all fish observed, with the most species-rich families being Labridae (16 species), Pomacentridae (10 species) and Gobiidae (7 species). Endemic species (Francis, 1993; Hobbs et al., 2009) detected in pools were *Coris bulbifrons* (detected at all sites), *Gymnothorax annasona* (Fig. 2d) and *Amphiprion mccullochi* (Fig. 2d) detected in pools at Johnsons Reef and Middle Beach and *Enneapterygius howensis* detected in pools at Middle Beach, Mermaid Pools and Signal Point.

### 3.1. Comparisons among sampling methods

Of the 65 species identified, 54 species were detected using mini-BRUVs (n = 24), 42 by OOVs (n = 12) and 36 by VCs (n = 12). Pairwise comparisons between mini-BRUVs and the other two methods, of their ability to measure fish species richness, found no significant difference between mini-BRUVs ($9.33 \pm 0.79$) and VCs ($9.67 \pm 1.30$, $P = 0.748$) and no significant difference between mini-BRUVs ($9.42 \pm 1.33$) and OOVs ($7.42 \pm 1.33$, $P = 0.200$). PERMANOVA analyses, with presence–absence data, found no significant difference in fish assemblages between mini-BRUVs and VCs ($P = 0.485$) or between mini-BRUVs and OOVs ($P = 0.513$), but found significant differences in assemblages between sites ($P < 0.001$, both tests). No significant interactions were found between methods and sites ($P > 0.532$, both tests), indicating that the similarities in the performances of the different methods were consistent across sites. While we made no direct comparison between VCs and OOVs, we expect that their ability to assess fish assemblages would be similar, as results from both methods were not significantly different from those obtained using mini-BRUVs. Visualisation of similarities in assemblages among methods and sites, using nMDS, showed clustering of similar assemblages by site, but no discernible differences among methods at each site (Fig. 3).

Comparisons of the total survey time, amongst methods, identified that mini-BRUVs required significantly more total survey time ($62.5 \pm 1.7$ min) than OOVs ($12.3 \pm 1.6$ min, $P < 0.001$) and VCs ($8.8 \pm 0.6$ min, $P < 0.001$).

### 3.2. Effect of pool volume

Regardless of the method used for sampling, species richness was found to increase significantly with the log of pool volume ($P < 0.001$, $R^2 = 0.467$, Fig. 4). Comparing the performance of differing methods for sampling in small pools (i.e. volume $< 25$ m$^3$) identified that mini-BRUVs were significantly more effective ($P < 0.001$, n = 13) than the alternate roving methods (VCs and OOVs). In contrast, roving methods detected a greater number of species ($12.4 \pm 1.1$), than mini-BRUVs ($11.6 \pm 1.2$) in the large pools (i.e. volume $\geq 25$ m$^3$), although these differences between methods were not significant ($P = 0.70$, n = 11).

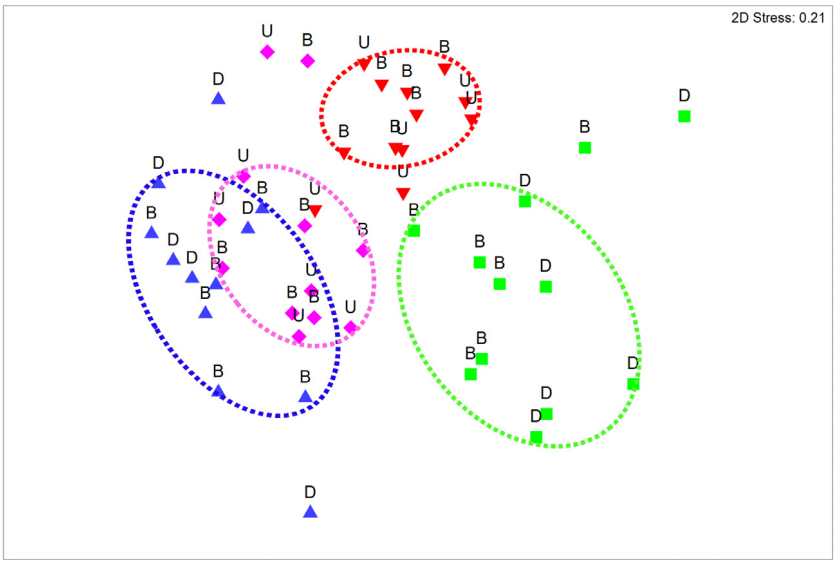


**Fig. 3.** Non-metric multidimensional scaling plot showing relative similarity of fish assemblages in rock pools at Lord Howe Island for data from mini-BRUV (B), VC (U), and OOV (D) surveys at 4 sites; Johnsons Reef (blue triangles), Middle Beach (red inverted triangles), Mermaid pools (Green squares) and Signal Point (pink diamonds). Ellipses enclose 83% of data points for pools at each site.

Combining data from stationary and roving methods was found to provide a better overall representation of the species richness in pools, with results for combined methods giving significantly higher average species richness per pool (11.67 ± 0.94) than that obtained using mini-BRUVs alone (9.38 ± 0.76, $P < 0.001$), with the combined methods capturing a broader range of shy, small and territorial species (e.g. Serranidae, Gobiidae and Muraenidae).

### 3.3. Effect of sampling duration

Examination of the time at which fish species were first detected by mini-BRUVs found that 72.2 ± 4.5% of fish species were detected within 5 min, 86.6 ± 2.6% within 10 min and 92.5 ± 2.1% within 15 min (Fig. 5). Data from mini-BRUVs, therefore, provided an approximation of the true number of species within each pool, with the quality of this approximation increasing as sampling effort increased (Fig. 5). PERMANOVA analysis, for assemblage data from 15 and 30 min mini-BRUV sample durations, however, found no significant differences between durations ($P = 0.135$), but found significant differences between sites ($P < 0.001$), with no significant interactions between sites and durations ($P = 0.977$). These results indicate that differences in fish assemblage data, between 15 min and 30 min sample durations, were small compared to the inherent variability that existed between pools and sites. Examining the total survey time for 15 min and 30 min mini-BRUVs identified that the average total survey time required for 15 min samples (38.7 ± 1.9 min) was significantly less than that required for 30 min samples (60.4 ± 1.8 min, $P < 0.001$).

### 3.4. Comparison of LHI with NSW coastal assemblages

Average species richness of fishes on LHI (22.33 ± 1.66) was comparable with that obtained at the same latitude on the NSW coast (i.e. 22.42 ± 0.33 at 31.55 °S). SIMPER analyses identified that species at sites on LHI were most similar to those from Flat Top Rock (30.14 °S) to Red Head (32.04 °S) on the NSW coast. However species assemblages on LHI were significantly different from those on the coast ($P = 0.002$, Fig. 6) with high levels of dissimilarity between fish assemblages at sites on LHI and those on the NSW coast (i.e. >72%). Only 17 of the 54 species found by mini-BRUVs on LHI were also found in pools on the NSW coast, with the three most common species on LHI (i.e. *G. cyanea*, *N. polyacanthus*, *S. fasciolatus*) not found in any pool on the NSW coast (Harasti et al., 2016). Similarly only 17 of the 113 species found on the NSW coast were also found at LHI, with the two most common species on the NSW coast (*Aldrichetta forsteri*, *Girella elevata*) absent on LHI.

## 4. Discussion

Due to their accessibility rock pools have been used to assess ecological patterns for shallow marine fish species at a range of spatial scales including; global shifts in species assemblages (Griffiths, 2003), regional latitudinal gradients (Okada et al., 2015; Harasti et al., 2016) and local variations influenced by: pool volume (Mahon and Mahon, 1994); height relative to low tide (Davis, 2000; Griffiths et al., 2003); and habitat complexity (Griffiths et al., 2006). The results from this study, and previous studies, indicate that the most appropriate method to apply in a study of rock pool fish assemblages depends on a range of factors including the ethical constraints imposed on survey methods, the size and location of pools to be examined and the behaviours of the species being studied.

This study demonstrated that the non-destructive survey methods tested (i.e. mini-BRUVS, VCs and OOVs) were all capable of providing valuable information on fish assemblages in rock pools. No significant differences were detected among assemblage data collected using these methods, while all methods were found to be capable of detecting significant differences in fish assemblages among sites. This is an important finding, as while more established methods for sampling fishes in rock pools, such as anesthetising fish and pumping pools dry, have been shown to be effective at capturing data on species richness and abundance in pools (Mahon and Mahon, 1994; Griffiths, 2000), these methods are destructive to pool ecosystems through intentional collection of fishes (González-Murcia et al., 2012), or unintentional fish mortality (Griffiths, 2000) and through their potential to harm other organisms in pools (Frisch et al., 2007; Robertson and Smith-Vaniz, 2008). In contrast, the non-destructive methods tested in this study generally had minimal impact on rock pool ecosystems, apart from some limited impacts at entry and exit points. The demonstrated ability of these non-destructive methods to provide valuable data on fish assemblages, therefore, means that these methods are

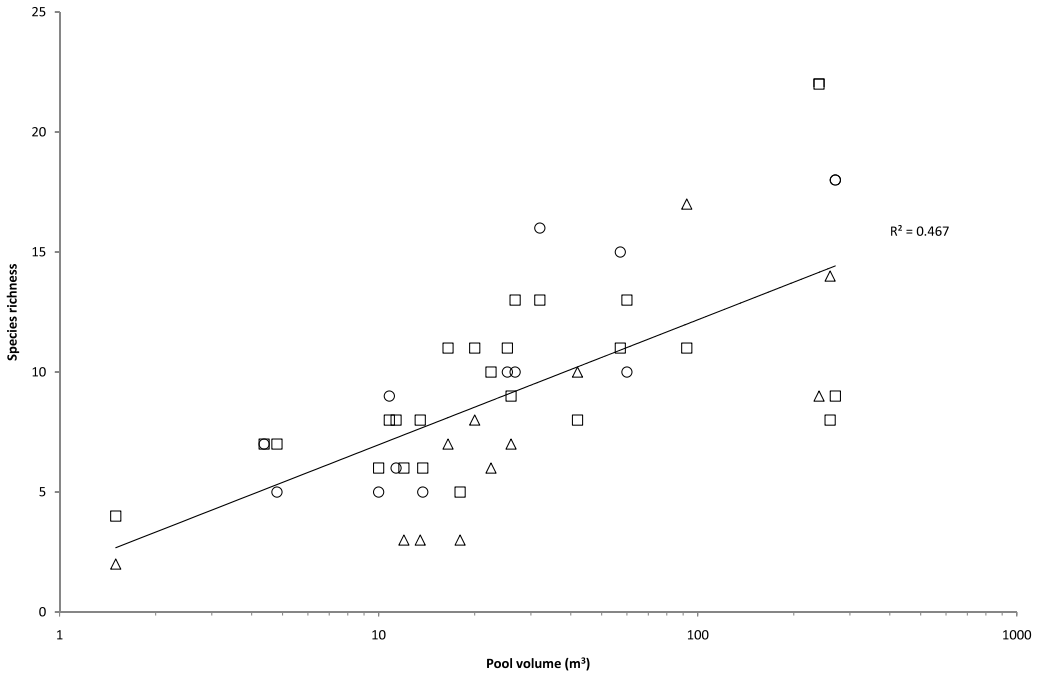


**Fig. 4.** Species richness versus pool volume for rock pools at Lord Howe Island, measured using mini-baited remote underwater videos (Squares), observer operated videos (triangles) and visual census (circles). Line indicates least squares logarithmic regression fit to data.

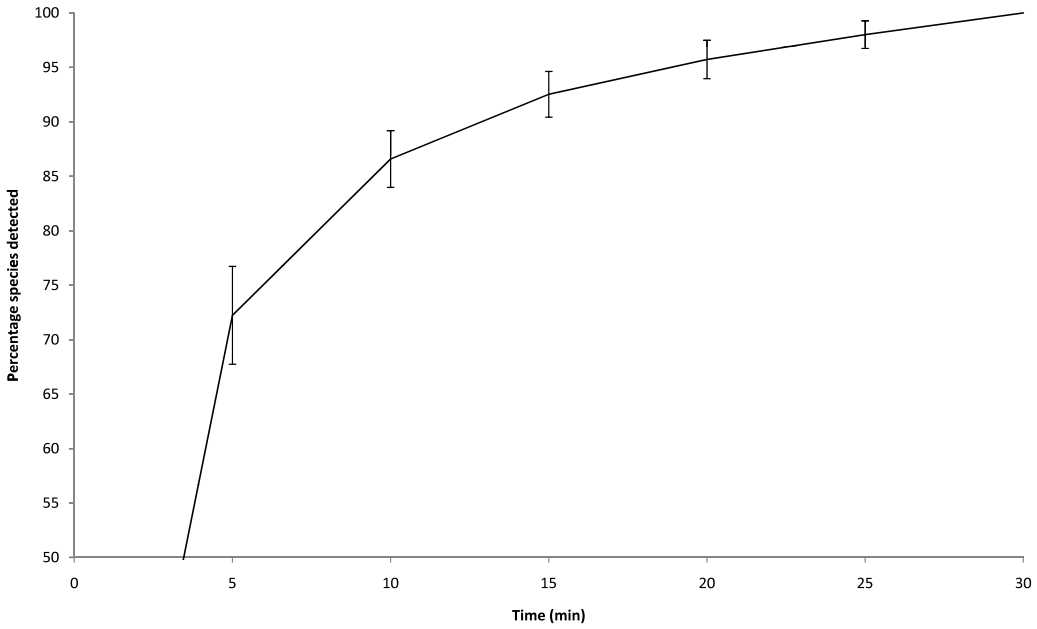


**Fig. 5.** Proportion of total fish species detected (±S.E.) on mini-baited remote underwater videos against elapsed time for 30 min sampling in rock pools on Lord Howe Island (n = 24).

better-suited for monitoring sensitive locations, such as in MPAs, where negative impacts on marine ecosystems are undesirable.

Data from pools at LHI showed high levels of variability amongst pools, with a strong influence of pool volume and significantly higher species richness in larger pools. This result supports previous research findings showing increased species richness in larger pools (Mahon and Mahon, 1994; White et al., 2015) with increases attributed predominantly to larger pools providing more locations for fish to shelter from predation (Mahon and Mahon, 1994). An additional factor that may also have contributed to the observed increase in species richness in larger pools was that larger pools often contained a wider range of benthic habitats (pers. obs.), with fish species richness known to increase as habitat complexity increases (Griffiths et al., 2006; Davis et al., 2016; Davis and Smith, 2017).

Another consideration when selecting the method for surveying fish in rock pools is, therefore, the volumes of the pools to be sampled. Methods such as anesthetising fish and pumping pools dry are constrained, in terms of the pool volume in which they can be applied, due to difficulties associated with anesthetising fish in larger pools or pumping larger pools dry. These methods have, therefore, typically only been applied in studies of very small pools (e.g. $<2.5$ m$^3$) with small pools specifically selected in many studies because of these limitations (Griffiths, 2000; Okada et al., 2015).

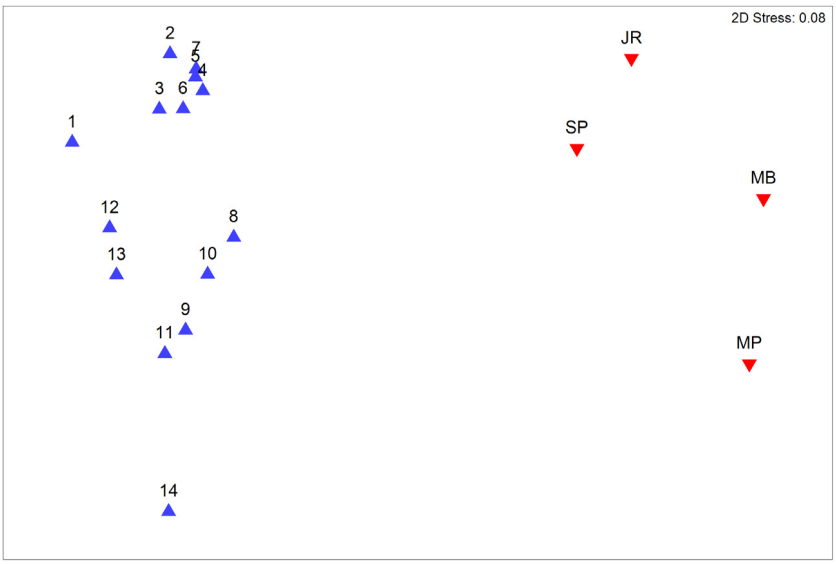


**Fig. 6.** Non-metric multidimensional scaling plot showing relative similarity of average fish assemblages in rock pools at Lord Howe Island (LHI). Inverted triangles with two letter codes indicate sites on LHI as per Fig. 1. Triangles with numbers indicate sites on the New South Wales coast from (1) Sandon, 29.67 °S to (14) Boat Harbour, 32.79 °S per Harasti et al. (2016).

In contrast, the non-destructive methods examined in the current study were found to perform adequately across a wide range of rock pool volumes (i.e. 1.5–270 $m^3$), supporting previous research that demonstrated that mini-BRUVS can successfully be applied to study ecological patterns across a wide range of pool volumes (Harasti et al., 2016). The non-destructive methods examined here are, therefore, well-suited to studying locations with large pools (i.e. $\geq$25 $m^3$), with some rock pools investigated up to 2 orders of magnitude larger than those typically surveyed by pumping pools dry, or using anaesthetics to capture fish.

Comparisons amongst the methods tested, at an individual pool level, identified that mini-BRUVs were more effective than VCs and OOVs at documenting fishes in the confined space of small pools (i.e. $<$25 $m^3$) and at detecting shy fishes (e.g. Serranidae, Muraenidae), potentially due to these fishes avoiding observers in pools. In a study of the presence of *E. daemelii*, in pools in NSW, Harasti et al. (2014) identified that mini-BRUVs were more effective than VCs at detecting cryptic Serranidae and also identified that mini-BRUVs were more effective than VCs at detecting species richness. Our study supports these findings for smaller pools, but we found that VCs and OOVs were generally more effective at measuring species richness in large pools (i.e. $\geq$25 $m^3$). We attribute this to the ability of these roving methods to survey the full extent of larger pools, with mini-BRUVs constrained to a fixed point and, therefore, unable to adequately sample species in larger pools that are not attracted to bait. This limitation of mini-BRUVs could, potentially, be addressed through the application of multiple cameras in large rock pools, an area of research that warrants further investigation. Importantly, VCs and OOVs were found to provide significant advantages, over mini-BRUVs, in terms of the total survey time required for fieldwork and post-processing. Overall, combining mini-BRUVs with one of the roving methods tested (i.e. VC or OOV) provided the most detailed information on species richness for each pool, with mini-BRUVs and roving methods complementing each other in larger pools, giving greater coverage and better data on the presence of shy and cryptic species.

Where there are high levels of variability among study replicates, as is the case for rock pools, it is important that studies have sufficient replication to test the ecological questions under investigation (Andrew and Mapstone, 1987). Minimising the time required for each replicate is, therefore, important as this allows more replicates to be collected in the typically limited time that is available for intertidal fieldwork. A previous study comparing 60 min and 30 min mini-BRUV sampling recommended the use of 30 min sampling for fish assemblages in rock pools (Harasti et al., 2014), but did not examine shorter sample durations. Here we demonstrated that, at LHI, 15 min sampling provided data comparable to 30 min sampling, for fish assemblages in pools, at reduced cost in terms of total survey time for fieldwork and post-processing. The ability to use shorter 15 min sampling is attributed to the relatively short time required for bait plumes to disperse in pools, combined with the short distances that fish need to travel to find mini-BRUVs, with bait plume dispersal linked to effectiveness in BRUV sampling of fish assemblages (Heagney et al., 2007). Use of 15 min mini-BRUV sampling in future studies, in place of 30 min sampling, would provide a range of benefits, allowing more replicates to be conducted on the low tide at each site and reducing costs in terms of fieldwork and video post-processing times. The resulting time savings would allow greater levels of replication which, in turn, would provide greater detail on local fish species diversity, through sampling a wider range of pools. Greater replication would also provide increased power in subsequent testing of ecological patterns. Increased replication, however, would only be possible for those sites where there are sufficient numbers of pools available for sampling, with replication at many sites constrained by the number of suitable pools that are present (Harasti et al., 2016).

Examination of fish species diversity from the replicate samples collected in pools on LHI (64 species in 24 pools), identified that fishes in pools on LHI were relatively diverse, when compared against studies examining similar numbers of pools in other locations, with studies in Japan finding 42 species in 30 pools (Okada et al., 2015), El Salvador finding 19 species in 29 pools (González-Murcia et al., 2012) and Barbados finding 63 species in 19 pools (Mahon and Mahon, 1994). The high species richness captured at LHI is thought to be predominantly due to the many large pools examined on LHI which, due to their size, contained highly diverse fish assemblages (i.e. up to 22 species in a single pool). This finding highlights the potential limitations of studies that only examine fishes in small pools (as is commonly the case due to restrictions imposed by the sampling methods used), with sampling of only small pools potentially leading to underestimation of local species

richness. Fish species richness in rock pools has been shown to decrease with increasing latitude (Okada et al., 2015; Harasti et al., 2016) and species richness on LHI was found to be similar to that measured, using the same methods, at the same latitude on the neighbouring NSW coastline (Harasti et al., 2016). The composition of the fish assemblages on LHI was, however, found to be significantly different from that occurring on the NSW coast, with only a small overlap in the number of species shared between pools at these two locations.

In addition to sheltering diverse fish assemblages, pools on LHI were found to provide refuges for juveniles of a number of endemic, threatened and fishery-targeted species, supporting previous studies that have indicated that rock pools play an important role as safe havens for juvenile fishes (Bennett, 1987; Griffiths, 2003; González-Murcia et al., 2012; Harasti et al., 2014). The detections of numerous juvenile *G. cyanea* and some juvenile *C. bulbifrons* in rock pools was an important study finding, with these species targeted by recreational fishers on LHI (Ferrell, 2005; Hobbs et al., 2009) and indicating that rock pools may play a role in the early development of these species. An additional important record was that of a juvenile Black Cod *E. daemelii*, in a pool at Mermaid Pools, with juveniles of this threatened species previously detected in rock pools along the NSW coast (Griffiths, 2003; Harasti et al., 2014), but not on LHI (Harasti et al., 2014). This detection indicates that juvenile *E. daemelii* may recruit into pools on LHI, although the frequency at which this occurs has not yet been established.

## 5. Conclusion

This study highlights the unique nature of fishes in rock pools on LHI, and demonstrates that rock pools on LHI shelter endemic and threatened species. For monitoring rock pools, the non-destructive methods investigated in this study were shown to provide practical and cost-effective approaches for gathering data on fish assemblages. These non-destructive methods were found to be particularly suited to studies examining larger rock pools and for use in locations where research permits require minimal impact on intertidal ecosystems.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

## Sampling and field studies

All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable.

## Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2018.09.002.

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